



UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO TECNOLÓGICO
PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA QUÍMICA

Clara Dourado Fernandes

**Enzymatic Synthesis of Aliphatic Polyesters for Application in Tissue
Engineering: Development and Characterization of Nanofiber Mats with
Bioactive Glasses**

Florianopolis-SC, Brasil

2025

Clara Dourado Fernandes

Enzymatic Synthesis of Aliphatic Polyesters for Application in Tissue Engineering: Development and Characterization of Nanofiber Mats with Bioactive Glasses

Tese submetida ao Programa de Pós-Graduação em Engenharia Química da Universidade Federal de Santa Catarina como requisito parcial para a obtenção do título de Doutora em Engenharia Química.

Orientador(a): Prof. Dr. Prof. Dr. Pedro H. H. Araújo
Coorientador(a) Prof. Dr. Bruno Francisco Oechsler

Florianópolis -SC

2025

Clara Dourado Fernandes

Enzymatic Synthesis of Aliphatic Polyesters for Application in Tissue Engineering: Development and Characterization of Nanofiber Mats with Bioactive Glasses

Research Project thesis submitted to the Graduate Program in Chemical Engineering at the Federal University of Santa Catarina and Engineering at the Friedrich-Alexander-Universität Erlangen-Nürnberg to obtain the title of Doctor in Chemical Engineering and Engineering respectively.

Advisor: Prof. Dr. Pedro H. H. Araújo

Prof. Dr.-Ing. habil Aldo Boccaccini

Co-advisor: Prof. Dr. Bruno Francisco Oechsler

Florianópolis-SC
2025

Dourado Fernandes, Clara

Enzymatic Synthesis of Aliphatic Polyesters for Application in Tissue Engineering : Development and Characterization of Nanofiber Mats with Bioactive Glasses / Clara Dourado Fernandes ; orientador, Pedro Henrique Hermes de Araújo , orientador, Aldo Boccaccini, coorientador, Bruno Francisco Oechsler, 2025.

172 p.

Tese (doutorado) - Universidade Federal de Santa Catarina, Centro Tecnológico, Programa de Pós-Graduação em Engenharia Química, Florianópolis, 2025.

Inclui referências.

Trabalho elaborado em regime de co-tutela.

1. Engenharia Química. 2. Engenharia de Tecidos. 3. Scaffolds Bioativos. 4. Vidros Bioativos. 5. Policondensação Enzimática. I. Hermes de Araújo , Pedro Henrique. II. Boccaccini, Aldo . III. Oechsler, Bruno Francisco. IV. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Engenharia Química. V. Título.

Clara Dourado Fernandes

Enzymatic Synthesis of Aliphatic Polyesters for Application in Tissue Engineering: Development and Characterization of Nanofiber Mats with Bioactive Glasses

O presente trabalho em nível de Doutorado foi avaliado e aprovado, em 17 de Setembro de 2025, pela banca examinadora composta pelos seguintes membros:

Profa Dra. Andrea Buettner

Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)

Prof. Dr. Dirk Schubert

Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)

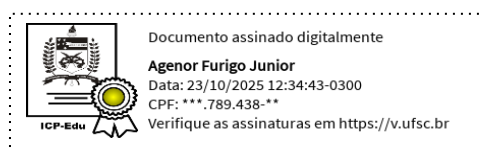
Prof. Dr. Diego Gomez Maldonado

Texas Tech University (TTU)

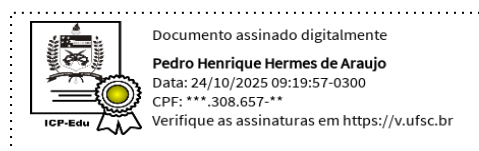
Profa. Dra. Ana Paula Serafini immich Boemo

Universidade Federal de Santa Catarina (UFSC)

Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado adequado para obtenção do título de Doutora em Engenharia Química



Coordenação do Programa de Pós-Graduação



Prof. Dr. Pedro H. H. Araújo

Florianópolis, 2025.

“Research is seeing what everybody else has seen and thinking what nobody else has
thought.” – Albert Szent-Györgyi

Acknowledgements

As I look back and see the path I've traveled to reach this point, I can truly say it was worth facing every difficulty, because it was through them that I became the Doctor I am today. In the heart of the northeastern backlands of Brazil, with no resources, no guidance, fueled only by the dreams of "Dona Nuce" and "Seu Paulo", I learned early on that life's battles are often cruel, but that daily effort can take us to places our ancestors never imagined setting foot in. Being the first in a family of farmers to earn a doctoral degree is a powerful reminder of the strength I carry from my roots.

To my "Painho", "Mainha", Lana, and Paulo, thank you for every sacrifice you made so I could get here. Your prayers, your blessings, and the financial hardships you endured have now transformed into the joy and pride of this historic day for our family. This achievement belongs to many hands, but above all, it stems from the solid foundation you gave me.

To my academic mentors in Brazil, Pedro, Bruno, Cláudia, and Débora, thank you for trusting me with a project that, at first, seemed so distant from my world as an environmental engineer. Thank you for your time, for your dedication to our weekly meetings, and for expanding my understanding of the world of enzymatic polymerization. More than knowledge, you gave me examples of true professionalism, always with respect, active listening, and patience. We grew together, and I will never forget that.

My doctoral journey began amid a pandemic: faces behind masks, social distancing, and empty laboratories. Yet I learned to smile with my eyes, to drink coffee in the open air, and to debate experiments that kept failing, until, slowly, they began to work. And by my side, through it all, was a wonderful person named Fernando Elias. He began his master's degree just as I started my PhD, and he taught me more than he knows. We shared two years of camaraderie and trust, the kind of bond only a pandemic lab could create.

This doctorate also gave me gifts far beyond titles and published articles. Cori, Ana, and Rica, thank you for running this marathon with me. To my soul sisters, Heloísa and Isabella, thank you for the memories we built between Brazil and Germany. You will always have a place in my heart.

Speaking of Germany, I am grateful to everyone at the Institute of Biomaterials, especially Sena, Meng, Iren, Zoya, Marcela, Amanda, and Isabela (again). Thank you for your warm welcome, the coffee breaks, the salsa classes, the beers, and the glühwein at the Christmas markets. You made the best year of my life even more special.

To Professor Boccaccini, my sincere gratitude for accepting me into the dual-degree program. Thank you for opening your lab, for sharing your scientific knowledge, and for teaching me to cultivate precision and seriousness in research. Your guidance was essential in making this work possible.

And finally, but with my whole heart, I thank Caio. My partner, my love, my home across all time zones. With you, every challenge felt softer and every achievement, sweeter. Even when oceans separated us, your presence was constant (in gestures, in words, in mornings of doubt and nights when certainty escaped me). You taught me to persist, to be brave, and to keep going. This victory is yours too. Thank you for walking beside me with so much love. This thesis, more than an ending, is the beginning of so many new dreams we are building together.

DISCLOSURE STATEMENT

Parts of this dissertation were published in:

Dourado Fernandes, C.; Francisco Oechsler, B.; Sayer, C.; Oliveira, D. De; Hermes De Araújo, P. H.

Recent advances and challenges on enzymatic synthesis of biobased polyesters via polycondensation.

European Polymer Journal, v. 169, p. 111132, 15 abr. 2022.

DOI: 10.1016/j.eurpolymj.2022.111132

ISSN: 0014-3057

Dourado Fernandes, C.; Oechsler, B. F.; Sayer, C.; Oliveira, D. De; Hermes De Araújo, P. H.
Enzymatic Synthesis of Hydroxy-Functionalized Glycerol-Based Polyesters: A Study of Functionalization, Regioselectivity Control, and Catalyst Reuse.

Macromolecules, v. 57, p. 456-469, 23 jan. 2023

DOI: 10.1021/acs.macromol.3c01533

ISSN: 15205835

Dourado Fernandes, C.; Grünewald, A.; Hadzhieva, Z.; Oechsler, B. F.; Sayer, C.; Hermes De Araújo, P. H.; Boccaccini, A. R*

Incorporation of Poly(propylene succinate-co-glycerol succinate) (PPSG) as a Renewable Additive in Electrospun PCL Fibers with Bioactive Glass Particles for Soft Tissue Engineering

ACS Applied Bio Materials, v. 8, p. 4791-4804, 03 jun. 2025

DOI: 10.1021/acsabm.5c00176

ISSN: 25766422

Dourado Fernandes, C.; Harmanci, S.; Grünewald, A.; Hadzhieva, Z.; Oechsler, B. F.; Sayer, C.; Hermes De Araújo, P. H.; Boccaccini, A. R*

Boron-Doped Mesoporous Bioactive Glass Nanoparticles (B-MBGs) in Poly(ϵ -caprolactone)/Poly(propylene succinate-co-glycerol succinate) Nanofiber Mats for Tissue Engineering

ACS Applied Bio Materials, v. 8, p. 5557-5567, 13 jun. 2025

DOI: 10.1021/acsabm.4c01871

ISSN: 25766422

RESUMO

A engenharia de tecidos busca regenerar tecidos danificados, promovendo um ambiente favorável à adesão, proliferação e diferenciação celular por meio da combinação de biomateriais, células e sinais biológicos. Entretanto, a seleção do biomaterial ideal ainda é um desafio, especialmente para a regeneração de tecidos moles. O poli(ϵ -caprolactona) (PCL) é amplamente utilizado devido à sua biocompatibilidade e processabilidade, mas sua elevada hidrofobicidade e lenta taxa de degradação limitam a aplicação em sistemas que exigem reabsorção controlada. Para superar essas limitações, este trabalho investiga a incorporação de poliésteres hidrofílicos de origem renovável, sintetizados por policondensação enzimática, como alternativa sustentável para modificar as propriedades das fibras de PCL. O poli(succinato de propileno-co-succinato de glicerol) (PPSG), obtido com catálise da lipase B de *Candida antarctica* (CALB), foi utilizado como aditivo polimérico para promover a hidrofiliidade, ajustar a taxa de degradação e modular as propriedades mecânicas, tornando o PCL mais adequado para aplicações biomédicas.

A síntese enzimática oferece controle sobre a estrutura do polímero sob condições brandas, evitando subprodutos tóxicos e alinhando-se aos princípios da química verde. Outro desafio relevante é a baixa bioatividade de polímeros sintéticos, que geralmente não promovem interações biológicas essenciais à regeneração tecidual. Para contornar essa limitação, este estudo investigou a incorporação de partículas de vidro bioativo (BG 45S5) e nanopartículas mesoporosas de vidro bioativo dopado com boro (B-MBGs) em fibras obtidas por eletrofiação da blenda de PCL/PPSG. Esses vidros são conhecidos pela por promover bioatividade indireta, através da formação de hidroxiapatita em contato com fluidos biológicos e pela liberação de íons que estimulam a adesão e proliferação celular.

Os resultados confirmaram a eficácia da catálise enzimática na síntese do PSG, com seletividade de 94% para hidroxilas primárias do glicerol, gerando um polímero hidrofílico de massa molar média de 10,5 kDa (dispersidade ± 2). A incorporação de PSG nas fibras de PCL/PPSG/BG 45S5 aumentou o diâmetro médio das fibras para $\sim 2,2 \mu\text{m}$, sem formação de esferas, indicando processamento homogêneo. A adição de 10% de PSG reduziu o ângulo de contato de 117° (PCL puro) para 26° , promovendo melhor molhabilidade e adesão celular. As propriedades mecânicas também melhoraram: o módulo de Young aumentou de 1,7 para 6,0 MPa e a resistência à tração de 0,4 para 1,5 MPa. A bioatividade foi confirmada pela formação de hidroxiapatita após três dias em fluido corporal simulado. A incorporação das B-MBGs

reduziu o diâmetro médio das fibras para $\sim 0,9 \mu\text{m}$ devido ao aumento da condutividade da solução polimérica. Concentrações elevadas diminuíram a ductilidade, indicando a necessidade de otimização da formulação. Ensaio de viabilidade celular com fibroblastos dérmicos humanos (NHDF) mostraram maior proliferação e morfologia bem espalhada nas amostras contendo B-MBGs ($50\text{SiO}_2\text{--}40\text{CaO--}10\text{B}_2\text{O}_3$). Em síntese, a incorporação de PPSG e nanopartículas de vidro bioativo em fibras de PCL resultou em scaffolds com propriedades físico-químicas e biológicas aprimoradas, confirmando o potencial dessa abordagem para o desenvolvimento de biomateriais sustentáveis e funcionais para engenharia de tecidos moles.

Palavras-chaves: Engenharia de Tecidos, Scaffolds Biomédicos, Vidro Bioativo, Policondensação Enzimática, Nanopartículas Mesoporosas de Vidro Bioativo Dopadas com Boro (B-MBGs).

RESUMO EXPANDIDO

Introdução

A crescente demanda por biomateriais sustentáveis e de alto desempenho para aplicações biomédicas impulsiona a busca por rotas de síntese que sigam os princípios da química verde, substituindo catalisadores tóxicos e condições de reação severas. A policondensação enzimática mediada pela lipase B de *Candida antarctica* (CALB ou N435) surge como uma alternativa tecnológica promissora, oferecendo alta seletividade na síntese de poliésteres funcionalizados sob condições brandas.

Polímeros sintéticos amplamente utilizados, como a poli(ϵ -caprolactona) (PCL), apresentam limitações intrínsecas, como elevada hidrofobicidade e lenta taxa de degradação, que restringem sua aplicação na regeneração de tecidos moles. Para superar esses desafios, este trabalho propõe a incorporação do poliéster hidrofílico de origem renovável poli(succinato de propileno-co-succinato de glicerol) (PPSG), sintetizado por rota enzimática, como aditivo em fibras de PCL. Espera-se que o PSG melhore a molhabilidade, acelere a degradação e module as propriedades mecânicas do scaffold.

Além disso, a baixa bioatividade dos materiais poliméricos é um obstáculo na engenharia de tecidos. A incorporação de partículas de vidro bioativo (BG), como o 45S5, e de nanopartículas de vidro bioativo mesoporoso dopado com boro (B-MBGs), pode aprimorar a biocompatibilidade. Os BGs são conhecidos por induzir a formação de hidroxiapatita (HAp) e liberar íons que estimulam a adesão e a proliferação celular — fenômenos essenciais para a engenharia de tecidos moles e a cicatrização de feridas crônicas.

Dessa forma, este trabalho propõe integrar o PSG, sintetizado de forma sustentável, em fibras de PCL fabricadas por eletrospinning e enriquecidas com BG 45S5 e B-MBGs, para desenvolver scaffolds híbridos e altamente funcionais.

Objetivo

Desenvolver scaffolds fibrosos avançados para engenharia de tecidos, integrando poliéster alifático sintetizado por rota enzimática em fibras de poli(ϵ -caprolactona) (PCL) e enriquecendo-os com vidro bioativo (BG 45S5) ou nanopartículas de vidro bioativo mesoporoso dopado com boro (B-MBGs), visando aprimorar as propriedades bioativas e de biodegradabilidade, com foco na regeneração de tecidos moles e na cicatrização de feridas crônicas.

Metodologia

O PPSG foi sintetizado por policondensação enzimática *in bulk* (sem solvente), utilizando succinato de dietila, 1,3-propanodiol e glicerol, catalisada pela N435 (CALB) sob pressão reduzida, garantindo alta regioseletividade para as hidroxilas primárias do glicerol. O produto foi caracterizado quanto à massa molar, grau de ramificação, reologia e hidrofilicidade.

O poliéster PPSG foi então adicionado à solução de PCL em ácido acético (solvente verde), e partículas de BG 45S5 foram incorporadas para o processo de eletrofiação. As fibras resultantes foram caracterizadas quanto à morfologia, diâmetro, molhabilidade (ângulo de contato), degradação hidrolítica em solução de PBS e propriedades mecânicas (módulo de Young e UTS). A bioatividade acelular e a viabilidade celular com fibroblastos NHDF foram avaliadas.

Por fim, nanopartículas de vidro bioativo mesoporoso dopado com boro (B-MBGNs) foram sintetizadas pelo método sol-gel assistido por microemulsão. Essas nanopartículas foram incorporadas às fibras de PCL/PPSG por eletrofiação, e os novos compósitos foram avaliados quanto à morfologia, propriedades mecânicas, bioatividade *in vitro* e citocompatibilidade com fibroblastos NHDF.

Resultados e Discussão

A policondensação enzimática catalisada pela N435 foi eficaz na síntese do PPSG, resultando em um poliéster com massa molar média em peso (M_w) de 10,5 kDa (para PPSG20), controlando a formação de ramificações indesejáveis. A alta regioseletividade da enzima (94%) pelas hidroxilas primárias do glicerol permitiu a obtenção de um polímero hidrofílico, essencial para aplicações biomédicas.

O aumento da concentração de glicerol elevou a viscosidade do meio reacional, dificultando a difusão dos oligômeros e a remoção do subproduto, o que limitou a reciclabilidade da enzima e o aumento da massa molar. No entanto, a incorporação do glicerol levou a uma diminuição acentuada no ângulo de contato do polímero, de $71 \pm 4^\circ$ (PPS) para $37 \pm 1^\circ$ (PPSG20), confirmando o aumento da hidrofilicidade.

Em relação à blenda polimérica integrada com o vidro bioativo comercial BG 45S5, observou-se que a incorporação de PPSG em fibras de PCL por eletrofiação gerou fibras homogêneas e livres de deformações. O PPSG modulou as propriedades do compósito, aumentando significativamente a molhabilidade das fibras e reduzindo o ângulo de contato de $117 \pm 8^\circ$ (PCL puro) para $40 \pm 5^\circ$ (PCL/10PPSG), o que promove uma superfície mais favorável à adesão celular. O módulo de Young aumentou de $1,7 \pm 1,0$ MPa para $6,0 \pm 0,8$ MPa

(PCL/10PPSG), indicando maior rigidez, enquanto a resistência à tração (UTS) aumentou de $0,4 \pm 0,1$ MPa para $1,5 \pm 0,1$ MPa. Contudo, a incorporação das partículas micrométricas de BG 45S5 reduziu a ductilidade e aumentou os pontos de estresse, diminuindo o alongamento na ruptura.

Quanto à bioatividade e citocompatibilidade, observou-se que a presença de BG 45S5 promoveu a bioatividade dos scaffolds, com formação de hidroxiapatita após 3 dias de imersão em SBF, evidenciada por picos característicos em difração de raios X. Os testes de viabilidade celular com fibroblastos NHDF demonstraram que a combinação PCL/PPSG/BG não é citotóxica e favorece o crescimento e a dispersão celular, sendo a amostra com 15% de BG a que apresentou maior proliferação.

A adição de nanopartículas de B-MBGs reduziu o diâmetro médio das fibras de 340 ± 150 nm (PCL/PPSG) para 100 ± 35 nm (PCL/PPSG/5B-MBGs), provavelmente devido ao aumento da condutividade da solução polimérica. A dispersão das nanopartículas foi homogênea, confirmada por mapeamento EDS. No entanto, em altas concentrações, observaram-se pontos de aglomeração, o que interferiu no módulo de Young, que aumentou de $3,8 \pm 0,3$ MPa para $11 \pm 1,2$ MPa, e no UTS, que atingiu $1,2 \pm 0,1$ MPa com a adição de 15% de B-MBGs (PCL/PPSG/15B-MBGs).

A formação de hidroxiapatita também foi confirmada após 3 dias de imersão em SBF para todas as concentrações de B-MBGs. Os ensaios de viabilidade celular com fibroblastos NHDF confirmaram a citocompatibilidade do material, com as células apresentando morfologia bem espalhada e citosqueleto organizado — o que reforça o potencial dessas fibras para regeneração de tecidos moles e cicatrização de feridas crônicas.

Considerações Finais

O trabalho demonstrou que a rota de policondensação enzimática utilizando CALB é uma estratégia sustentável e eficiente para a síntese controlada do PPSG a partir de monômeros renováveis, com alta seletividade para hidroxilas primárias.

A incorporação do PPSG e das partículas de vidro bioativo (BG 45S5 e B-MBGs) em fibras de PCL resultou em scaffolds com propriedades aprimoradas para engenharia de tecidos, particularmente em aplicações voltadas a tecidos moles e feridas crônicas. A combinação do controle estrutural proporcionado pela rota enzimática, o aumento da hidrofilicidade e biodegradabilidade pelo PPSG, e a melhoria da bioatividade e do desempenho mecânico pela

adição das nanopartículas de vidro bioativo valida a estratégia para o desenvolvimento de biomateriais sustentáveis e altamente funcionais.

ABSTRACT

Tissue engineering aims to regenerate damaged tissues by creating a favorable microenvironment for cell adhesion, proliferation, and differentiation through the combination of biomaterials, cells, and biological cues. However, selecting the ideal biomaterial remains a major challenge, particularly for soft-tissue regeneration. Poly(ϵ -caprolactone) (PCL) is widely used due to its biocompatibility and excellent processability, but its high hydrophobicity and slow degradation rate limit applications requiring controlled resorption.

To overcome these limitations, this work investigates the incorporation of renewable hydrophilic polyesters synthesized via enzymatic polycondensation as a sustainable alternative to modify the properties of PCL fibers. The poly(propylene succinate-co-glycerol succinate) (PPSG), obtained through catalysis by *Candida antarctica* lipase B (CALB), was employed as a polymeric additive to enhance hydrophilicity, tune the degradation rate, and modulate mechanical performance, thereby rendering PCL more suitable for biomedical applications. The enzymatic synthesis route provides structural control under mild conditions, avoiding toxic by-products and aligning with the principles of green chemistry.

Another critical limitation of synthetic polymers is their inherently low bioactivity, which often prevents the establishment of essential biological interactions for effective tissue regeneration. To address this issue, this study explored the incorporation of bioactive glass (BG 45S5) particles and boron-doped mesoporous bioactive glass nanoparticles (B-MBGs) into electrospun PCL/PPSG blends. These glasses are known for their ability to induce indirect bioactivity through the formation of hydroxyapatite (HAp) upon exposure to biological fluids and for releasing therapeutic ions that promote cell adhesion and proliferation.

The results confirmed the efficiency of the enzymatic catalysis in PSG synthesis, with 94% selectivity toward primary hydroxyl groups of glycerol, yielding a hydrophilic polymer with a weight-average molecular mass of 10.5 kDa (dispersity ± 2). Incorporation of PSG into PCL/PPSG/BG 45S5 fibers increased the average fiber diameter to approximately 2.2 μm , without bead formation, indicating homogeneous electrospinning. The addition of 10 wt% PSG reduced the contact angle from 117° (pure PCL) to 26°, enhancing wettability and cell adhesion. Mechanical properties were also improved: the Young's modulus increased from 1.7 to 6.0 MPa, and the ultimate tensile strength (UTS) from 0.4 to 1.5 MPa. Bioactivity was

confirmed by the formation of hydroxyapatite after three days of immersion in simulated body fluid (SBF).

The incorporation of B-MBGs further reduced the average fiber diameter to approximately 0.9 μm due to the increased conductivity of the polymer solution. However, high nanoparticle concentrations decreased ductility, suggesting the need for formulation optimization. Cell viability assays with normal human dermal fibroblasts (NHDF) showed enhanced proliferation and well-spread morphology in samples containing B-MBGs ($50\text{SiO}_2\text{--}40\text{CaO--}10\text{B}_2\text{O}_3$).

In summary, the incorporation of PPSG and bioactive glass nanoparticles into PCL fibers resulted in scaffolds with enhanced physicochemical and biological properties, confirming the potential of this sustainable approach for the development of functional biomaterials aimed at soft-tissue engineering and chronic wound healing.

Keywords: Tissue Engineering; Biomedical Scaffolds; Bioactive Glass; Enzymatic Polycondensation; Boron-Doped Mesoporous Bioactive Glass Nanoparticles (B-MBGs)

ZUSAMMENFASSUNG

Die Gewebetechnik zielt darauf ab, geschädigtes Gewebe zu regenerieren, indem ein günstiges Umfeld für Zelladhäsion, -proliferation und -differenzierung geschaffen wird. Dies wird in der Regel durch die Kombination geeigneter Biomaterialien in Form von dreidimensionalen Gerüsten mit Zellen und biologischen Signalen (z. B. Wachstumsfaktoren) erreicht. Die Auswahl des idealen Biomaterials stellt jedoch nach wie vor eine erhebliche Herausforderung dar, insbesondere im Hinblick auf die Regeneration von Weichgewebe. Poly(ϵ -caprolacton) (PCL) ist ein weit verbreiteter, biologisch abbaubarer, synthetischer Polymer, der aufgrund seiner Biokompatibilität und Verarbeitbarkeit häufig für die Herstellung von Gerüsten verwendet wird. Seine intrinsischen Eigenschaften können jedoch seine Anwendung einschränken. Beispielsweise weist PCL eine hohe Hydrophobie auf, was die Zelladhäsion und -proliferation erschwert. Darüber hinaus weist PCL eine relativ langsame Abbaurate auf, was das Gleichgewicht zwischen Gerüst und neuem Gewebe stören kann, wodurch das Material für Anwendungen, die eine kontrollierte Resorption erfordern, ungeeignet ist.

Um diese Einschränkungen zu überwinden, untersucht diese Arbeit die Integration von hydrophilen Polyestern auf Basis nachwachsender Rohstoffe, die durch enzymatische Polykondensation synthetisiert wurden, als nachhaltige Alternative zur Modifikation von PCL-Fasern. Die Einführung des polymeren Additivs Poly(Propylensuccinat-co-Glyzerolsuccinat) (PPSG) verbessert die Benetzbarkeit, passt die Abbaurate an und ermöglicht eine feine Modulation der mechanischen Eigenschaften, wodurch das Material für biomedizinische Anwendungen im Kontakt mit Weichgewebe besser geeignet wird. Die enzymatische Polykondensation zur Synthese von PSG wird durch die *Candida antarctica* Lipase B (CALB) katalysiert. Dieses Verfahren ermöglicht nicht nur eine größere Kontrolle über die Funktionalisierung des Polymers, sondern erfolgt auch unter milden Bedingungen, wodurch toxische Nebenprodukte vermieden werden und das Verfahren nachhaltiger und mit den Prinzipien der Grünen Chemie vereinbar wird.

Eine weitere Herausforderung in der Gewebetechnik ist die geringe Bioaktivität polymerer Materialien, die für sich genommen weder die Bildung mineralischer Phasen (wie bei der Knochenregeneration) anregen noch essenzielle biologische Interaktionen zur Gewebevaskularisierung fördern. In dieser Studie werden bioaktive Glaspartikel (BG) mit der Zusammensetzung 45S5 (Gew.-%: 45 % SiO₂, 24,5 % Na₂O, 24,5 % CaO und 6 % P₂O₅) sowie borgepumpte mesoporöse bioaktive Glasnanopartikel (B-MBGNs) in Fasern eingebracht, die

durch Elektrosponnen aus PPSG-PCL-Lösungen hergestellt wurden. BGs sind bekannt für ihre Fähigkeit, bei Kontakt mit biologischen Flüssigkeiten die Bildung von Hydroxylapatit zu fördern und Ionen freizusetzen, die die Zelladhäsion stimulieren.

Die Ergebnisse bestätigten die Wirksamkeit der enzymatischen Katalyse bei der Synthese von PPSG mit Selektivität für primäre Hydroxylgruppen. PPSG zeigte eine mittlere molare Masse (M_w) von 5,8 kDa, eine Dispersität von ± 3 und einen durchschnittlichen Polymerisationsgrad von ± 9 , was Stabilität und Verarbeitungsfähigkeit gewährleistet. Die hohe Selektivität der CALB-Enzymkatalyse (94 %) für die primären Hydroxylgruppen des Glycerins führte zu einem Polymer mit hydrophiler Struktur, was entscheidend für die Interaktion mit Zellen und biologischen Flüssigkeiten ist. Die Integration von PPSG in elektrogesponnene PCL/BG-45S5-Fasern führte zu einer Erhöhung des durchschnittlichen Faserdurchmessers auf $2,2 \pm 0,4 \mu\text{m}$ (5 % PPSG) bzw. $2,2 \pm 0,2 \mu\text{m}$ (10 % PPSG), ohne Perlenbildung – ein Hinweis auf einen homogenen Spinnprozess. Die Zugabe von PPSG führte zu chemischen Modifikationen, die die Benetzbarkeit deutlich verbesserten, wobei der Kontaktwinkel von 117° (reines PCL) auf ca. 26° bei PCL/10PPSG sank, was eine zelladhäsionsfreundlichere Oberfläche erzeugte. Darüber hinaus erhöhte sich durch die Zugabe von 10 % PPSG der Young'sche Modul der Fasern von $1,7 \pm 1,0 \text{ MPa}$ auf $6,0 \pm 0,8 \text{ MPa}$. Auch die Zugfestigkeit (UTS) nahm von $0,4 \pm 0,1 \text{ MPa}$ auf $1,5 \pm 0,1 \text{ MPa}$ zu, was eine Verbesserung der mechanischen Eigenschaften durch die Copolymerintegration zeigt. Die Bildung von Hydroxylapatit wurde nach 3 Tagen Immersion in simuliertem Körperfluid (SBF) für die Proben PCL/PPSG/5BG, PCL/PPSG/15BG und PCL/PPSG/30BG durch charakteristische Röntgenbeugungspeaks bestätigt, was die Bioaktivität der Gerüste belegt.

Die Einbringung von B-MBGs in die PCL/PPSG-Fasern reduzierte den mittleren Faserdurchmesser auf $0,9 \pm 0,4 \mu\text{m}$, was auf die erhöhte Leitfähigkeit der Polymerlösung zurückzuführen ist. Das Vorhandensein dieser Nanopartikel wirkte sich positiv auf die mechanischen Eigenschaften aus, wobei ein Young-Modul von $4,7 \pm 0,3 \text{ MPa}$ (5 % B-MBGs) bzw. $11 \pm 1 \text{ MPa}$ (15 % B-MBGs) erzielt wurde; zusätzlich stieg die Zugfestigkeit auf $1,2 \pm 0,1 \text{ MPa}$ bei 5 % B-MBGs in der PCL/10PPSG-Zusammensetzung. Höhere Konzentrationen an B-MBGs reduzierten jedoch die Duktilität der Fasern, was die Notwendigkeit eines ausgewogenen Formulierungsansatzes verdeutlicht. Bioaktivitätstests zeigten die Bildung von Hydroxylapatit nach 14 Tagen Immersion in SBF, während Zellviabilitätstests mit NHDF-Fibroblasten (normale humane dermale Fibroblasten) eine verstärkte Zellproliferation in

Proben mit B-MBGs (nominale Zusammensetzung $50\text{SiO}_2\text{--}40\text{CaO--}10\text{B}_2\text{O}_3$) belegten. Die Zellen zeigten eine gut ausgebreitete Morphologie und eine ausgeprägte Zytoskelettorganisation, was das Potenzial dieser Fasern für die Geweberegeneration unterstreicht.

Die Ergebnisse zeigen somit, dass die Integration von PPSG und bioaktiven Glasnanopartikeln in PCL-Fasern zur Herstellung von Gerüsten mit verbesserten Eigenschaften für die Gewebetechnik führt. Die Kombination aus struktureller Kontrolle durch enzymatische Polykondensation, verbesserter Benetzbarkeit und Bioaktivität sowie der Anpassung mechanischer Eigenschaften bestätigt die Eignung dieser Strategie für die Entwicklung nachhaltiger und hochfunktioneller Biomaterialien für medizinische Anwendungen.

Schlüsselwörter: Gewebeengineering; Biomedizinische Gerüste; Bioaktives Glas; Enzymatische Polykondensation; Bor-dotierte mesoporöse bioaktive Glasnanopartikel (B-MBGs).

List of Figures

Figure 1. Summary of the research tasks carried out in the present work focused on the investigation of the use of PPSG and inorganic particles (BG, MBGNS) as an additive in electrospun fibers.....	34
Figure 2. Synthesis of polyesters in (I) by transesterification of dimethyl with a diol; (II) direct esterification of the diacid with a diol, followed by the polycondensation reaction	38
Figure 3. Enzymatic route for polyester synthesis by esterification.....	40
Figure 4. Illustrative diagram of the polymer jet formation process for the electrospinning of nanofibers.	51
Figure 5. Schematic representation of the production of boron-doped MBGNs by the microemulsion-assisted sol-gel technique. Adapted by Zahir, A. et al. 11	55
Figure 6. Enzymatic synthesis of polyesters using N435: a) Effect of the initial comonomer ratio (glycerol content) on the kinetic behavior; b) Mw during reactions without glycerol (PPS) and with 20% glycerol (PPSG20); c) Molar mass distributions during reactions .	67
Figure 7. ¹ H NMR proton assignments for linear, terminal and dendritic glycerol units contained in the polymers.....	72
Figure 8. ¹ H NMR spectra of PSG20 synthesized with 20% of glycerol in the diol fraction and 10% of N435 and 80°C. The monomers used for this synthesis were: Diethyl succinate (DS); 1,3-propanediol (1,3-PDO); Glycerol (Gly).	73
Figure 9. Curves of the apparent viscosity at different reaction times: (a) PSG20 and (b) PPS	77
Figure 10. Contact angle of PPS and PSG20 synthesized at 10% of N435, and 90°C	79
Figure 11. Analysis of: (a) average molar mass correlated with the enzymatic activity of N435 during the reuse of the biocatalyst for the synthesis of PSG20 and the relationship of the enzyme activity with the polydispersity Đ, and (b) Molar mass distributions.....	80
Figure 12. SEM images of the surface of immobilized lipase N435; before the reaction (a;b;c); after the first cycle (d;e;f) at the end of the third cycle (g;h;i).....	81
Figure 13. SEM microscopy of the electrospun fibers and their respective average diameter distribution: (a-h) PCL/PSG flow rate (F.R) 0.2 mL/h; (j-q) PCL/PSG F.R 0.4 mL/h; (r-y) PCL/PSG F.R 0.8 mL/h	89

Figure 14. SEM/EDX showing BG particle distribution in the fiber mats: (a) PCL/PPSG/5BG, (b) PCL/PPSG/15BG, (c) PCL/PPSG/30BG. All compositions contain 10% PSG.	92
Figure 15. FTIR spectroscopy of fiber mats in the 400 to 3500 cm ⁻¹ wavenumber range. Highlights are indicated in purple and detailed in Table S3.....	93
Figure 16. Degree of wettability of electrospun mats, as determined by contact angle measurement. (a) Composite mats containing PCL/PPSG blends and BG 45S5 bioactive glass. (b) Electrospun PCL mats with varying PSG fractions.....	95
Figure 17. Weight loss during submersion in PBS for 35 days (a) and SEM micrograph of samples after 35 days of immersion PCL/10PPSG fiber (b).	96
Figure 18. SEM images of fiber with BG under SBF and PBS: (a,b,c) PCL/10PPSG; (d,e,f) CL/10PPSG/5BG; (g,h, i) PCL/10PPSG/15BG; (j,k,l) PCL/10PPSG/30BG. The red arrow indicates Hydroxyapatite.	97
Figure 19. FTIR spectra of samples immersed in SBF over 14 days.	98
Figure 20. XRD patterns of samples immersed in SBF after 14 days. The presence of hydroxyapatite (♦) is marked by the peaks at $2\theta = 32^\circ$ and 46.7° . The crystallinity of PCL (●) is marked by the peaks at $2\theta = 21.4^\circ$ and 23.7°	99
Figure 21. Results of (a) Young's Modulus values and (b) stress-strain curves of PCL/PSG fibers and fibers with bioactive glass PCL/PPSG/BG samples.....	100
Figure 22. (a) Cell viability analysis was measured by cellular dehydrogenase staining assay at a wavelength of 450 nm (* $p < 0.05$). (b) Fibroblasts cultured on fiber blends were analyzed by fluorescence, DAPI in blue (cell nuclei), and rhodamine-phalloidin in red (actin filaments).	104
Figure 23. SEM image and corresponding EDX analysis of B-MBGs (a), XRD pattern (b), and FTIR (c) spectra of the B-MBGs.	113
Figure 24. SEM micrographs of the electrospun samples and their respective distribution of average fiber diameter, without B-MBGs (a) PCL/PPSG, and with 5 wt% (b) PCL/PPSG/5B-MBGs; with 10 wt% (c) PCL/PPSG/10B-MBGs; and 15 wt% (d) PCL/PPSG/15B-MBGs	115
Figure 25. EDS mapping of electrospun nanocomposite scaffold PCL/PPSG/5B-MBGs showing distribution of Ca and Si elements on the surface.....	117

Figure 26. FTIR spectra of fiber mats in the 400 to 3500 cm ⁻¹ wavenumber range. The peaks are discussed in the text.	118
Figure 27. Weight loss during immersion of the fiber mats in PBS at 37 °C for 30 days (a), Contact angle of electrospun mats with varying amounts of B-MBG (b), and SEM microscopy image of samples after 30 days of immersion of the fiber mats PCL/PPSG (c); PCL/PPSG/5B-MBGs (d); PCL/PPSG/10B-MBGs (e); PCL/PPSG/15B-MBGs (f).	121
Figure 28. XRD spectra of B-MBGs electrospun fibers incubated in SBF solution over 14 days. The peaks 21.4° and 23.8° indicate the crystal structure of PCL (●), and the peak 32.2° indicates the presence of hydroxyapatite (◆).	122
Figure 29. FTIR analysis of electrospun fiber mats containing B-MBGs immersed in SBF solution over 7 days and their respective SEM images	124
Figure 30. Metabolic activity measured by WST-8 analysis at 450 nm for all seedings (* p < 0.05). Fluorescence images of fibroblast cells on fibers after 1 day. PSC/PPSG/5B-MBGs (a); PSC/PPSG/10B-MBGs (b); PSC/PPSG/15B-MBGs (c); PSC/PPSG (d).	125
 Figure S 1. 1H-NMR spectra of PPS synthesized without glycerol and 10 wt% of N435 in relation to the monomers at 90°C for 24h.	164
Figure S 2. 1H-NMR spectra of PPSG10 synthesized with 10% of the glycerol and 10 wt% of N435 in relation to the monomers at 90 °C for 24 h.	165
Figure S 3. 1H-NMR spectra of PPSG20 synthesized with 10% of the glycerol and 10 wt% of N435 in relation to the monomers at 90 °C for 24 h.	165
Figure S 4. 1H-NMR spectra of the soluble fraction of PPSG50 synthesized with 10% of the glycerol and 10 wt% of N435 in relation to the monomers at 90 °C for 24 h.	166
Figure S 5. 13C-NMR spectra of PPS(a), PPSG10 (b) and PPSG20 (c) synthesized with 0, 10 and 20% of the glycerol respectively and 10 wt% of N435 in relation to the monomers at 90 °C for 24 h	168
Figure S 6. FTIR analysis of the synthesized samples: (A) Verification of synthesized PPS polyester, and PPS with 10% of glycerol (PPSG10) and 20% of glycerol (PPSG20); (B) Verification of synthesized PPSG20 polyester over time.	169
Figure S 7. DSC thermograms of electrospun fibers of PCL and PCL/PPSG blends.	170

Figure S 8. EDX analysis of electrospun fiber with PCL/PPSG/5BG bioglass	171
Figure S 9. Polymer solutions of PCL/PPSG/B-MBGs with 5, 10, and 15 wt% B-MBGs, showing increased nanoparticle agglomeration at 15 wt%.	171
Figure S 10. SEM image of the dispersed B-MBGs nanoparticles.	172

List of Tables

Table 1. Solvents used in polycondensation reactions, concerning Log P: Degree of hydrophilicity and boiling temperature.	44
Table 2. Enzymatic synthesis of aliphatic and aromatic polyesters by esterification.....	48
Table 3. Formulations of the enzymatic polycondensations in bulk conditions.....	61
Table 4. Effect of initial comonomer ratio on average molar masses and gel content.	70
Table 5. Effect of comonomer ratios (90 °C, 24 h, in bulk) on the distribution of glycerol structural units, end-group functionality, enzyme regioselectivity and degree of branching calculated based on ¹ H-NMR and ¹³ C-NMR spectra	74
Table 6. Correlation of tensile test results with fiber mat diameter.....	102
Table 7. Physical characterization of B-MBGs	113
Table 8. Mechanical properties of composite fibers with B-MBG.....	119
Table S 1. Representation of peaks in ppm of carbons and their respective integrals of PPS, PPSG10 and PPSG20 found in ¹³ C NMR.	167
Table S 2. Thermal properties of electrospun PCL and PCL/PPSG fibers determined by DSC analysis. The table presents the melting temperature (T_m), crystallization temperature (T_c), and enthalpy of fusion (ΔH) for electrospun fiber samples of pure PCL and PCL/PPSG blends (5%, 10%, and 20% PPSG).....	169
Table S 3. Characteristic FTIR absorption bands of PCL electrospun mats with the “green” PPSG and BG bioactive glass and their designation according to 188,207	Error!

Bookmark not defined.

List of Abbreviations

- ¹H NMR** – Nuclear Magnetic Resonance of Hydrogen-1
- ¹³C NMR** – Nuclear Magnetic Resonance of Carbon-13
- AMPs** – Antimicrobial Peptides
- B-MBGs** – Boron-doped Mesoporous Bioactive Glass Nanoparticles
- BG** – Bioactive Glass
- CALB** – *Candida antarctica* Lipase B
- CCK-8** – Cell Counting Kit-8 (WST-8 Assay)
- CLSM** – Confocal Laser Scanning Microscopy
- CTAB** – Cetyltrimethylammonium Bromide
- DAPI** – 4',6-Diamidino-2-Phenylindole (Fluorescent Stain)
- DLS** – Dynamic Light Scattering
- DMEM** – Dulbecco's Modified Eagle Medium
- DSC** – Differential Scanning Calorimetry
- EDX** – Energy Dispersive X-ray Spectroscopy
- ECM** – Extracellular Matrix
- FTIR** – Fourier-transform Infrared Spectroscopy
- GPC** – Gel Permeation Chromatography
- HAp** – Hydroxyapatite
- ICP-MS** – Inductively Coupled Plasma Mass Spectrometry
- ICP-OES** – Inductively Coupled Plasma Optical Emission Spectrometry
- ILs** – Ionic Liquids
- MBGs** – Mesoporous Bioactive Glass Nanoparticles
- M_w** – Weight Average Molar Mass
- M_n** – Number Average Molar Mass
- N435** – Novozyme 435 (Immobilized *Candida antarctica* Lipase B)
- NHDF** – Normal Human Dermal Fibroblast
- PBS** – Phosphate-buffered Saline
- PCL** – Poly(ε-caprolactone)
- PLA** – Poly(lactic acid)
- PPSG** – Poly(propylene succinate-co-glycerol succinate)

ROP – Ring-opening Polymerization
SBF – Simulated Body Fluid
scCO₂ – Supercritical Carbon Dioxide
SEM – Scanning Electron Microscopy
TGA – Thermogravimetric Analysis
TEOS – Tetraethyl Orthosilicate
TMS – Tetramethylsilane
UV – Ultraviolet
XRD – X-ray Diffraction

TABLE OF CONTENTS

Chapter 1 Introduction	31
1.1 Objectives	32
1.1.1 Main Objective	32
1.1.2 <i>Specific Objectives</i>	32
1.2 Thesis Structure	33
Chapter 2 Fundamentals	35
2.1 Aliphatic polyesters for tissue engineering.....	35
2.2 The role of polymerization techniques in synthesizing functional materials	37
2.2.1 <i>Polyester by enzymatic esterification</i>	39
2.2.2 <i>Influence of water on enzymatic polyesterification with lipases.</i>	41
2.2.3 <i>Influence of solvents on enzymatic polyesterification with lipases.</i>	42
2.2.4 <i>Biologically-based building blocks for enzymatic polyesterification</i>	46
2.3 Electrospinning Technology for Fiber Fabrication.....	50
2.4 Bioactive Glasses in Regenerative Medicine	52
2.5 Boron-Doped Bioactive Glasses.....	55
2.6 Final Remarks	57
Chapter 3 Hydrofunctionalized Polyester Synthesis	58
3.1 Introduction.....	58
3.2 Materials and Methods	60
3.2.1 <i>Materials</i>	60
3.2.2 <i>Lipase-catalyzed hydro-functionalized polyester synthesis</i>	61
3.2.3 <i>Enzyme Stability</i>	62
3.2.4 <i>Polymer Characterization</i>	63
3.3 Results And Discussion	65
3.3.1 <i>Synthesis of glycerol hydroxyl functionalized polyesters.</i>	65

3.3.2	<i>Evaluation of Polymer Architecture</i>	71
3.3.3	<i>Rheological Behavior</i>	76
3.3.4	<i>Hydrophilicity</i>	78
3.3.5	<i>Enzyme reuse</i>	79
3.4	Conclusion	82
Chapter 4 Incorporation of Poly(Propylene Succinate-Co-Glycerol Succinate) PPSG as a Renewable Additive In Electrospun PCL Fibers With Bioactive Glass Particles For Soft Tissue Engineering		83
4.1	Introduction.....	83
4.2	Materials and Methods	85
4.2.1	<i>PPSG Samples</i>	85
4.2.2	<i>Polymer Solution Preparation and Electrospinning Process</i>	85
4.2.3	<i>Characterization</i>	86
4.2.4	<i>Statistics</i>	87
4.3	Results And Discussion	88
4.3.1	<i>Electrospinning of PPSG-PCL</i>	88
4.3.2	<i>Chemical characterization</i>	92
4.3.3	<i>Wettability</i>	94
4.3.4	<i>Degradation in PBS Solution</i>	95
4.3.5	<i>In vitro Acellular Bioactivity</i>	97
4.3.6	<i>Mechanical Properties</i>	100
4.3.7	<i>Cell viability</i>	103
4.4	Conclusions	106
Chapter 5 Boron-Doped Mesoporous Bioactive Glass Nanoparticles (B-MBGs) in (Poly(E-Caprolactone)/Poly (Propylene Succinate-Co-Glycerol Succinate) Nanofiber Mats For Tissue Engineering		107
5.1	Introduction.....	107

5.2	Materials And Methods	108
5.2.1	<i>Synthesis of Boron-doped bioactive glass nanoparticles</i>	108
5.2.2	<i>Electrospinning process</i>	109
5.2.3	<i>Characterization</i>	110
5.2.4	<i>Mechanical Characterization</i>	110
5.2.5	<i>Degradation tests</i>	111
5.2.6	<i>Hydroxyapatite (HAp) Formation In Vitro</i>	111
5.2.7	<i>In vitro Cell viability evaluation</i>	111
5.2.8	<i>Statistics</i>	112
5.3	Results and Discussion	112
5.3.1	<i>Characterization of B-MBGs</i>	112
5.3.2	<i>Chemical characterization of PCL/PPSG/B-MBGs electrospun mats</i>	114
5.3.3	<i>Mechanical Properties</i>	118
5.3.4	<i>Wettability and Degradation in PBS Solution</i>	120
5.3.5	<i>In vitro Acellular Bioactivity</i>	122
5.3.6	<i>Cell viability</i>	124
5.4	Conclusions	126
Chapter 6 General Conclusions And Suggestions For Future Work		127
6.1	General Conclusions.....	127
6.2	Suggestions For Future Work.....	128
References.....		131
Supplementary material		146

Chapter 1 Introduction

The growing demand for sustainable and high-performance biomaterials for biomedical applications, combined with the urgent need to replace fossil raw materials with renewable resources, constitutes one of the main challenges for science and engineering in the 21st century ^{1,2}. Biodegradable and biocompatible materials, such as aliphatic polyesters, emerge as promising alternatives, allowing the creation of innovative devices for tissue engineering and other biomedical applications ^{3,4}. However, the limitations associated with traditional polyester synthesis methods, which often involve toxic catalysts and harsh reaction conditions, highlight the importance of developing routes that follow the principles of green chemistry ^{5,6}.

Enzymatic catalysis, using *Candida antarctica* lipase B (CALB), emerges as a sustainable technological solution, offering high selectivity for the synthesis of functionalized polyesters under mild conditions ⁷⁻⁹. Nevertheless, the scarcity of studies on the integration of these polyesters into biomedical scaffolds restricts the advancement of the field. Furthermore, widely used synthetic polymers, such as poly(ϵ -caprolactone) (PCL), have intrinsic limitations, including high hydrophobicity and low degradation rate, which compromise their application in soft tissue regeneration ^{10,11}. To overcome these drawbacks, the incorporation of bio-based hydrophilic aliphatic polyesters, such as the PGS analogue, poly(propylene succinate-co-glycerol succinate) (PPSG), represents a promising strategy^{7,12,13}. It is expected that these polyesters increase the wettability of electrospun fibers, accelerate degradation, and modulate mechanical properties to better mimic the extracellular matrix. Furthermore, its enzymatic synthesis allows sustainable production under mild conditions, ensuring high functionalization without toxicity.

In addition, the incorporation of bioactive particles, such as bioactive glass (BG) 45S5 (wt% 45% SiO₂, 24,5% Na₂O, 24,5% CaO, 6% P₂O₅), offers new opportunities to improve the biocompatibility of biopolymers applied to tissue engineering. BGs are materials capable of forming a hydroxyapatite layer when in contact with body fluids, promoting direct interaction with biological tissues ¹⁰. Commercial BG 45S5, for example, has been widely used in PCL fibers for applications in bone regeneration due to its osteogenic and bioactive properties ^{11,14}. Recently, doping these glasses with biologically active ions, such as boron, has expanded their applications to soft tissues and chronic wound healing ^{15,16}. Boron-doped mesoporous bioactive glass nanoparticles (B-

MBGNs) have also been shown to positively influence cell proliferation, angiogenesis, and tissue regeneration, in addition to reducing inflammatory responses, consolidating their potential in tissue repair, allowing the development of scaffolds with greater functionality and efficiency^{17,18}. However, there is significant room for innovations that integrate renewable polymers and bioactive particles in hybrid systems for applications in soft tissue engineering and chronic wound regeneration.

Motivated by these gaps in current biomaterial technologies, this work seeks to contribute to the evolution of sustainable and technologically advanced biomaterials, proposing the synthesis of functionalized aliphatic polyesters by an enzymatic route and their integration into PCL electrospun fibers enriched with BG 45S5 and B-MBGNs. This approach represents a significant step towards the creation of engineered biomaterials that combine sustainability, technological innovation, and potential clinical applicability, offering promising solutions to the challenges of tissue engineering.

1.1 Objectives

1.1.1 Main Objective

To develop advanced fibrous scaffolds for tissue engineering, integrating functionalized aliphatic polyester, synthesized by enzymatic route from renewable monomers, in electrospun fibers of poly(ϵ -caprolactone) (PCL), enriched with bioactive glass (BG 45S5) and boron-doped mesoporous bioactive glass nanoparticles (B-MBGNs), aiming to improve the mechanical, bioactive and biodegradability properties, with a focus on soft tissue regeneration and chronic wound healing.

1.1.2 Specific Objectives

- Investigate the synthesis of functionalized aliphatic polyesters via enzymatic polycondensation using renewable monomers, such as diethyl succinate, 1,3-propanediol and glycerol, and the enzyme *Candida antarctica* lipase B (CALB).
- Characterize the synthesized polyesters, evaluating their chemical, structural, thermal, mechanical and hydrophilicity properties using techniques such as NMR, FTIR, GPC, TGA, DSC and rheometry tests.
- Evaluate enzymatic recyclability in the synthesis process, focusing on identifying the efficiency and feasibility of reusing the CALB biocatalyst.

- Study the use of functionalized polyesters as additives in PCL electrospun fibers, analyzing the effects on the wettability, biodegradability, mechanical properties and bioactivity of the fibers.
- Integrate bioactive glass (BG) particles of micrometric size into fibers (PCL/PPSG), investigating the impact on bioactivity, mechanical properties and cell viability in NHDF fibroblasts.
- Synthesize and integrate boron-doped bioactive glass nanoparticles (B-MBGs) into fibers, investigating the impact on bioactivity and cell viability in NHDF fibroblasts.
- Perform *in vitro* bioactivity tests, including the evaluation of hydroxyapatite formation in simulated body fluid (SBF), as an indicator of biological interaction.
- Evaluate the cellular compatibility of fibers, verifying cell adhesion and proliferation for future application in soft tissue engineering and chronic wound healing.
- Contribute to the development of sustainable scaffolds, combining bio-based polyesters and bioactive inorganic particles in composite structures to overcome the limitations of traditional materials in biomedical applications.

1.2 Thesis Structure

The structure of this doctoral thesis features 5 chapters. Chapter 1 addresses the research motivation, introduction and objectives. Chapter 2 presents a literature review on enzymatic polycondensation and the use of renewable monomers for the synthesis of biopolymers for biomaterial applications. The objective of this chapter is to identify the challenges and gaps in the development of polyester additives by an environmentally friendly route. It also discusses the production of electrospun fibers for tissue regeneration, as well as highlights advances in the functionalization of these scaffolds with B-MBGs. Chapter 3 studies tools for the development of hydroxyl-functionalized polyesters by enzymatic catalysis. Chapter 4 investigated the use of the renewable polyester used in Chapter 3 (Poly(propylene succinate-co-glycerol succinate) PPSG) as an alternative additive for the production of PCL fibers by electrospinning for tissue engineering. In Chapter 5, B-MBGs were synthesized and added to electrospun PCL/PPSG fibers to investigate possible future applications in soft tissue engineering. Part of this thesis was developed in partnership with Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Germany, under a co-supervision regime. A schematic

overview of the research conducted in this project is shown in Figure 1.

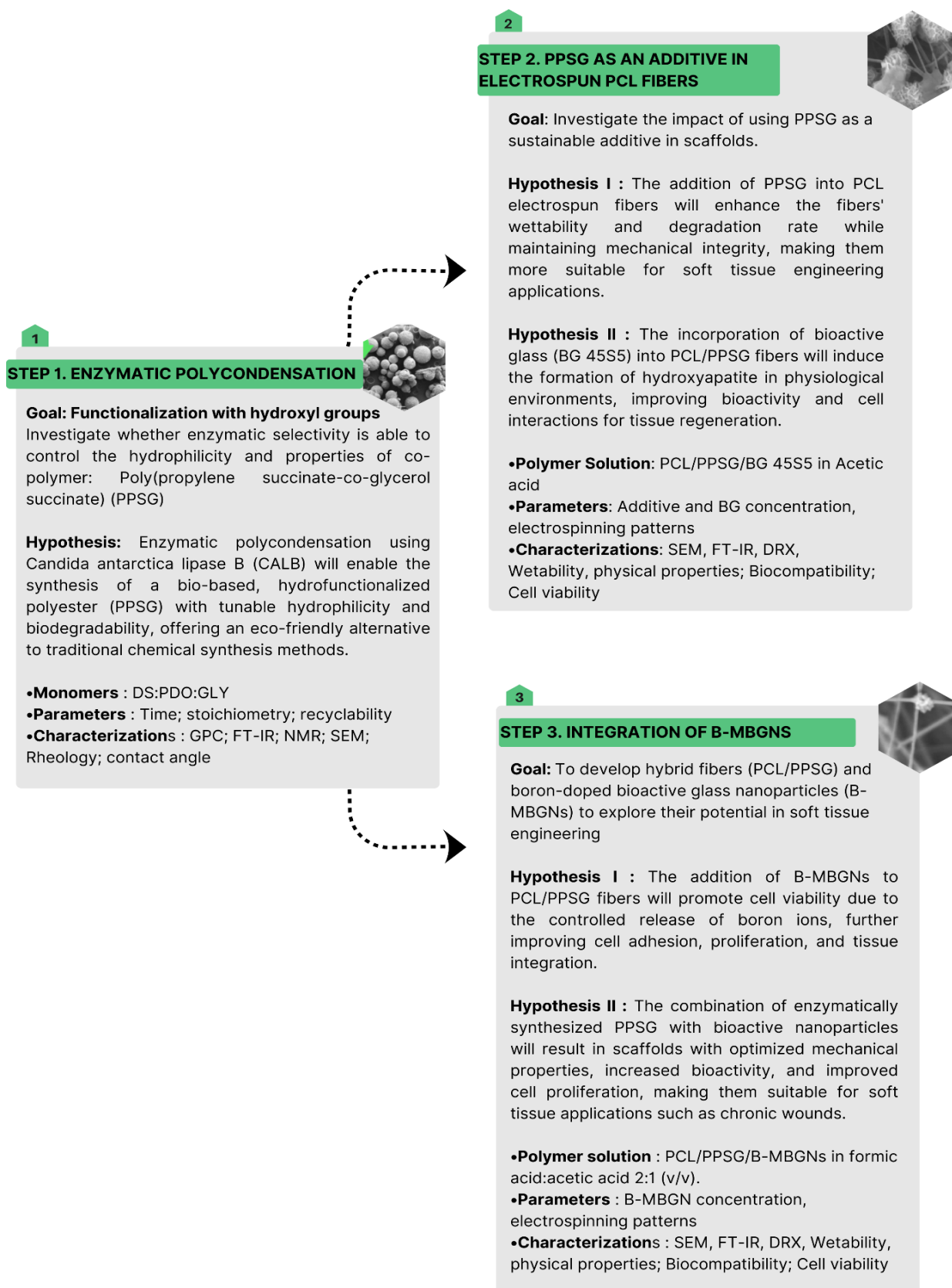


Figure 1. Summary of the research tasks carried out in the present work focused on the investigation of the use of PPSG and inorganic particles (BG, MBGNS) as an additive in electrospun fibers.

Chapter 2 Fundamentals

This session contains a review of the literature for the development of bio-based aliphatic polyesters and their application in tissue engineering. It explores bio-based monomers, enzymatic polymerization routes, and the role of aliphatic polyesters in biomedical applications, highlighting their biodegradability and biocompatibility. Special emphasis is given to enzymatic catalysis using *Candida antarctica* lipase B (CALB) as a sustainable alternative for polyesters synthesis. Additionally, the session discusses the incorporation of bioactive glass and boron-doped mesoporous bioactive glass nanoparticles (B-MBGs) in polymeric scaffolds, emphasizing their impact on bioactivity, hydroxyapatite formation and cellular interactions. Part of the bibliographic survey carried out was published as a review in the European Polymer Journal in March 2022 with the title “Recent advances and challenges on enzymatic synthesis of biobased polyesters via polycondensation” (<https://doi.org/10.1016/j.eurpolymj.2022.111132>), in which the candidate was the first author. The chapter was organized according to the norms proposed by the publication journal, and the bibliographic references were added at the end of the thesis.

2.1 Aliphatic polyesters for tissue engineering

With advancements in biotechnology, biomaterials are designed to interact with biological systems, playing a crucial role in tissue engineering by providing structural, functional, and biochemical support for cell growth and regeneration^{19–21}. Initially, these materials were evaluated primarily on their ability to avoid significant immune rejection. However, with scientific progress, additional criteria such as biodegradability, immune response modulation, and extracellular matrix (ECM) mimicry have become essential^{19–21}. These advancements have enabled the development of materials that not only provide physical support but also promote effective integration with host tissues and stimulate regeneration.

Among biodegradable polymers, aliphatic polyesters have emerged as promising candidates to meet the demands of tissue engineering. Derived from monomers such as lactic acid, glycolic acid, and ϵ -caprolactone, these materials are widely used in scaffolds, drug delivery systems, and sutures^{22–24}. Their controlled biodegradability and tunable

physical properties, such as elasticity and hydrophilicity, make them suitable for specific applications, including bioactive scaffolds for cardiac, muscular, and bone tissue regeneration ^{25,26}.

The selection of these materials depends on factors such as: (I) biocompatibility, avoiding adverse reactions ⁴; (II) Bioactive particle incorporation, accelerating regeneration ²⁷; (III) controlled degradability, ensuring gradual integration with regenerating tissue ^{3,28}; and (IV) processability, enabling fabrication into different shapes, such as fibers and membranes ^{21,29}.

The biodegradation of aliphatic polyesters occurs through the hydrolysis of ester bonds, a reaction catalyzed by esterases in physiological environments ^{21,29}. This feature allows a gradual transfer of functionality from the biomaterial to the regenerating tissue, promoting efficient and functional integration ³⁰. However, commercial polyesters such as poly(ϵ -caprolactone) (PCL) exhibit limitations, including high hydrophobicity and slow degradation (up to 4 years), which restrict their applicability in some tissue engineering areas ³¹.

To address these challenges, functionalization strategies, such as reducing crystallinity and increasing hydrophilicity, have shown promise in accelerating degradation, enhancing cell adhesion, and mitigating local acidification caused by degradation byproducts ^{32,33}. Various polyesters have been modified with functional groups (NH₂, COOH, OH) to improve their properties ^{7,34,35}. However, their biomedical applications remain underexplored.

Therefore, the development of new functionalized polyesters represents a class of materials with great potential for tissue regeneration. Their applications include bioactive scaffolds for bone, cartilage, nerve and muscle tissues, as well as advanced biomedical devices ³⁶. Ongoing research underscores their role in regenerative therapies, paving the way for materials that better address current demands.

2.2 The role of polymerization techniques in synthesizing functional materials

Aliphatic polyesters can be synthesized for two primary methods: ring-opening polymerization (ROP) and polycondensation. Both approaches enable the production of polymers with controlled properties, with key examples being PCL and Poly (lactic acid) (PLA), which are prominent in biomedical applications ³⁷.

Ring-opening polymerization (ROP) is widely used to synthesize aliphatic polyesters with high molecular mass and low polydispersity. In this process, cyclic monomers are opened through reactive centers, such as ionic, cationic, or radical species ³⁸. A well-documented example is the synthesis of PLA, achieved by polymerizing L-lactide using tin octoate as a catalyst and an alcohol as an initiator. This method provides excellent reaction control, resulting in linear or branched structures, and is widely employed on an industrial scale due to its efficiency and reproducibility ³⁹. Similarly, FDA approved PCL, a biocompatible polymer, which is synthesized from ϵ -caprolactone using the same catalyst, making it preferable for producing high molar mass materials with controlled mechanical properties, essential for biomedical device applications ⁴⁰.

Conversely, polycondensation is a traditional and extensively used method for synthesizing polyesters, typically occurring through two mechanisms: (I) Transesterification: A dimethyl ester reacts with a diol, forming a diester (monomer) and oligomers, followed by polycondensation to produce the polymer; (II) Direct esterification: A diacid reacts with a diol, followed by polycondensation ⁴¹ (Figure 2). During the transesterification process, the corresponding diol is released and as soon as the distillation of the diol is stopped, the monomer synthesis process is completed. In the case of monomer production by Fischer esterification, water is released as a byproduct of the formation of the corresponding monomer ⁴².

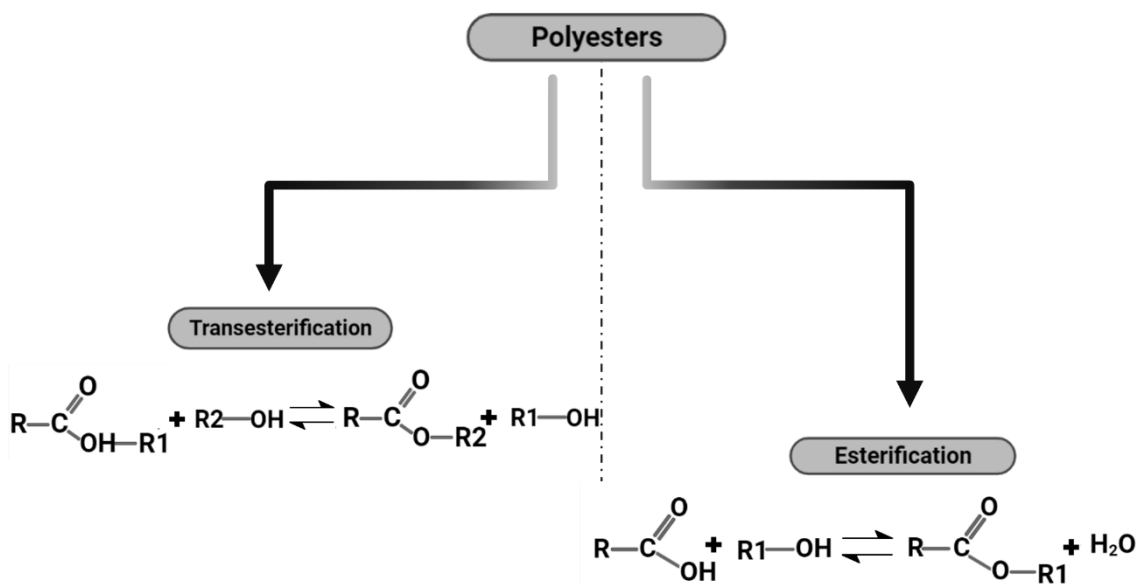


Figure 2. Synthesis of polyesters in (I) by transesterification of dimethyl with a diol; (II) direct esterification of the diacid with a diol, followed by the polycondensation reaction. Reproduced from ⁴³.

After the synthesis of the monomers, the formation of polymers occurs in the same way in both cases. The next stage is polycondensation, which is conducted with increased temperature, reduced pressure, and in the presence of conventional or enzymatic catalysts to decrease the reaction time. The monomer molecules react with each other releasing the corresponding diol with the formation of chains that subsequently react increasing the chain size. The polymer is formed by a step-growth polymerization mechanism ¹.

With the advancement of green technologies and the search for more sustainable and cleaner processes for biomedical applications, enzymatic polycondensation has emerged as a promising alternative to conventional methods. In this approach, enzymes act as biocatalysts, allowing the reaction to occur under milder temperature and pressure conditions. During synthesis, the enzyme promotes the selective formation of ester bonds, resulting in polymers with a linear structure and precise adjustment of the degree of branching. For example, polyglycerol sebacate (PGS) can be synthesized by the reaction between glycerol and sebacic acid at moderate temperatures, such as 90 °C, in a process that can last up to 24 hours in the presence of CALB ⁴⁴.

Enzymatic polycondensation has several advantages over conventional methods. First, it occurs under milder conditions, which prevents thermal degradation of the monomers and makes it possible to obtain polymers with better thermal and mechanical

properties. In addition, enzymatic selectivity favors the formation of aliphatic structures, with a lower occurrence of undesirable secondary reactions, such as excessive branching. Another important advantage is the sustainability of the process, which eliminates the use of toxic metal catalysts and solvents, avoiding the toxic residue from conventional catalysts. Finally, the ability to customize the polymer, through the adjustment of parameters such as temperature, enzyme quantity and monomer proportion, allows the production of materials with specific properties, such as controlled molecular mass and degree of branching ⁴⁵.

2.2.1 Polyester by enzymatic esterification

The synthesis of aliphatic polyesters by esterification in the presence of chemical catalysts is already well documented. Polymers such as poly(1,3-propylene adipate) PPA_d, poly(1,3-propylene succinate) PPS_u, poly (1,3-propylene sebacate) PPSe, as well as their copolyesters poly(1,3-propylene sebacate-co-1,3-propylene adipate) PPSe-co-PPA_d, poly(1,3-propylene sebacate-co-1,3-propylene succinate) PPSe-co-PPS_u, poly (1,3-propylene adipate-co-PPA_d-co-PPS_u of 1,3-propylene succinate) were synthesized by bulk esterification and transesterification in the presence of Ti(OBu)₄ or titanium isopropoxide with 95% average yield ⁴⁶⁻⁴⁸. The reactions conducted in bulk with the chemical catalyst Ti(OBu)₄ from adipic acid, succinic acid, sebacic acid, and 1,3-propanediol reached a number average molecular mass (M_n) of 5,100-5,800 g/mol. When incorporating toluene as a solvent in the reaction, Debuissy et al. ⁴⁶ reached a M_w of 35,100 g/mol in the synthesis of polyesters from succinic and adipic acids with 1,3-propanediol using titanium isopropoxide as catalyst. Syntheses by the chemical route are carried out at high temperatures (170–280 °C) to reduce viscosity and, thus, favor mass transfer during the formation of polymer chains ⁴⁹. However, despite having a good yield, the catalysts used in the chemical route have metallic structures that cause undesirable side effects due to low selectivity, generation of toxic by-products that limit their application, in addition to being carried out at high temperatures, which is unfavorable for thermally and chemically unstable monomers ⁵⁰. Due to these aspects, there is an increase in industrial and scientific interest in using alternative catalysts in the synthesis of polyesters.

Thus, the application of enzymes as biocatalysts in polymer synthesis (Figure 3) offers several advantages compared to traditional chemo-catalysts, such as milder reaction conditions in terms of temperature (usually T <100 °C), pressure, and pH

(usually 3-8). Such conditions generally lead to remarkable energy efficiency, high enantio-, regio-, and chemo-selectivities, as well as stereochemical control. These characteristics allow the development of new functional compounds for pharmaceuticals, agrochemicals, and polymers using non-toxic natural catalysts with a significant "green" appeal, which brings satisfactory benefits to ecological requirements ⁹.

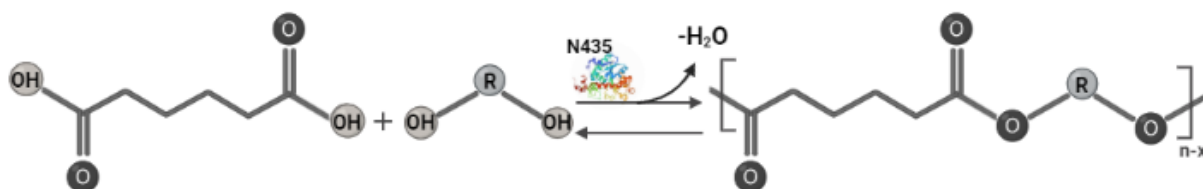


Figure 3. Enzymatic route for polyester synthesis by esterification. Reproduced from ⁴³.

Among the various enzymes that can be used for this purpose, hydrolases stand out as versatile catalysts in the synthesis of water-insoluble polyesters. Lipases (E.C.3.1.1.3) are hydrolase enzymes that can originate from different microbial sources, such as *C. rugosa* (CR), *A. niger* (AN), *P. species* (PS) among others ⁵¹. However, as noted during the survey, one of the most used is lipase B from *C. antarctica* in its immobilized version (commercially known as CAL B –N435). Its favoritism is associated with its high catalytic potential, enantioselectivity, and the ability to maintain its enzymatic activity in several catalytic systems with organic solvents or in the aqueous phase ⁵².

C. antarctica can produce two types of lipases. Lipase A has good thermal stability, resistance to acidic pH, and is capable of accepting tertiary alcohols. However, it presents reduced activity, due to its structure that prevents the substrate from accessing the active site. On the other hand, CAL-B is well characterized as a biocatalyst, as it is versatile in several organic syntheses. Another difference that distinguishes CAL-B is the fact that the active site of the enzyme is not covered by a cap, and the site of attachment of the hydrophobic substrate is directly exposed to the solvent ⁵³. CAL-B has favorable working conditions between pH 6.0 to 8.0 and temperatures ranging from 25-50 °C. To improve its catalytic performance such as thermal stability, enantioselectivity, and specificity, the CAL-B lipase is immobilized and widely marketed to industries. Novozym 435 (N435) is the most used immobilized enzyme in polymerization processes and a reflection of its widespread use is shown in Table 3. Its production by submerged

fermentation, through the genetically modified microorganism *Aspergillus oryzae*, is adsorbed on a macroporous acrylic polymer. Its immobilization guarantees a good enzymatic activity up to 80-90 °C in addition to allowing its recyclability and viability in industrial processes ⁵⁴.

In addition to the recyclability potential of the biocatalyst, the enzyme also guarantees regioselectivity in maintaining functional groups of interest. Hydroxy functional polyesters with a number average molecular mass (M_n) of 53,937 g/mol were obtained through the solution esterification reaction between glycerol, 1,8-ODO, and adipic acid using N435 and diphenyl ether as a solvent ⁶. A similar reaction was performed by Zeng et al. ⁵⁵ showing that the polycondensation of glycerol, 1,8-ODO and adipic acid catalyzed by N435 produced a polyester where the acylation selectivity at the primary hydroxyl sites of glycerol was 74.9% ⁵⁵. According to the authors, the new approach can facilitate the incorporation and bioavailability of molecules of pharmaceutical interest, in addition to increase the biodegradability, due to the presence of hydroxyl groups at the polymer chain.

2.2.2 Influence of water on enzymatic polyesterification with lipases.

Although enzymatic catalysts are interesting alternatives to surpass several drawbacks of organometallic catalysts, some points need to be taken into account to ensure their proper performance. In general, lipases are stable in a nonpolar medium, which suggests the possibility of carrying out reactions that would not be possible in the aqueous medium, having high activity in organic solvents, often hydrophobic ⁵⁶. Despite their stability in non-aqueous media, lipases depend on a minimal amount of hydration that allows the mobility of the enzymatic structure. In 2001 Warwel, Demes, and Steinke studied the polyesterification of 1,3-propanediol with diacids C_{18} , C_{20} , C_{26} and catalyzed by N435 ⁵⁷. Despite the synthesis occurring with a long chain and symmetrically unsaturated diacids, the work did not take into account the removal of excess water, resulting in low molar mass polyesters (M_w values of 1950-3300 g/mol). Binns ⁵⁸ also observed that the water content reduces the reaction rate of the enzyme-catalyzed (N435) synthesis of in the synthesis of polyester with adipic acid, 1,4-butanediol, reaching M_w and M_n values of 1341 and 532 g/mol, respectively. These phenomena were probably caused by water molecules that change the balance of polyester synthesis, reducing

product yield and molecular weight, since excess water causes the reaction to shift, in addition to forming several hydrogen bonds that restrict the structural mobility of the active conformation of hydrolytic enzymes. Although a minimum amount of water is required to perform enzymatic catalysis, polyester synthesis is favored under water restrictions (low water amounts)⁵¹. Since the synthesis of esters and enzymatic hydrolysis are reversible processes, the balance can be shifted by the excess of one of the substrates or by lack of control of the water content of the reaction system.

To remove the “excess” of water formed during the reaction, molecular sieves or evaporation under vacuum, are coupled to the systems⁵⁶. In this way, the presence of water in the medium is decreased, allowing the formation of polymers with high molecular weight. For example, when adding molecular sieves, Lee et al.⁵⁹ reached M_n and M_w of 21000 and 27000, respectively, in polyesterification of 1,8-octanediol, and 1,6-hexanediol with azelaic acid. In the vacuum system (10 mmHg), the enzymatic polymerization of 1,18-cis-9-octadecenedioic acid (ω -carboxyl OA), 1,22-cis-9-docosenoic acid (ω -carboxyl EA), 1,18-cis-9,10-epoxy-octadecanoic acid (ω -carboxyl epoxy SA) and 1,8-octanediol resulted in polyester with M_w and polydispersity index ($\bar{D}$) from 25000 to 57000 g/mol and 2.1, respectively⁵⁴.

2.2.3 Influence of solvents on enzymatic polyesterification with lipases.

Another important factor that can improve the performance of the biocatalyst is the use of organic solvents capable of dissolving the monomers to make them more available to the active site of the enzyme. This practice can interfere with the molecular mass and physicochemical characteristics of polyester⁵¹. In general, the effects of organic solvents on biocatalytic activity arise from direct action on the native conformation of the enzyme, or interaction of water essential to the hydration layer. Its classification can be performed according to the degree of hydrophilicity on the effect of catalytic activity, with $\log P$ being used as a qualitative parameter. Hydrophilic or polar solvents (with a low $\log P$ value) are those that generally show some enzymatic inhibition, while hydrophobic solvents (with a high $\log P$ value) favor high enzymatic activity in nonpolar substances⁵⁹. Although polar solvents facilitate the solubility of some monomers, their more hydrophilic characteristics can cause the removal of the enzyme's hydration layer,

which leads to its denaturation or inactivation, which compromises its catalytic activity⁶⁰.

During immobilized CAL-B catalyzed polymerization of azelaic acid and 1,8-octanediol, the solvents toluene and diphenyl ether were tested at 75 °C in the presence of molecular sieves as H₂O scavengers⁵⁹. Though toluene (log P = 2.68) is not classified as one of the main solvents that respect the principle of green chemistry, Lee et al.⁵⁹ concluded that the diphenyl ether brought better results for Mn and Mw (that is, 21,000 g/mol and 27,000 g/mol after 72 h of reaction). Mahapatro⁶¹ also points out that diphenyl ether, due to its high boiling point, favors the synthesis of polyesters with a high Mn of 28,500 g/mol when adipic acid and 1,8-octanediol were used as monomers. Diphenyl ether was also indicated as the best solvent between xylene, tetra(ethylene glycol), dimethyl ether, and 2-methoxyethyl ether in a previous study⁶² about the polyesterification of adipic acid and 1,8-octanediol.

The combination of solvents can be a feasible alternative. In a study by Cruz-Izquierdo et al.⁶³, the reactions between α , ω -diols (C₂, C₃, C₄, C₆, C₈, C₁₀, C₁₂) and 2,5-FDCA-diMe were performed using the immobilized CAL-B enzyme as catalyst in an anhydrous reaction medium composed of 70% (by weight) of toluene and 30% (by weight) t-butanol. This solvent mixture combines the stabilizing effect of toluene, a hydrophobic solvent that promotes polycondensation reactions catalyzed by lipase⁶⁴, with the greater solubility of the monomers in hydrophilic t-butanol (log P = 0.35)⁶⁵. The results were also compared with bulk polyesterification. In a preliminary study of the esterification of adipic acid and 1,4-butanediol (complex AB), Binns⁵⁸ pointed out that a step-growth mechanism based only on the sequential addition of an AB synthon by the esterification mode occurred in conditions without solvent. On the other hand, when toluene was used as a solvent, there was a change in the favoured esterification mode, according to the conventional polyesterification procedure, pointing to a change in the specificity of the lipase. Thus, the bulk polymerizations reached higher molecular weights (2,227 Mw; and 1,490 Mn) when compared with the solution polymerization with the addition of toluene (1,341 Mw and 532 Mn). Therefore, the molecular characteristics of the polymers were influenced by the solvents used. Table 1 presents the typical solvents used in polycondensation reactions and the respective LogP values.

Table 1. Solvents used in polycondensation reactions, concerning Log P: Degree of hydrophilicity and boiling temperature. Reproduced from ⁴³.

Solvent	Log P	Tb (°C)	Reference
Diphenyl ether	4.21	258.00	66
<i>p</i> -Cymene	4.10	177.00	67
n-heptane	4.00	98.42	68
Cyclohexane	3.71	80.74	66
n-hexane	3.50	68.73	66
Toluene	2.50	110.60	63
2-MeTHF	1.85	80.20	69
T-butyl methyl ether	1.43	55.20	68
Tetrahydrofuran (THF)	0.53	66.00	70
Cyrene	-1.52	226.00	67
Dimethyl sulfoxide	-1.35	189.00	68
Ethyl ether	-0.83	34.60	68
Acetone	-0.21	56.00	70

Although solution polycondensation improves the interchain transesterification rate when N435 is the catalyst, many solvents deviate from the principle of green chemistry for the synthesis of bio-based polyesters ⁶. In this context, new solvent alternatives are being investigated. 2-Methyltetrahydrofuran (2-MeTHF) is a neoteric solvent (with Log P of 1.85) that shows to be a green alternative for the substitution of hexane, toluene, and THF which are more environmentally aggressive ⁷¹. 2-MeTHF is a volatile ether that can be produced by the catalytic conversion of biomass, and its use has been demonstrated in several applications, such as the enzymatic synthesis of poly(1,4-butylene adipate) and poly(1,8-octylene adipate) with $M_n > 2,000$ g/mol and conversions of more than 80% ⁷². When compared to THF during enzymatic synthesis of PGAs, 2-MeTHF was shown to have the same efficiency and M_n values of 3,000 g/mol were reached ⁷¹.

Another alternative in prospecting is ionic liquids (ILs) that have a low vapour pressure, high chemical stability, and favourable solvation properties for many organic or inorganic substances, which allows their application for enzymatic polycondensation reactions under mild conditions ⁷³. Among the different ILs, the ammonium-based ILs

have the lowest viscosity (12 mPa.s at 30 °C) and are highly compatible with N435, due to their “water-like” structure capable of transposing both the ester group (bond acceptor and hydrogen) and the tert-alcohol group (hydrogen bond donor) ³⁷. Thus, these characteristics enabled the use of ammonium-based ILs with dual functionality for the enzymatic ring-opening polymerization (e-ROP) of ϵ -caprolactone reaching Mw up to 18,000 g/mol and high yields (up to 74%) ³⁷.

Studies performed by Yang ⁶ also indicated that N435 has greater activity and stability in ILs with $[\text{Tf}_2\text{N}]^-$ anions than in $[\text{PF}_6]^-$ or $[\text{BF}_4]^-$. This is because ILs containing $[\text{PF}_6]^-$ or $[\text{BF}_4]^-$ anions are more nucleophilic than those containing $[\text{Tf}_2\text{N}]^-$, which reduces the activity of N435 ⁶. In addition to the affinity with the biocatalyst, the relationship between the structure of the polyesters must also be taken into account in the analysis of ILs viability. The enzymatic polycondensation of adipic acid, glycerol and 1,8-ODO was performed at 60 °C ⁶; however it was found that the high viscosity of the reaction medium limited the efficiency of ILs when using $[\text{Tf}_2\text{N}]^-$. The increased viscosity hindered the diffusion of the monomers, reducing the rate of glycerol incorporation.

Supercritical fluids such as carbon dioxide (scCO_2) could also be a promising alternative for enzymatic polycondensation. scCO_2 is a supercritical fluid that has low toxicity and high availability, in addition to having low surface tension and high diffusivity that can be manipulated by changing the temperature or pressure of the reaction system ^{74,75}. Studies indicate that scCO_2 applied in enzymatic polycondensation brings benefits to the synthesis of telechelic polymers since it allows reactions at low temperatures (35 - 60 °C) that avoid enzyme inactivation, and side reactions that cause ramifications and cross-links ^{74,76}. Curia et al., ⁷⁶ performed the enzymatic polycondensation of azelaic acid and 1,6-hexanediol in scCO_2 at 35 °C and 275 bar, reaching Mn values of 2,400 g/mol. On the other hand, the enzymatic polycondensation of divinyl adipate and 1,4-butanediol in scCO_2 at 60 °C and 275 bar resulted in polyesters with Mn of 3,900 g/mol. The scCO_2 can be a good solvent for enzymatic polycondensation reactions, but still little explored, probably due to the high pressures that should be attained ⁷⁴.

Another alternative solvent that is emerging in the literature is the dihydrolevoglucosenone (CyreneTM). CyreneTM is a non-toxic, biodegradable and bio-

based solvent derived from lignocellulosic biomass feedstock, produced by Circa Group⁷⁷. This solvent was shown to be able to solubilize polyesters such as poly(3-HydroxyButyrate-co-3-HydroxyValerate) and form stable and homogeneous solutions. Cyrene™ is a polar aprotic solvent with a chemical-physical profile comparable to traditional solvents such as N-dimethylacetamide and N-dimethylformamide⁷⁷. However, the effect of Cyrene on enzymatic activity in polycondensation reactions is still to be elucidated.

2.2.4 Biologically-based building blocks for enzymatic polyesterification

In addition to being used as a renewable source for obtaining diols, research has been extended regarding the application of polyols in esterification processes^{78,79}. When glycerol and azelaic acid were used for the synthesis of poly(glycerol azelate) in the absence of catalysts at 140 °C, there was an increase in the degree of branching and cross-linking of the chains, with an weight-average molecular mass and $\bar{D}$ of 6981 g/mol and 2.43, respectively, and crosslink density of 7.60 Kmol/cm³. The presence of branched and cross-linked chains improved the mechanical properties of the polymer that presented a rubbery consistency at room temperature with a glass transition temperature in the range of -28.43 to -23.40 °C⁴⁵. On the other hand, when glycerol and adipic acid were used as monomers in the chemical route, hyper-branched polymers were synthesized (23% branching degree) obtaining Mw of 4900⁸⁰.

The diarylborinic acids have also been reported as an option for polymerization by enzymatic catalysis, with high regioselectivity capable of activating the OH group used to control microstructures in the synthesis of Poly(glycerol sebacate), obtaining a 93% yield with Mn of 24.4 kDa and $\bar{D}$ of 1.4⁸¹. However, branched polymers can reach the extreme form of lattices that have as main consequence the alteration of properties in the product, especially concerning melting temperature and solubility properties, in addition to hindering the approximation of the polymer chains, reducing the molecular interaction. In contrast to reactions without catalysts or those carried out by the chemical route, immobilized Cal-B proved to be an excellent catalyst for the synthesis of aliphatic polyesters, such as poly(1,12-dodecanedioic acid-co-1-thioglycerol) with an Mw of 5380 g/mol⁸²; and polyesters of glycerol and oleic diacid, with Mn values from 6000 to 9100 g/mol and low degrees of branching (13 to 16%)⁵⁴.

Table 2 summarizes the recent and relevant studies that used biological-based building blocks for the synthesis of polyesters by enzymatic polycondensation. It is possible to observe that although the survey corroborates the position of Yu et al.⁵⁰ that the length of the monomer chain implies in obtaining higher molecular weights, monomers with a short chain and the odd number of methylene units, such as 1,3-propanediol, can result in several excellent properties, such as high elasticity and capacity of dyeing, which makes it suitable for the textile market⁸³. Poly(propylene terephthalate) (PPT), which is composed of 27% bio-based and is produced by polycondensation of 1,3-PDO and TPA is an example that has already been successfully applied in the market, to replace PET⁸⁴. Usually, this reaction occurs in the presence of metallic catalysts, and its products are not considered biodegradable. In an attempt to build a greener polyester with good properties, transesterification reactions are being considered in new studies.

Table 2. Enzymatic synthesis of aliphatic and aromatic polyesters by esterification. Reproduced from ⁴³.

Diacid	Diol	Catalyst	Synthesis T (°C)	By-product removal Strategy	Solvent	Time (h)	Mw (g/mol)	Đ	Yield (%)	Reference
1,9-nonanedioic acid; 1,10-decanedioic; 1,11-undecanedioic	1,8-ODO, 1,6- HDO	IM-CAL-B	75	Molecular sieves	DPE; Toluene	24;48;72	21,000– 27,000	1.21- 1.27		59
Malic acid	1,3-PDO; 1,5- pentanodiol; 1,8- ODO	N435	80	Vaccum	DPE	24;48;72	48,00- 8,100	1.3-1.4	82	85
Adipic acid	1,4-BDO	N435	60	Vaccum	Bulk/Toluene	24	1,341- 2,227	1.1-1.7		58
1,18-cis-9- octadecenedioic;1,22- cis-9- docosenedioic;1,18- cis-9,10-epoxy- octadecanedioic acids	1,3-PDO 1,8- ODO 1,16- hexadecandiol.	N435	90	Vaccum	Bulk/DPE	24	25,000- 57,000	2.0-2.3		54
1,18-cis-9- Octadecenedioic (oleic diacid, ~99% pure)	Glycerol	N435; Dibutyltin oxide	90 – 150	Vaccum	Bulk	24	6,000 a 9,100	3.3-9.8		86
1,12-dodecanedioic	1-thioglycerol	N435	80	Vaccum/ potassium hydroxide pellets	Bulk	96	5,380	2.9	89	82
Sebacate acid	Glycerol	Diarylborinic acids	140		THF	48	24,400	-	60-80	81

Favoritism for enzymatic synthesis of polyesters by direct esterification is also related to catalyst selectivity and the ability to operate at low temperatures. Thus, the enzymatic catalysis contributes to obtaining polymers with narrow molecular mass distributions, indicating that the reactions are well controlled with the absence of parallel reactions during polycondensation such as cyclization and degradation, reactions typically observed at high temperatures. Given the above, Yang et al.,⁸⁶ reports that when comparing N435 with Dibutyltin oxide, for the synthesis of Poly(oleic diacid-co-glycerol), the enzyme was able to reduce the $\bar{M}_w$ from 9.8 to 3.3, in addition to avoiding cross-linking between monomers.

Lipase/hydrolase catalyzed polycondensation reactions have proven to be a powerful tool for synthesizing aliphatic polyesters by esterification. The high enantio-, regio-, and chemoselectivity, as well as the stereochemical control of these catalysts, are characteristics that allow the development of new functional compounds for products with biomedical applications. In addition to providing satisfactory benefits to ecological requirements, enzymatic catalysts are applicable with many thermally or chemically unstable monomers, including hydroxyl moieties, which provide an important option for the synthesis of diverse polymers. In addition to the assertive choice of enzymatic catalysts, the reaction method is also taken into consideration. The application of solvents during the reaction is indicated as facilitators of diffusivity, viscosity reducers, and increase of homogeneity that allow the improvement of the achievement of high molecular weights. However, given the difficulty in removing the solvent from the reaction product, bulk polymerization is gaining prominence for application in new studies, due to the possibility of forming high molecular mass polymers without interfering with enzymatic activity. The many advantages of enzymatic catalysis in bulk polycondensations, together with the choice of sustainable building blocks, form alternative technological tools for the commercial production of future biomaterials. The few studies found on short-chain bio-based building blocks, such as 1,3-PDO, show a gap that should be evaluated in future research, since this monomer has proven to be versatile for esterification reactions.

The new polyesters confer characteristics that offer significant improvements in the mechanical properties and biodegradability of traditional scaffolds, such as those based on PCL and PLA, contributing to overcoming limitations associated with tissue engineering. By integrating enzymatically produced biopolyesters into scaffolds, it is possible to explore unique

properties such as elasticity, hydrophilicity and the ability to carry bioactive molecules, which are fundamental characteristics for mimicking the extracellular matrix. Thus, sustainably obtained polyesters, free from aggressive chemical catalysts, can be electrospun as additives in three-dimensional nanostructured structures, expanding their applications as innovative biomaterials and contributing to advances in tissue regeneration.

Therefore, this study aims to investigate the use of renewable monomers (Diethyl succinate, 1,3-propanediol and Glycerol) to obtain aliphatic polyester by enzymatic route as a promising additive for biomaterials in tissue engineering.

2.3 Electrospinning Technology for Fiber Fabrication

The electrospinning (ES) technique is extensively employed in fabricating scaffolds for tissue repair purposes, due to its unique capability to create nanofiber-based architectures that resemble the extracellular matrix (ECM)⁸⁷. This structural similarity, combined with the large surface area and high porosity of the nanofibers, promotes an environment favorable to proliferation, differentiation, and cell adhesion, fundamental characteristics for tissue regeneration⁸⁸. Historically, the technique has its roots in the 17th century, but it was from the 1930s and 1990s that its industrial and biomedical potential was consolidated, culminating in the recognition of its applicability in areas such as filtration, sensors, drug delivery systems, and tissue engineering⁸⁹.

The ES process is based on electrohydrodynamic phenomena that involve the application of an electrical voltage to a polymeric solution⁸⁹. This solution, consisting of synthetic or natural polymers dissolved in volatile solvents, is extruded through a syringe fitted with a metallic needle. Once the applied voltage surpasses the surface tension of the polymer solution, an electrically charged fluid jet emerges from the Taylor cone end of the needle⁸⁹. During the path of this jet to the metal collector, the solvent evaporates and the jet undergoes elongation, leading to the deposition of solid nanofibers (Figure 4).

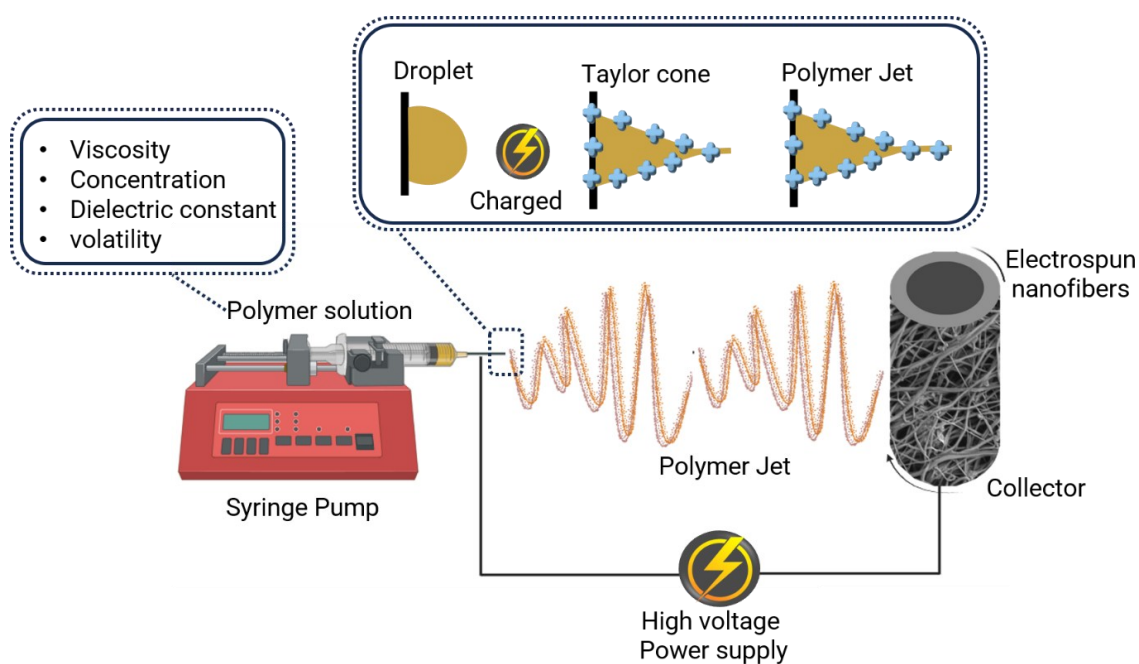


Figure 4. Illustrative diagram of the polymer jet formation process for the electrospinning of nanofibers.

The control of the properties of the obtained nanofibers depends on three sets of interdependent parameters (Figure 4): (i) polymer solution parameters, such as solvent viscosity, concentration and volatility; (ii) process parameters, including solution flow, gap from the needle to the collector, electric potential applied; and (iii) environmental parameters, for example relative humidity and temperature. Changes in these elements can notably affect fiber morphology, directly impacting their uniformity, the formation of defects (such as beads) and the final properties of the scaffold⁹⁰.

Several polymers have been employed in producing fibrous scaffolds for biomaterials engineering through the electrospinning process. Natural polymers such as chitin, collagen, and chitosan are known for their biocompatibility, although they often present limitations in the mechanical properties required for specific applications^{91–93}. Conversely, synthetic polymers frequently utilized in electrospinning include PLA, PCL, among others⁹⁰. However, despite its excellent processability and mechanical stability, pure poly(ϵ -caprolactone) (PCL) presents intrinsic limitations, such as high hydrophobicity and a slow degradation rate, which can hinder cell adhesion, proliferation, and integration with regenerating tissues⁹⁴. These drawbacks make PCL less suitable for applications that require enhanced bioactivity and controlled degradation to match tissue regeneration rates.

Among the polymers aimed at tissue engineering, poly(glycerol sebacate) (PGS) is notable for its tunable properties, excellent biocompatibility, and ideal degradation rate, which makes it attractive for soft tissue engineering^{95–97}. However, the electrospinning of pure PGS fibers is challenging due to the insolubility of cured PGS^{94,98,99}. On the other hand, at room temperature, the PGS prepolymer has high viscosity, which makes it difficult to form continuous filaments due to its low molecular mass⁹⁹. To overcome these barriers, PGS and its analogues can be electrospun as additives in already consolidated structures such as PCL, allowing the development of new scaffolds with tunable properties⁹⁰.

This technique involves combining PGS or its analogues with solutions of other compatible polymers, allowing the mixture to be processed and electrospun. During the process, the auxiliary polymers act as carriers that facilitate fiber formation⁹⁰. Moreover, the addition of these auxiliary materials allows adjusting and improving the properties of the obtained fibers.

One example of a highly used degradable polymer is PCL, often used in mixtures with PGS for biomedical applications. Luginina et al, for example, used the PGS prepolymer to improve the stiffness of PCL and obtain fibers composed of bioactive silicate glass (BG 13-93) or borosilicate glass (BG 13-93BS) aiming at application in the regeneration of soft tissues¹⁰⁰, such as native human myocardial tissue²⁸. Najafabadi et al. developed elastic PGS-PCL nanofibers by electrospinning, which were applied in flexible electronic devices, such as smart dermal patches¹⁰¹. More advanced studies have also demonstrated the advantage of using PGS-analogous polyesters obtained by the enzymatic route, such as poly(glycerol-1,8-octanediol-sebacate), which allows obtaining adjustable compositions, improving the biodegradability and mechanical properties of the fibers¹⁰². Thus, with the application of this technique, PGS-based materials allow expanding tissue engineering investigations for chronic wounds, soft tissues, cardiac, vascular, tendons, and ligaments, for example⁹⁰.

2.4 Bioactive Glasses in Regenerative Medicine

With advances in tissue engineering, bioactive glasses play a crucial role as scaffold components. Scaffolds, such as electrospun mats, act as temporary matrices that guide bone repair by offering structural integrity along with a biochemical setting that promotes cellular growth and vascularization¹¹. In parallel, bioactive glasses can be combined with electrospun polymers, creating composites that integrate bioactivity properties, making them ideal for advanced applications in tissue regeneration³⁰.

The first bioactive glass (BG) was obtained and reported by Larry Hench in 1969¹⁴. Hench et al. investigated several materials that could bind to bone and selected a degradable glass that had a composition near a ternary eutectic point on the Na₂O-CaO-SiO₂ phase diagram [wt%]: 45.0 SiO₂, 24.5 CaO, 24.5 Na₂O, 6.0 P₂O₅, later named Bioglass® 45S5¹⁴. This discovery marked the beginning of the field of bioactive ceramics, leading to the development of numerous materials and applications based on bioactive glass systems.

Bioactive materials are described as those that elicit a favorable physiological reaction, notably through attachment to the recipient's tissue¹⁰. The tissue binding mechanism is attributed to the development of hydroxyapatite (HA) on the BG surface after its initial dissolution. HA has a mineral composition similar to the mineral phase of bone, therefore capable of interacting with collagen fibrils, binding to host tissue. This characteristic makes HA a suitable surface for the proliferation and fixation of osteogenic cells¹⁰ thus being one of the indicators of biocompatibility.

BGs are generally synthesized through either melt-derived or sol-gel processing routes. Bioglass® 45S5 and other commercial compositions are commonly produced via the melt method, which involves heating a mixture of oxides to high temperatures (usually between 1300–1500°C) in platinum crucibles, followed by quenching in graphite molds or water¹⁰³. Alternatively, the sol-gel approach allows the production of BGs nanoparticles at a low energy cost due to the processing temperature occurring between 600-800°C, allowing greater control over composition and morphology¹⁰⁴. This technique uses soluble organic or inorganic precursors (such as TEOS, nitrates or acetates) that undergo hydrolysis and polycondensation reactions in a liquid medium, forming a three-dimensional gel network. The main advantage of the sol-gel route is its ability to produce materials with high surface area and porosity at lower temperatures, which provides desirable characteristics for interaction with biological fluids, HA formation, in addition to making it possible to incorporate therapeutic compounds, such as ions and bioactive molecules¹⁰⁵.

BGs are amorphous materials composed of network formers, modifiers, and intermediate oxides whose functions define their physicochemical and biological properties. Silica (SiO₂) is the main network former, forming a continuous matrix of tetrahedrons linked by oxygens¹⁶. Modifiers such as Na₂O and CaO interrupt these bonds, creating non-binding oxygens that increase the solubility and bioactivity of the material. Intermediate oxides, such as pentoxide (P₂O₅) and boron trioxide (B₂O₃), can affect the structure in a variable way,

contributing to the formation of calcium phosphate and the modulation of the glass network^{16,106}. The proportion between these components is essential to control the reactivity, the rate of manipulation and the integration with biological tissues.

The sol-gel method consists fundamentally of precursor hydrolysis and condensation reactions, followed by drying and calcination steps. Precise control of processing parameters allows adjusting properties such as morphology and final chemical composition of BGs. In the synthesis of bioactive glass nanoparticles (BGNs) by this route, tetraethyl orthosilicate (TEOS) is widely used as a silica source, while water and/or alcohols, such as methanol or ethanol, act as solvents¹⁰. The technique can be carried out under acidic or basic conditions, which directly affect the structural and functional characteristics of the material obtained. During the process, TEOS undergoes hydrolysis and condensation in the presence of appropriate catalysts, yielding SiO₂ nanoparticles (NPs)¹⁰⁴. Hydrolysis promotes the replacement of alkoxide groups by hydroxyls, allowing partially hydrolyzed molecules to join by condensation of silanol groups (Si–OH), forming siloxane bonds (Si–O–Si), with the release of water or alcohol as a byproduct. Metal ion precursors can be incorporated during or after the formation of silica NPs. The particles formed are subsequently dried and calcined to obtain BGNs.

Silicon alkoxides, such as TEOS, tend to generate a three-dimensional gel structure in an acidic medium or isolated particles in a basic medium¹⁰. Based on this property, spherical and monodisperse nanoparticles, with diameters between 0.05 and 2 µm, can be produced through the base-catalyzed sol-gel route, known as the Stöber method¹⁰⁷.

In Stöber method, TEOS and metal precursors are added to a solution containing water, alcohol, and ammonium hydroxide, under constant stirring. Variables such as the TEOS/water ratio, the pH of the solution, and the nature of the precursors directly influence the size and shape of the particles. The presence of OH groups on the surface favors repulsive forces between the nanoparticles, resulting in spherical and uniform morphologies. Studies show that lower concentrations of TEOS and water favor the production of particles with a narrower size distribution¹⁰⁸. while increasing the concentrations of these reagents, as well as ammonia, leads to the formation of larger particles. In methanol-based systems, BGNs tend to have smaller dimensions, increasing proportionally to the length of the carbon chain of the alcohol used^{109,105}.

Mesoporous bioactive glass nanoparticles (MBGNs) are synthesized by the addition of surfactants to the sol-gel technique^{110,111}. This accessible approach enables the production

of MBGs with different morphologies, suitable for the controlled release of ions in biological environments¹¹⁰.

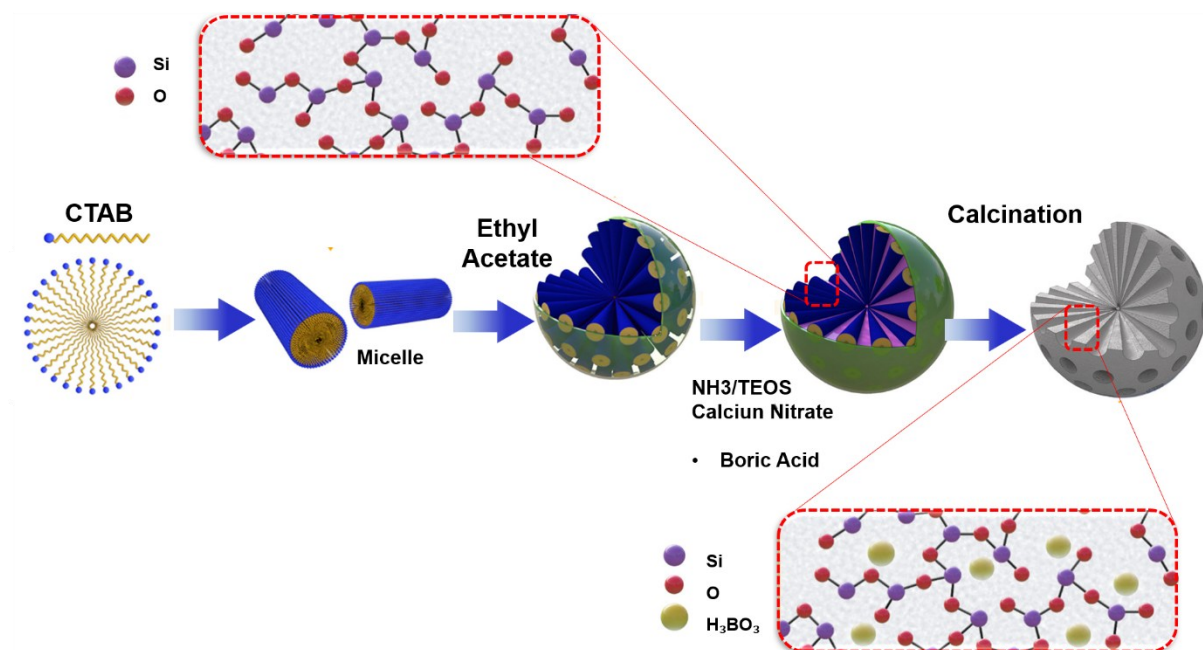


Figure 5. Schematic representation of the production of boron-doped MBGs by the microemulsion-assisted sol-gel technique. Adapted by¹¹².

To obtain MBGs, microemulsion droplets are formed as a liquid template from a mixture of ethyl acetate with the surfactant cetyltrimethylammonium bromide (CTAB) and water, thus creating mesoporous channels within the particles¹¹⁰ (Figure 5). The organization of the pores and the final mesostructure depend on the concentration of the surfactant and the dynamics of the microemulsion. After synthesis, the templates are eliminated by calcination, consolidating the mesoporous structure. The hydrolysis of ethyl acetate is promoted by the addition of aqueous ammonia, which acts as a catalyst¹¹⁰. The combination of nanometric scale, mesoporosity, and bioactivity gives MBGs a high surface area and excellent capacity for incorporation and release of therapeutic molecules, fundamental properties for applications in biomaterials.

2.5 Boron-Doped Bioactive Glasses

BGs promote therapeutic responses mainly through the gradual release of ions into the biological environment. Several elements, such as copper (Cu), magnesium (Mg), silver (Ag), zinc (Zn), and boron (B), have been incorporated into the BG structure to enhance biological

properties, including osteogenesis, angiogenesis, antimicrobial activity, and anti-inflammatory effects¹⁸. As BGs dissolve in body fluid, ions are released by ion exchange, reaching the site of action directly. This maximizes therapeutic benefits while minimizing side effects^{113–116}.

Furthermore, BGs allow a continuous release of ions due to their controlled degradation. The release rate is adjusted by altering the connectivity of the glass network and the types of modifiers or intermediate ions added^{117–119}. However, to achieve the desired effects, it is essential that the ions are released in a controlled manner and in optimized concentrations, ensuring an environment conducive to cell proliferation without inducing toxicity¹¹⁸.

Among the elements that can be added, B stands out as an ion of great relevance^{16,120}. Naturally present in nature, boron is essential for regulating biological functions¹²¹. B is capable of modulating structural components of the cell membrane, such as glycoproteins and glycolipids in addition to influencing biochemical pathways associated with calcium absorption and vitamin D metabolism, contributing to bone mineralization processes and protection against¹²².

The addition of boron to the composition of bioactive glasses not only enhances their bioactivity but also stimulates regenerative responses, such as bone formation, angiogenesis, and soft tissue regeneration. A notable example was observed in mesoporous bioactive glass nanoparticles (MBG) containing boron silicate, synthesized with sizes between 100 and 300 nm. These nanoparticles showed high bioactivity, the ability to reduce inflammatory responses without causing cytotoxicity at adequate concentrations, proving to be suitable for bone regeneration¹²³. The effects of boron go beyond bone regeneration, extending to chronic wound healing and soft tissue regeneration. Boron exhibits antibacterial and angiogenic properties, accelerating blood vessel formation and promoting healing. Studies have shown that boron stimulates the proliferation, migration, and tubule formation of human umbilical vein endothelial cells (HUVECs) was enhanced, along with an increased release of cytokines involved in angiogenesis^{30,124}. Other investigations have demonstrated that HS-2 epithelial cells treated with boron-containing composite mats exhibited significantly enhanced wound healing attributed to the modulation of oxidative stress pathways and attenuation of the inflammatory process¹²⁵.

Furthermore, boron applications in soft tissues have shown promising results for example, when applying boron to poly(L-lactide) (PLLA) mats containing Boron, the ability to

stimulate myogenic differentiation of C2C12 cells was demonstrated, with greater myotube formation and an increase in cells positive for sarcomeric myosin ^{126,127}. Likewise, Ciriza et al. ¹²⁸ showed that Boron supplemented in culture medium induced the activation of NaBC1 protein and integrins, promoting the fusion and maturation of myotubes of C2C12 cells ^{30,128}. These results highlight the role of boron in stimulating the regeneration of skeletal muscle tissues.

Additionally, the stimulating effect of boron on soft tissues was associated with the upregulation of key angiogenic mediators, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) ¹²⁹. These molecules facilitated the development of newly formed microvasculature and the expansion of rough endoplasmic reticulum, thereby enhancing the applicability of bioactive glass-based systems in the regeneration of both soft and hard tissues ¹²⁹.

Thus, the incorporation of boron into tissue engineering systems presents itself as a promising approach to treat chronic wounds, promote angiogenesis, regenerate and restore the functionality of various types of tissues. The use of this element is a field to be explored to develop new advanced biomaterials with applications aimed at tissue engineering.

2.6 Final Remarks

Although significant advances have been made in the development of biomaterials for tissue engineering, challenges remain in the development of new polyesters through environmentally friendly routes that act as additives to control the degradation and hydrophilicity of conventionally used polymers such as PCL. Furthermore, the lack of studies integrating bioactive nanoparticles with biobased aliphatic polyesters in hybrid scaffolds represents a research gap.

This study aims to address these challenges by developing enzymatically synthesized functionalized polyesters and incorporating them into PCL electrospun fibers enriched with bioactive glass and boron-doped mesoporous bioactive glass nanoparticles. This approach represents a step forward towards sustainable, bioactive, and highly functional scaffolds for regenerative medicine applications.

Chapter 3

Hydrofunctionalized Polyester Synthesis

Following the initial discussion on the importance of renewable polymers for biomedical applications, this chapter focuses on the controlled synthesis of functionalized aliphatic polyesters. This section contains results on the investigation of the synthesis of hydrofunctionalized polymers from glycerol (GLY), 1,3-propanediol (1,3-PDO), and diethyl succinate (DS). The polymers were obtained by solvent-free enzymatic polycondensation, and the selectivity of N435 was investigated. The results obtained were published in *Macromolecules Journal*, 2024 under the title “*Enzymatic Synthesis of Hydroxy-Functionalized Glycerol-Based Polyesters: A Study of Functionalization, Regioselectivity Control, and Catalyst Reuse.*” (DOI: 10.1021/acs.macromol.3c01533), of which I am the first author.

3.1 Introduction

Aliphatic polyesters are widely used in the construction of electronics, pharmaceuticals, packaging, and healthcare products because they have a biocompatible and tunable properties structure, as well as adjustable physicochemical properties¹³⁰. However, the lack of modifiable pendant functional groups on the main chain limits the large-scale application of these polyesters. In this sense, efforts have focused on synthesizing functionalizable polyesters from polyols with free hydroxyl groups for applications such as micropatterned elastomers¹³¹, salt-leached scaffolds¹³², and electrospun fibers¹³³. These functional groups also enhance biodegradability, post-functionalization, and crosslinking, which is desirable for drug delivery systems¹³⁴.

Among polyols, glycerol is promising due to its natural abundance and production as a biodiesel by-product. It is also used as a substrate for the biosynthesis of succinic acid and 1,3-propanediol^{135,136}. Glycerol's trifunctionality enables its use in esterification and transesterification reactions⁶. It is recognized as a chemical building block by the US Department of Energy and is considered safe by the FDA.¹³⁷ However, the synthesis of linear polyesters functionalized by glycerol remains a major challenge due to its multiple hydroxyl groups of different reactivity¹³⁸. This complexity demands precise control over reaction conditions to avoid branching and gelation^{130,139}.

To address this, models such as Martin-Smith have been applied to define optimal monomer ratios for non-gelling hyperbranched polymers^{140–142}. Another strategy involves selective protection of hydroxyl groups, yielding tailored polyols¹⁴³.

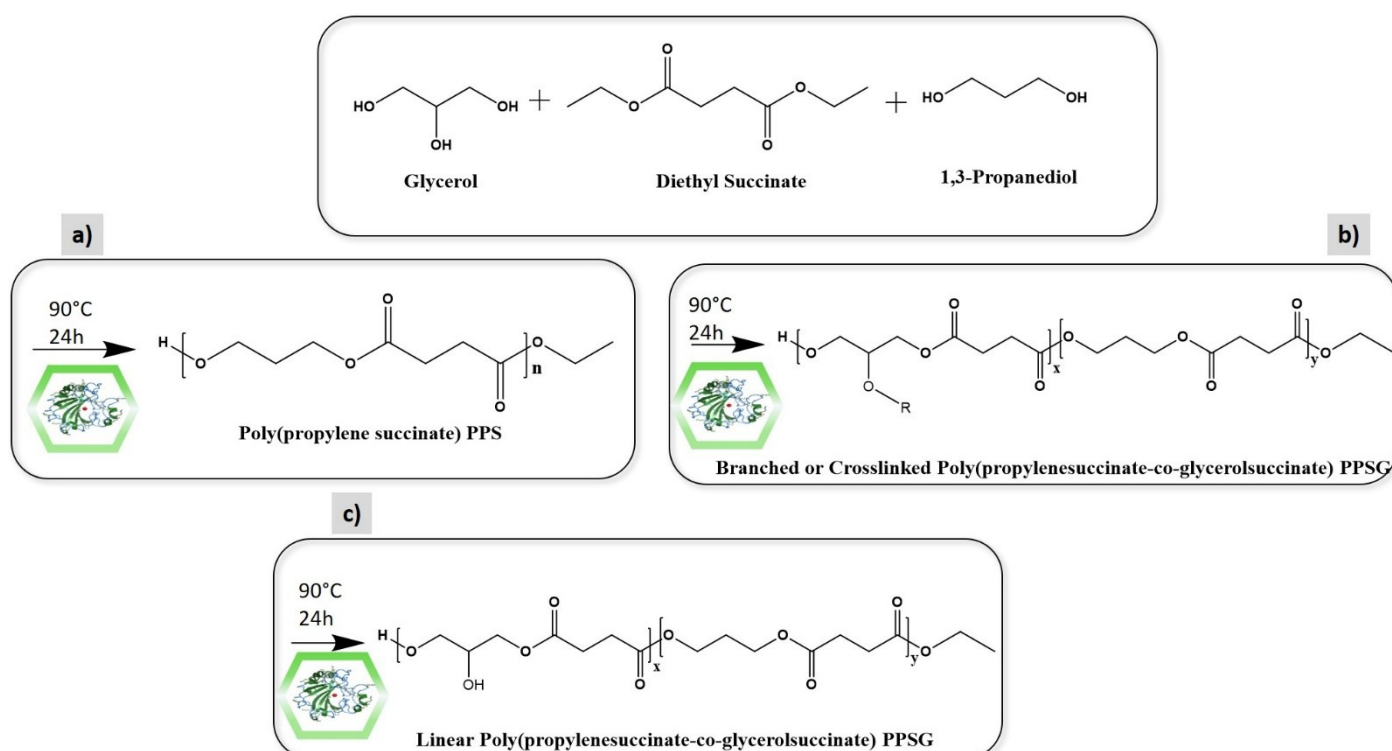
Enzymatic catalysis has emerged as a selective and eco-friendly alternative to traditional methods^{2,102,134}. Enzymes offer high activity under mild conditions, regioselectivity, and low toxicity^{2,144}. When immobilized (e.g., Novozyme N435®10), they allow reuse and recovery, supporting green chemistry principles^{145–147}.

Lipases have been used in direct esterification, transesterification, and ring-opening polymerizations¹⁴⁸. Direct esterification may present low reaction rates due to solubility restrictions of succinic acid. Transesterification, using diesters like diethyl succinate, circumvents such limitations at 40–90 °C¹⁴⁹. Enzyme-catalyzed processes are carried out at lower temperatures than conventional catalytic systems, avoiding undesired crosslinking^{150,151}. Temperature, monomer ratios, and enzyme type influence polymer properties, including in reactions using divinyl sebacate and glycerol^{152–154}.

Similar behaviors were observed in the synthesis of poly(octamethylene adipate-co-glycerol adipate). Hydroxy-functionalized polyesters with high molar mass ($M_n = 53,937 \text{ g.mol}^{-1}$ in 24 h) were obtained using ionic liquids as solvent¹³⁴. Bulk enzymatic synthesis of poly(glycerol adipate) under varying conditions showed increased branching with temperature¹⁵⁵. While immobilized lipases like N435 tolerate higher temperatures, stability drops above 90 °C, while lower temperatures increase viscosity and mass transfer limitations⁸.

Diols influence polyester properties: 1,3-propanediol (PDO), from glycerol fermentation, reduces crystallinity and melting point and enhances elasticity and degradation^{46,156,157}. It is also used in PTT synthesis^{2,158}. However, although the synthesis of polymers with pendant hydroxyl groups has been extensively studied, there remains limited research on copolyesters with tailored architectures for diverse applications.

To address this gap, this work developed an enzymatic polymerization strategy for producing hydroxy-functionalized copolyesters. The optimal glycerol concentration, reaction time, and N435 reuse were evaluated using transesterification of DS, PDO, and glycerol under solvent-free conditions (Scheme 2). The DS/PDO/Gly molar ratios tested were 1:0.5:0.5; 1:0.8:0.2; and 1:0.9:0.1. Polymer structure and enzyme stability were analyzed using FT-IR, NMR, GPC, SEM, and enzymatic activity. This approach enables the synthesis of high molar mass, low-branched copolyesters with selective reaction at primary hydroxyls.



Scheme 2. Synthesis of: PPS (without glycerol) (a); PPSG when all the three hydroxyls of glycerol are reactive, rendering a branched or crosslinked copolymer represented by **R** (b); PPSG when only the primary hydroxyls of glycerol are reactive rendering a linear copolymer (c). Reproduced from¹⁵⁹.

3.2 Materials and Methods

3.2.1 Materials

Candida antarctica lipase *B* immobilized on the acrylic resin (N435) was cordially provided by Novozyme (EC 3.1.1.3). 1,3-propanediol, (PDO, purity 98%), Diethyl Succinate (DS purity $\geq 99\%$) was purchased from Sigma-Aldrich. Chloroform (purity, 99%) was obtained from Dinamica, (Brazil). Chloroform (HPLC, 99%) was provided by J.T Baker Co., Ltd (Brazil). Deuterated chloroform (99%) was purchased from Sigma-Aldrich. Oleic acid (OA, purity $\geq 99\%$), and ethanol (EtOH, purity 95%) were obtained from Neon. All reagents were used without further purification.

3.2.2 Lipase-catalyzed hydro-functionalized polyester synthesis

Poly (propylene succinate-co-glycerol succinate) (PPSG) was synthesized by polycondensation in bulk with different monomer ratios. For this synthesis, the desired amounts of monomers (as detailed in Table 3) were added into a 50 mL round bottom flask with one mouth. 10 wt % of N435 was used as a catalyst in all reactions and these were performed at 90°C, under stirring (500 rpm), and at reduced pressure (0.1 atm) for 24 h.

Table 3. Formulations of the enzymatic polycondensations in bulk conditions. Reproduced from¹⁵⁹.

Entry	Succinic Acid (mol)	Diethyl Succinate (mol)	1,3-PDO (mol)	Glycerol * (mol)	COO: OH (mol ratio)
PPS	-	1	1	-	1: 1
PPSG ₁₀	-	1	0.9	0.1	1: 1.05
PPSG ₂₀	-	1	0.8	0.2	1: 1.1
PPSG ₅₀	-	1	0.5	0.5	1: 1.25
PPS _A G ₂₀	1	-	0.8	0.2	1: 1.1

*The amount of glycerol was based on the assumption that only 2 of the 3 available OH groups would react.

In sequence, the flask was placed in an oil bath on a heating plate with magnetic stirring (C-MAG HS 7, IKA), under N₂ flow at 90 °C and 700 rpm for 30 min to homogenize the system. Then, the catalyst (N435) (10% by total weight of monomers) was added to the reaction flask, at reduced pressure (0.1 atm, provided by a vacuum pump, I2P-740/SCN, Indutec) to remove the by-product (ethanol). Reactions were performed at 90 °C and 500 rpm for 24 h.

The by-product vapors were condensed in a column condenser (kept at 5 °C by a thermostatic bath, model MQBTC99-2, Microquímica), collected to a dean stark and weighed during the reaction. According to the stoichiometric equation of these reactions between a diester and polyols, the formation of by-products (ethanol) is expected during polycondensation. Thus, from the quantification of the ethanol collected in the dean stark during the reaction, it is possible to calculate the conversion of the ester groups, as described by Equation (1):

$$\text{Ester Group Conversion (\%)} = \frac{m_{\text{ethanol}}}{(2 \times n_{DS} \times MM_{\text{ethanol}})} \times 100 \quad (1)$$

where $m_{ethanol}$ (g) is the mass of ethanol collected throughout the reaction, n_{DS} (mol) is number of moles of diethyl succinate (DS) in the reaction and $MM_{ethanol} = 46.06$ (g/mol) is the molar mass of ethanol.

The final product obtained through enzymatic polycondensation was cooled and dissolved in chloroform to reduce the viscosity and remove N435 by vacuum filtration. Next, the filtrate (including the gel and soluble polymer fractions) was evaporated on a heating plate at 50 °C under stirring (200 rpm) to remove excess solvent¹³. Finally, the samples were taken to a forced convection oven (DL – AF, De Leo) and left for 72 h at a temperature of 60 °C to ensure the total solvent evaporation. The same process was followed for all formulations shown in Table 1. As a control of enzymatic catalysis, a thermal reaction was carried out with succinic acid, 1,3-propanediol, and glycerol as monomers. The direct esterification reaction was started at 150 °C, above the melting temperature of succinic acid, and 500 rpm under a stream of nitrogen at a flow rate of 3 mL.s⁻¹ for 1 h at atmospheric pressure. Next, the pressure was reduced to 0.1 atm and the temperature increased to 180 °C and the reaction was maintained for an additional 12 h under the constant stirring rate of 500 rpm without enzyme addition¹⁶⁰

3.2.3 Enzyme Stability

After the end of each reaction, the biocatalysts were washed with chloroform and dried in a forced convection oven at 60 °C for 24 h to evaluate the enzymatic stability through reuse cycles. Subsequently, the enzymatic activity of the immobilized Lipase was determined based on the esterification between OA and EtOH, measuring the acid concentration before and after the test. Thus, 0.045 mol (12.771 g) of OA, 0.045 mol (2.071 g) of EtOH, and 0.013% w/w (0.195 g) of N435 were added to a magnetic stirring system, at a temperature of 40 °C, for 40 min. Aliquots (0.150 µL) from the initial time and after 40 min of reaction were diluted in 20 mL of EtOH (to stop the reaction). Then, the diluted samples were titrated using a NaOH solution (0.05 mol.L⁻¹) until reaching pH 11 to measure the concentration of non-reacted OA. Equation (2) is applied to calculate the enzymatic activity in U.g⁻¹.

$$Lipase\ Activity\ (U.g^{-1}) = \frac{(V_i - V_f) \times [NaOH] \times 10^3}{m \times t} \quad (2)$$

where V_i and V_f are, respectively the initial and the final titrant volumes (mL); $[NaOH]$ is the concentration of the NaOH solution (mol.L⁻¹); m is the mass of N435 (g), and t is the reaction time (min).

Regarding enzymatic activity and stability, when performing the reuse of the biocatalyst, the kinetics of ester groups conversion and molar mass distributions of polymer samples at the reaction end were also measured to verify the performance of lipase during reuse cycles in these polycondensations. Scanning electron microscopy (SEM) was used to evaluate the morphology of the enzymatic support before and after polycondensation reactions. Therefore, N435 samples, washed and dried after the reaction, were dispersed on carbon tape. The samples were coated with a thin layer of gold to avoid the accumulation of electrical charge in the primary beam and to improve the level of secondary electron emission during the analysis. The tests were carried out in a Scanning Electron Microscope (JSM – 6390 LV, Jeol). The evaluation of the results was performed using micrographs with magnifications between 25-200 times and an electron beam with an energy of 10 kV. In addition, confocal laser microscopy (CLSM) analysis was performed to qualitatively verify the amount of enzyme adsorbed on the N435 support before and after the polycondensation reaction, using DAPI (4',6-diamidino-2-6 phenylindole, Molecular Probes®, USA), as a protein indicator. Fluorescence verification was performed using a DMI6000 B (Leica®) with a 63x objective lens with image processing in the software Leica Application Suite X (LAS X)

3.2.4 Polymer Characterization

3.2.4.1 Average Molar Mass

Number (M_n) and weight (M_w) average molar masses, degree of polymerization (DP_n), molar mass dispersity ($\mathcal{D}$) and molar mass distributions were evaluated through the technique of Gel Permeation Chromatography (GPC) (SHIMADZU, model LC-20AD), equipped with a MiniMIX PL gel precolumn (5 μ m, 50 x 4 mm), two MiniMIX PL gel columns (5 μ m, 250 x 4.6 mm) in series, and a RID-10A refractive index detector. Aliquots of 0.05 g of the polymer were dissolved in 4 mL of chloroform. The polymer solution was filtered through a nylon membrane, with a pore size of 0.45 μ m. Chloroform (HPLC, 99%) was used as a solvent of the mobile phase with a flow rate of 0.3 mL.min⁻¹ at 40 °C. For the calibration curve of the equipment, polystyrene standards were used with molar masses ranging from 580 to 9.835 x 10⁶ g mol⁻¹. Then, the data were analyzed using Trisec GPC version 3 software.

3.2.4.2 Polymer Architecture.

Nuclear Magnetic Resonance (NMR), ^1H NMR and ^{13}C NMR, spectroscopies were performed to investigate the polymer architecture of glycerol-based PPS regarding the branching and crosslinking degree. ^1H NMR and ^{13}C NMR analyses were performed in deuterated chloroform, CDCl_3 , using a 400 MHz and 700 MHz Bruker Ascend 600 spectrometer. Chemical responses are reported in parts per million (ppm) relative to the internal standard tetramethylsilane (TMS) used in calibration. Sample preparation consisted of the dissolution of polymer samples in deuterated chloroform at a concentration of approximately 40 mg.mL^{-1} for ^1H NMR and 80 mg.mL^{-1} for ^{13}C NMR. The distribution of glycerol structural units, end-group functionality, enzyme regioselectivity and degree of branching were calculated based on ^1H -NMR and ^{13}C -NMR spectra, as presented in Supplementary Material.

To perform Fourier transform infrared (FT-IR) analyses, the samples diluted in chloroform were dropped onto KBr pellets and dried with a hot air blower to evaporate the solvent. The hydroxyl functional groups incorporated into the polymeric chains were investigated. The analyses were performed on a spectrometer (AGILENT TECHNOLOGIES – Cary 660 FT-IR), and the spectra were recorded between 4000 to 400 cm^{-1} accumulating 32 scans at a resolution of 4 cm^{-1} .

3.2.4.3 Gel content

The gel content was measured by the addition of 0.020 g of polymer and 4 mL of CHCl_3 (PA 99%) in a flask, and subjected to solubilization for 48 h at rest. Subsequently, the solution was filtered through a glass syringe and a $0.45 \text{ }\mu\text{m}$ pore nylon filter. The flask and filter containing the crosslinked polymer fraction were dried in a forced convection oven at $60 \text{ }^\circ\text{C}$ for 48 h to remove residual solvent. The gel content (%Gel_Content) was calculated using Equations 3 and 4:

$$m_{pr} = (m_{df} + m_{dfi}) - (m_f + m_{fi}) \quad (3)$$

$$\% \text{Gel_Content} = \frac{m_{pr}}{m_p} \times 100 \quad (4)$$

where m_{pr} is the dried mass of the crosslinked polymer, m_{df} and m_{dfi} are the masses of the flask and the filter containing the dried crosslinked polymer, m_f is the initial mass of the flask, m_{fi} is

the initial mass of the filter, m_p (0.020 g) is the mass of polymer added to the solvent (4 mL). The gel fraction includes crosslinked polymeric species of infinite length.

3.2.4.4 Rheological Behavior

The analysis of the rheological behavior of the polymeric aliquots at different times in the linear viscoelasticity regime were carried out in a way that corresponds to slow deformations, in which the polymer molecules are not removed from their equilibrium state and thus, the rheological properties of the polymeric materials in this regime can be correlated with its structure. The analyzes were performed in the rotational rheometer (Haake Mars, Thermo Scientific). The geometry used was parallel plates with a diameter of 60 mm. The samples were kept at 50°C over a slit of 0.3 mm and the shear rate was changed from 10 s⁻¹ to 500s⁻¹⁰.

3.2.4.5 Hydrophilicity

The contact angles of the copolymers were measured using a Ramé-Hart model 260 goniometer at 25°C to evaluate the hydrophilicity of polymer samples. Copolymer films were fixed on microscopy slides in the dimensions of 1 cm width by 3 cm length. The contact shape and angle were analyzed by DROPImage Advanced software. Each sample was measured at six different locations and then the mean and standard deviation of the contact angle were calculated.

3.3 Results And Discussion

This work focused on the synthesis of PPSG functional polyester (Scheme 2) by N-435 catalyzed in solvent-free polycondensations. Of particular interest, the synthesis of the PPSG was intended under different conditions of initial glycerol ratios as well as analyzing the reaction kinetics. Other studies were carried out to understand the characteristics of the catalyst with potential recyclability.

3.3.1 Synthesis of glycerol hydroxyl functionalized polyesters.

For the synthesis of functional aliphatic polyesters, it is conceivable to replace some diols with glycerol to synthesize copolyesters with pendant hydroxyl groups. Thus, polyester poly(propylene succinate) (PPS) was selected according to the literature ² as the base polymer to receive glycerol as a comonomer and synthesize the copolymer poly(propylene succinate-co-glycerol succinate) (PPSG). In this sense, the study aims to evaluate the selectivity of the

enzyme N435 towards primary hydroxyl groups of glycerol during these enzymatic transesterifications.

For the synthesis of PPSG, different glycerol:PDO ratios were analyzed. In addition, the reaction time is a crucial parameter to be analyzed for the formation of a linear copolymer. Long reaction times can lead to the consumption of the secondary hydroxyl, increasing the gel content by the formation of polymeric species with high branching or crosslinking density¹³⁴. However, short reactions can compromise the molar mass of the polyester. Therefore, a detailed investigation of the optimal reaction time was necessary to develop a procedure capable of obtaining polyesters of relevant molar mass with low crosslinking and branching degree. Thus, the average molar masses, the dispersity, and the gel content were compared, considering the standard condition of PPS synthesis (blank test) and the polymerizations with the addition of glycerol.

Thus, to compare the selectivity of the N435 catalyst, the reaction temperature and the catalyst dosage were kept constant. The temperature of 90 °C allows the monomers to be in a homogeneous phase, favoring mass transfer rates without interfering with the catalytic activity. In addition, the pressure was reduced during the transesterification reaction to improve the removal of the ethanol as a by-product.

When comparing the reactions with different glycerol:PDO ratios in Figure 6(a), it is noted that the increase in glycerol concentration directly affects the kinetic behavior of the reaction. The increase in the glycerol concentration impacts the increase in the viscosity of the reaction medium, which makes it difficult to remove the by-product and consequently the conversion decreases with the increase in the glycerol concentration (Figure 6a). For this reason, PPSG₁₀ (DS:PD:G of 1:0.9:0.1) exhibited a higher conversion in comparison with the other samples of PPSG, and lower Mw (6 kDa, in Table 4) was achieved. On the other hand, glycerol influenced the molar mass. With the high concentration, the consumption of the 3 hydroxyls is more likely to occur, leading to the formation of more branched polymers and, therefore, higher molar masses, greater polydispersity, and gel content. This behavior becomes clear when the dispersity is evaluated over the reaction time for the PPSG₂₀, presenting a final $\bar{D}$ of 2.29 and a low gel content (13%, Table 4). On the other hand, the highest weight average molar mass was obtained with monomer initial ratios of PPSG₅₀ (1:0.5:0.5, Table 4) reaching 32 kDa and high $\bar{D}$ (7.88).

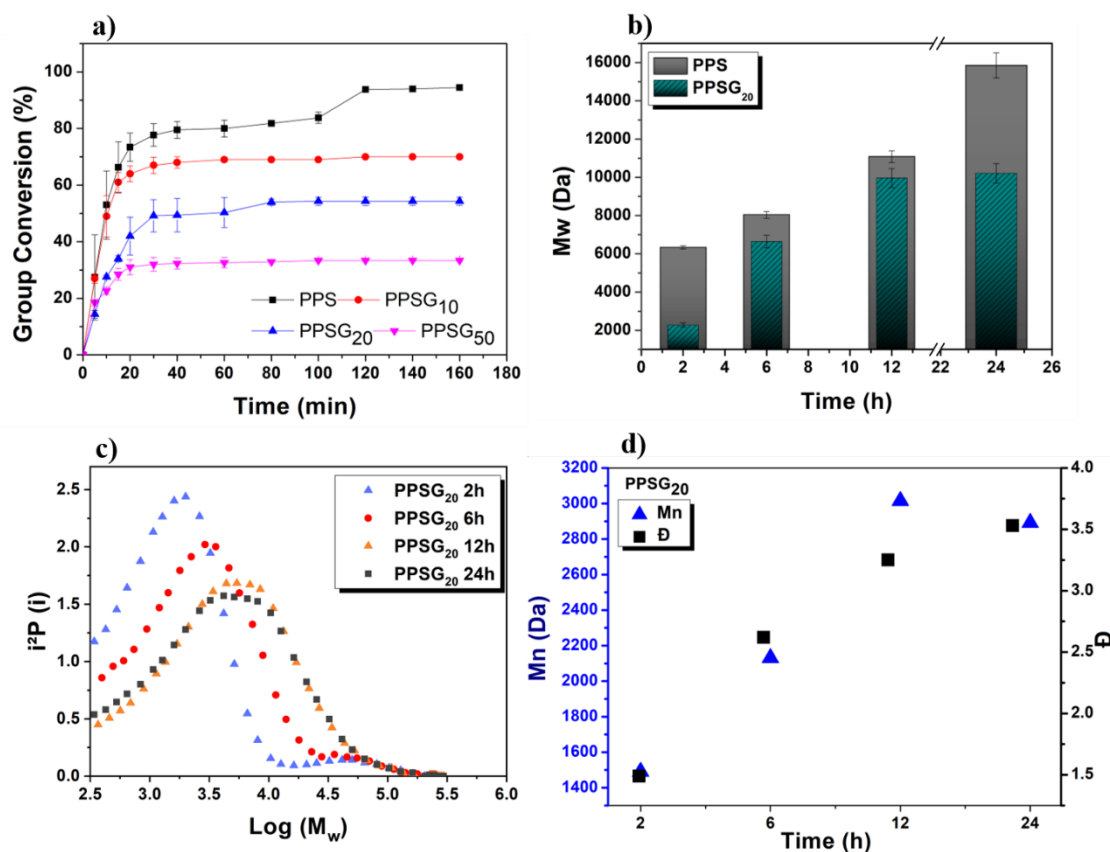


Figure 6. Enzymatic synthesis of polyesters using N435: a) Effect of the initial comonomer ratio (glycerol content) on the kinetic behavior; b) Mw during reactions without glycerol (PPS) and with 20% glycerol (PPSG₂₀); c) Molar mass distributions during reactions.

Reproduced from¹⁵⁹.

The high molar mass and dispersity ($\bar{D} = 7.88$, Table 2) obtained for PPSG₅₀ reflects the occurrence of branching due to the consumption of all the 3 hydroxyl groups of glycerol and also of crosslinking, indicated by the gel content of 89.5%. In addition, the high viscosity of the reaction medium, which influences the diffusion of polymeric species to reach the active sites of the immobilized enzyme, may also lead to a broadening of the molar mass distribution. The broadening of the molar mass distributions of the soluble fraction of PPSG₂₀ (as can be seen in Figures 6(c) and (d)) over time can also be attributed to the increased branching and crosslinking degree: polymeric species with higher lengths have high amounts of available hydroxyl groups which can react forming crosslinked chains becoming insoluble on solvents. In contrast, the linear chains and/or branched, but non-crosslinked chains, remain in the soluble fraction. Therefore, Figure 6(c) shows that over the reaction time, there is an increase in the branching density, indicated by the widening in the distribution of molar masses of the soluble fraction

that is reflected in the dispersity. Figures 6(b)-(d) also suggested that molar masses did not change after 12 h indicating diffusional hindrance. In particular, PPSG₂₀ presented an average molar mass of 3 kDa with a dispersity of 2.3. In contrast, previous studies required up to 71 h in enzymatic synthesis of poly(glycerol sebacate) (PGS) to obtain a Mn of 3.7 kDa and a dispersity of 17¹⁰².

Previous studies have demonstrated that it is possible to avoid gelation in thermal polymerization between glycerol and adipic acid following the Martin-Smith approach, resulting in a weight average molar mass (Mw) of about 2 kDa¹⁴². Conversely, studies show that for large-chain comonomers such as 1,8-octanediol and sebacic acid, the increase of glycerol fraction did not influence the gel content^{86,161,162}.

Therefore, when decreasing the glycerol content from 0.5 to 0.2 (PPSG₅₀ and PPSG₂₀ respectively) there is a decrease in the gel content and consequently in the values of Mw (10,356 Da) and Đ (2.29) (Table 2), while Mn in the condition of 0.5 decreases, which is a clear indication of the high crosslinking and branching, corroborating as measures of gel content.

Additionally, the degree of polymerization is influenced by several factors, such as the reactivity of the monomers, the length of the chains and the reaction conditions. The literature already solidly demonstrates that linear alkane- α,ω -aliphatic diols with longer alkylene chains exhibit greater enzymatic reactivity, resulting in number average molar mass (Mn) of around 2200 g.mol⁻¹ and an average degree of polymerization of around 6¹⁶³.

The length and concentration of the monomers are critical factors that directly influence the properties of the formed polymers. Previous studies have already demonstrated that varying the length of the monomer chain affects the number average molar mass (Mn) of the resulting polymers. For example, in the enzymatic synthesis of polyesters using diacid esters, a significant increase in Mn was observed, ranging from 3,000 to 21,000 g/mol with the increase in the chain length of the monomers from C2 to C4¹⁶³. Similarly, the use of diols with longer chains, such as C6, C8, and C10, resulted in higher Mn values compared to shorter-chain diols⁶³.

Conversely, the length of the monomers can also affect the cross-linking or branching of the polymers. In some scenarios, monomers with shorter chains can lead to the formation of dendritic units or branching in the polymer structure^{164–167}. For example, in the synthesis of poly(glycerol sebacate) using glycerol and sebacic acid as monomers, the increase in the amount of enzyme resulted in a higher content of dendritic units in the polymer¹⁶¹. In addition,

the decrease in glycerol levels in the comonomer ratio (glycerol, 1,8-octanediol, and sebacic acid) led to a decrease in the average molar mass of polyol-polyester and its dispersity, justified by the decrease in steric restrictions at the site CALB access^{161,168}.

Furthermore, the influence of monomer length has been investigated in the enzymatic synthesis of copolyesters. Studies demonstrated that increasing the length of the diol and diacid led to greater enzymatic activity. In the case of the reactivity of diethyl succinate and diethyl adipate with 1,3-PDO and 1,4-BDO, the average degree of polymerization reached the value of 29 and an average molar mass (M_n) of $12,800 \text{ g.mol}^{-1}$ ¹⁵⁷. Likewise, when using larger diacids such as oleic acid in the enzymatic synthesis of poly(oleic diacid-co-glycerol), M_n was $9,000 \text{ g.mol}^{-1}$ (M_n) in 24 h with a branching degree of 16% and dispersity of 5¹⁶⁹. These observations reflect and corroborate the results found in the present study, where it was observed that the degree of polymerization increases as glycerol is incorporated into the chain, changing from 9 to 15, together with a corresponding increase in M_w (from 6 to 11 kDa) for samples PPSG₁₀ and PPSG₂₀, respectively.

Monomers with trifunctionality can generate highly branched structures, subject to the gelation process¹⁶⁵. Gel formation implies substantial changes in the properties of the polymer, such as viscosity and solubility. Some works suggest that the gel formation is influenced by the processing conditions and the composition of the monomers used¹⁶².

In particular, in the enzymatic polycondensation between oleic diacid and glycerol, it was observed that increasing the reaction time and monomer feed ratio has a significant role in gel formation¹⁶⁹. Reactions with times longer than 6 h resulted in gel content that reached 50 %, especially in the feed proportions of 1.3:1.0 and 1.5:1.0, also presenting a high dispersity of 10.5 and 11.1, respectively¹⁶⁹. Furthermore, the fraction of glycerol units in the structure of these products was 32 and 36 %, respectively. Conversely, equimolar reactions exhibited reduced gel fractions, with corresponding values of $1,700 \text{ g.mol}^{-1}$ for M_n , 8.9 for $\bar{D}$, and 17 % for the gel fraction¹⁶⁹. This indicates that gel formation is directly related to the composition of the reactions

Table 4. Effect of initial comonomer ratio on average molar masses and gel content. Reproduced from¹⁵⁹.

Sample	DS:PDO:G Initial ratio	Mw (kDa)	Mn (kDa)	Đ	DP _n	Gel Content (%)
PPS	1:1:0	15.9 ± 0.5	3.5 ± 0.8	4.6	26	-
PPSG ₁₀	1:0.9:0.1	5.8 ± 0.8	1.7 ± 0.2	3.3	9	10
PPSG ₂₀	1:0.8:0.2	10.5 ± 0.4	2.9 ± 0.6	2.3	15	13
PPSG ₅₀	1:0.5:0.5	31.6 ± 4.0	1.8 ± 0.7	7.9	10	89
PPS _A G ₂₀	1:0.8:0.2		1,3 ± 0.6		6	97

DS is Diethyl Succinate, PDO is 1,3-Propanediol, G is glycerol; DP_n is the average degree of polymerization and Đ is the dispersity. The reactions took place with 10% catalyst (wt%) at 90 °C for 24 h. The reaction control was performed with succinic acid at 180 °C for 12 h, without catalyst in bulk (PPS_AG₂₀).

Therefore, the processing conditions clearly influence the formation of gel in the investigated systems. However, it is important to note that, even in reactions with up to 20% glycerol, the polyesters produced through enzymatic polycondensation presented gel fractions of approximately 10% by weight. Therefore, even though partially cross-linked, the polymer samples retained available hydroxyl groups within the polymeric chains. The proper adjustment of these factors plays a fundamental role in preventing or promoting gelation in polycondensation reactions. It can be regulated through adjustments in reaction conditions such as time, temperature, and concentration of monomers and enzymes.

A non-enzymatic esterification reaction at 180 °C with the same comonomer ratio as in reaction PPSG₂₀, was carried out for comparison (Table 2). This specific reaction system was chosen because esterification is self-catalyzed by the acid group, while transesterification reaction requires catalysts such as enzymes or organometallic materials^{150,160}. The non-enzymatic reaction at 180 °C yielded an extremely sticky polymer insoluble in chloroform after 12 h of reaction with a gel content of 97%, illustrating the low selectivity of non-catalyzed polycondensation regarding the reactive hydroxyl groups from glycerol. Therefore, the presence of an enzyme was essential to achieve the purpose of this work, related to the synthesis of succinic copolyesters of glycerol with tunable degrees of hydrophilicity.

The use of reduced pressures, as typically performed in industrial sites, is employed to remove by-products when the viscosity of the reaction medium is high, favouring the increase of the polymerization degree. The gel content can also increase due to branching and crosslinking reactions as the reaction proceeds. It is important to emphasize that despite the reduced pressure, enzymatic transesterification presented a lower gel fraction than non-enzymatic esterification, illustrating the high selectivity of enzymes regarding to reaction of pendant hydroxyl of glycerol.

All reported copolymers were tested for their gel fraction according to their CHCl_3 solubility with low evidence of a gel fraction for PPSG₁₀, and PPSG₂₀. PPSG₅₀ presented an 89.5% gel fraction, so the analyses refer to the soluble aliquot. At room temperature, the PPS polymer appears as soft translucent wax. In contrast, PPSG₅₀ is opaque and brittle, while PPSG₂₀ and PPSG₁₀ are a high viscosity sticky liquid and optically translucent.

3.3.2 Evaluation of Polymer Architecture

Previous investigations have shown that analyses of ^1H -NMR spectra can indicate end groups and repeating units of low molar mass polymers¹³⁴. Therefore, the polymer was analyzed by ^1H -NMR to provide an approximation of the configuration of the glycerol units in the polymeric chains. Figure 7 shows the PDO and DS protons with emphasis on the assignments of the terminal glycerol, linear and dendritic glycerol units. Figure 8 shows a representative ^1H -NMR spectrum of the polymer of reaction PPSG₂₀ with 20% glycerol, performed at 90 °C in the presence of 10% by weight of N435 and without solvent.

The sections that represent the repeating units of diethyl succinate in the chain refer to the protons of the methylene group $-\text{OC}-\text{CH}_2\text{CH}_2\text{CO}-$ (q) which are identified between 2.73 and 2.63 ppm. The presence of peaks around 1.26 ppm indicates the chain termination with the DS fraction referring to $\text{CH}_3\text{CH}_2\text{O}-$ (o). The repeating diol PDO units are represented in the fractions around 1.94 ppm $-\text{O}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{O}-$ (b) and in the peak 3.69 ppm to 3.72 ppm referring to the protons $-\text{CH}_2\text{CH}_2\text{O}-$ from the PDO (p). In addition, signal assignments corresponding to glyceride single bonds have been highlighted based on previous publications^{23,44,170} around 3.8 and 5.7 ppm.

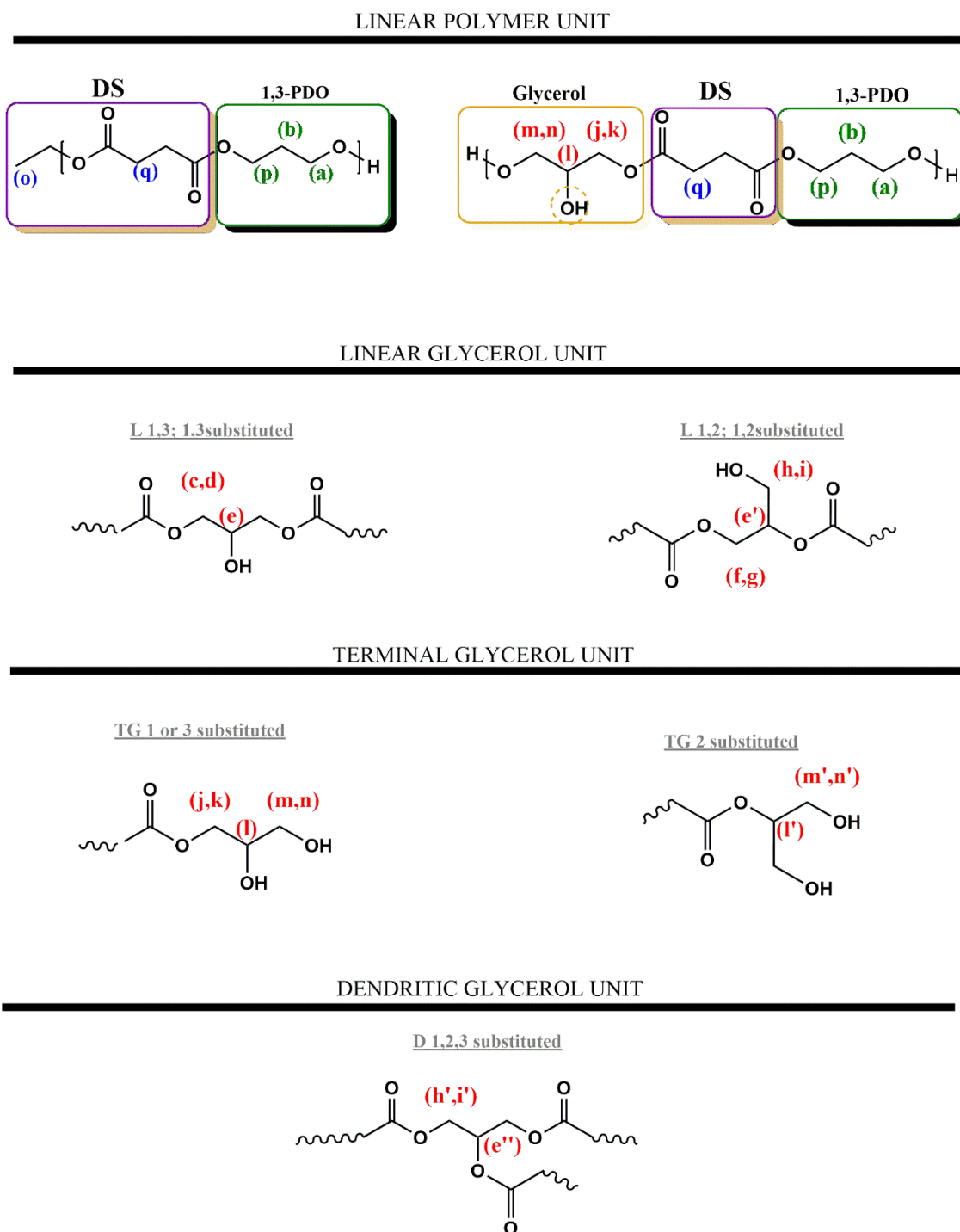


Figure 7. ^1H NMR proton assignments for linear, terminal and dendritic glycerol units contained in the polymers. Reproduced from¹⁵⁹.

The terminal glycerol units are multiplets that should appear at 3.8 ppm (l). The appearance of peaks at 5.1 and 5.3 ppm are related to 1,2-substituted (e') and 1,2,3-substituted (e'') glycerides, indicating the lack of regioselectivity of the enzyme during the reaction.

Therefore, the dendritic units that correspond to the degree of branching (1,2,3-substituted) and 1,2-substituted, were calculated from ^1H -NMR and ^{13}C -NMR to determine the enzymatic affinity for the hydroxyls of glycerol following a previous methodology as described in the supplementary material ¹³⁴.

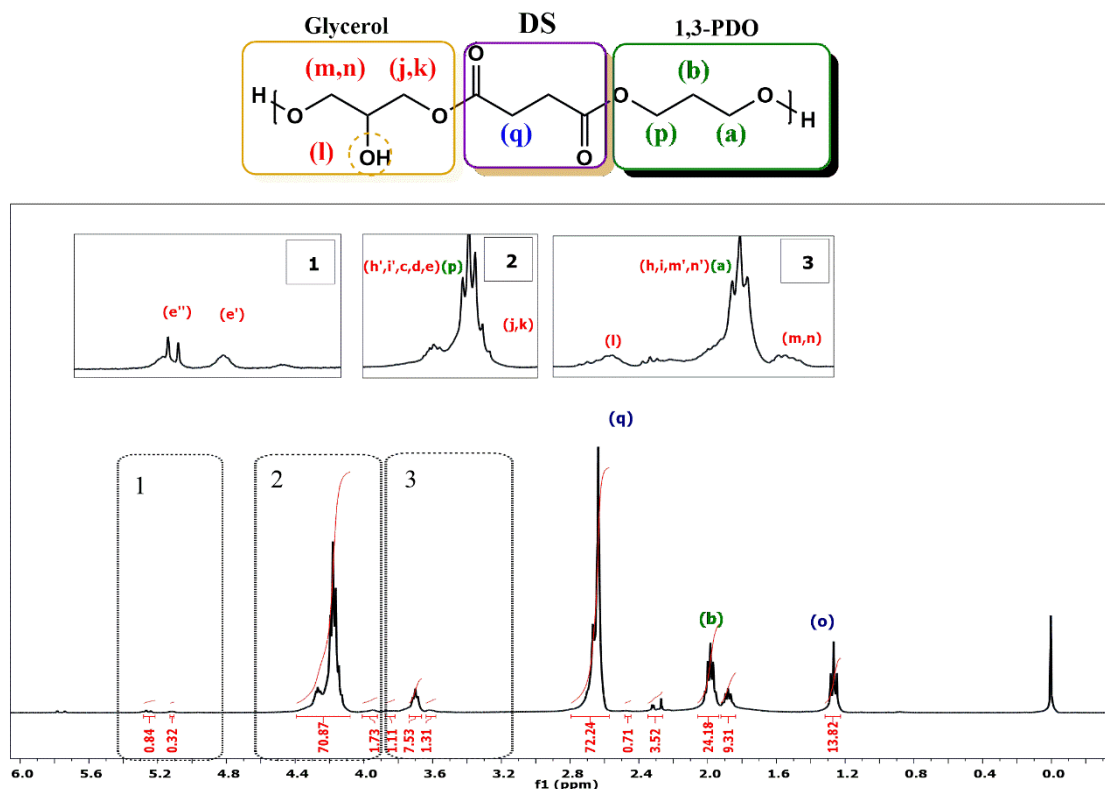


Figure 8. ^1H NMR spectra of PPSG20 synthesized with 20% of glycerol in the diol fraction and 10% of N435 and 80°C. The monomers used for this synthesis were: Diethyl succinate (DS); 1,3-propanediol (1,3-PDO); Glycerol (Gly). Reproduced from¹⁵⁹.

Table 5 shows the relative proportion of conformation of glycerol groups and abundance of final groups depending on comonomer ratios (see information in Eqs. (E1)–(E16) in Supplementary Material). According to the data collected in Table 3, the relative abundance of terminal groups is predominated by DS and PDO for PPSG₁₀ and PPSG₂₀. The results corroborate previous works, in which for equimolar initial monomer concentrations, the relative amount of terminal groups of diesters and diols were almost balanced (57.8% for the glycerol and 42.2% for the divinyl adipate) ¹³⁴. It is observed that for 50% glycerol (PPSG₅₀), a high gel content was obtained and therefore the soluble fraction evaluated is not representative of the polymeric conformation.

Table 5. Effect of comonomer ratios (90 °C, 24 h, in bulk) on the distribution of glycerol structural units, end-group functionality, enzyme regioselectivity and degree of branching calculated based on ¹H-NMR and ¹³C-NMR spectra. Reproduced from¹⁵⁹.

Entry	Relative abundance of the glycerol structural unit (%)					Relative abundance of end groups (%)			Regio (%)	Regio (%) (*CNMR)	DB (%)
	TG% (1 or 3-sub)	TG2% (2-sub)	L1,3% (1,3-sub)	L1,2% (1,2-sub)	Den% (1,2,3-sub)	G	PDO	DS			
PPS	-	-	-	-	-	-	35.8	64.2	-	-	-
PPSG ₁₀	3.1	4.7	79.1	5.7	7.4	2.8	33.3	63.9	90.5	88.6	16.5
PPSG ₂₀	3.6	3.0	84.7	3.9	4.7	3.8	33.9	62.3	93.7	94.6	10.8
PPSG ₅₀ *	4.8	7.6	64.9	12.3	10.4	7.8	43.3	51.1	-	-	-

*This analysis corresponds to a soluble PPSG50 recipe and does not represent the polymer conformation

To determine whether a change in the initial comonomer ratio will generate branched structures, the degree of branching (DB) of the polymers was also evaluated by ¹H NMR and their values are also presented in Table 3 (see Equation 17 Supplementary material). The degree of branching is directly related to the formation of dendritic structures; therefore, it is a measure of the dendritic character of polymeric structures. The high regioselectivities, above 90%, for the primary hydroxyls of the PPSG₁₀ and PPSG₂₀ reactions indicated that the frequency of branching reactions was kept low, mainly leading to the formation of linear polymeric structures with low Đ, also corroborating the relatively low gel contents (Table 2). These results were also confirmed by ¹³C-NMR analysis. The regioselectivity of lipases towards primary alcohols in polyols has previously been reported to occur within a specific range of time and temperature¹³⁴. Thus, the regioselectivity results align with previous observations made under similar conditions. In a study using divinyl ester and glycerol with *Candida antarctica* immobilized on microporous acrylic resin at 40 °C for 24 h in THF, a yield of 65 % of polymer (after dissolution in acetone and precipitation in diethyl ether) was achieved^{6,134}. It is important to emphasize that for the reactions with 10 and 20 mol % of glycerol in relation to the polyols, the regioselectivity of N435 was not affected by the initial comonomer ratio, and the incorporation of glycerol in the chain mainly occurred by the L1,3-substituted (79.1-84.7%).

Acyl migration is a phenomenon that occurs during the synthesis of polyesters and acylglycerols, affecting their properties and functionality¹⁷¹. This process results in

rearrangements in the structure of the polymers, affecting the molar mass, distribution of acyl groups, and formation of branches and terminal groups. Factors such as reaction temperature, solvents, and reactant concentration influence acyl migration¹⁷². These changes can affect the physical and mechanical properties of the final products, making acyl migration an important aspect to be considered. Previous work has shown that higher glycerol concentrations and higher temperatures can increase the rate of acyl migration¹⁷³. Therefore, the % regioselectivity (Regio%) in the primary sites of the glycerol units calculated from the normalized ¹H-NMR intensity (Table 3) can also include the possible acyl migration reactions and should be interpreted as an apparent regioselectivity.

Given these observations, it is clear that in the condition of higher glycerol concentration (PPSG₅₀), crosslinking and branching (which are undesired) become accentuated, while in the condition of lower concentration, the amount of pendant intermediate hydroxyls is low. At an intermediate concentration (20%), it is possible to evaluate a compromise relation in terms of the concentration of pendent hydroxyls performed that crosslinking or branching. Therefore, based on these results, accurate synthesis of copolyesters following a solvent-free pathway at low temperatures (90°C) was demonstrated using diethyl succinate (DS), 1,3-propanediol (PDO), and glycerol (Gly). By adjusting the glycerol initial concentration, it was possible to produce mainly linear PPSG as confirmed by the degree of branching calculated based on the ¹H-NMR spectra.

FTIR spectra of the synthesized polymers are shown in Figure S6 (Supplementary material). The functional groups of PPSG₂₀ synthesized over time were investigated to verify the maintenance of the hydroxyl groups bound to the polymer chain during the reaction, and their respective spectra are represented in Figure S6. Spectra of the synthesized polyesters have a typical band around 3500 cm⁻¹ related to the axial deformation of the O-H bond. However, the quite low band intensities around 3500 cm⁻¹ for samples synthesized with 10% glycerol (PPSG₁₀), indicate that at low glycerol concentration, hydroxyls are easily reacted to form ester bonds. Furthermore, this result corroborates the branching degree (Table 3) observed for this reaction, which is a direct consequence of the reaction of the hydroxyls of glycerol. Therefore, the permanence of this band in the spectra of the samples with the highest concentration of glycerol (PPSG₂₀), must be related to free O-H groups (terminal or intermediate) belonging to the glycerol which did not react over the reaction time making it a hydroxy-functionalized polyester.

All synthesized polyesters also presented peaks in the bands around 2935 cm^{-1} and 1465 cm^{-1} , characteristic of the asymmetric deformation of the C-H bond and absorption into bending of CH_2 , respectively, however in the PPSG₂₀ sample this band is more intense, confirming the incorporation of glycerol into the polymeric chain. The characteristic band of the ester bond is composed of the axial strains of C-O in two coupled asymmetric vibrations: (i) the axial strain of C(=O)-O is considered the most characteristic vibration of diethyl succinate esters and appears around 1295 cm^{-1} and was detected in all the spectra of the synthesized polyesters. In the same way, the vibrations of axial deformation in the C=O group of diesters occur around 1680 cm^{-1} ^{174,175}, as also observed for the spectra of all samples. In addition to these, (ii) the peaks present in the O-C-C vibration of esters appear around 1040 cm^{-1} ²⁵. In the vicinity of this peak, the peaks of esters of primary alcohols in the O-C-C band, referring to the axial deformation of the carbon-oxygen bond of “alcohols”, occur between 1164 cm^{-1} and 1031 cm^{-1} . And the peak of secondary alcohol esters appears at $\sim 1100\text{ cm}^{-1}$ ²⁵.

Therefore, the appearance of characteristic bands of esters, discussed in this section, as well as the permanence of typical bands of OH in alcohols, comprise strong pieces of evidence that the esterification reaction between the monomers happened with the desired selectivity for the permanence of the intermediate hydroxyl groups.

3.3.3 Rheological Behavior

Another important characteristic of enzymatic polymerizations is the viscosity that affects the mass transfer rates of polymeric chains between the bulk phase and the enzyme support, the internal diffusion rates through the porous support and the by-product remotion rates. Generally, a polycondensation reaction medium contains a combination of monomers and polymeric chains with increasing viscosities with the polymer conversion, ranging from Newtonian to non-Newtonian behaviour. Viscosity depends on factors such as polymer volume fraction, viscosities of the phases involved, and reaction temperature. In this regard, the rheological behaviour in the synthesis of polyesters has been extensively investigated ^{176,177}. Nevertheless, to the best of our knowledge, there are no available experimental data in the literature investigating the relationship between viscosity and the diffusional effects in enzymatic polycondensation. Therefore, analysis of apparent viscosity changes with shear rates

(SR) may be of interest for potential practical applications because it offers important insights into the external and internal mass transfer rates of monomers and oligomers, which are mainly controlled by viscous effects in bulk polycondensation.

Figure 9 shows the rheological behavior related to the effect of glycerol incorporation in the copolymer on the polymeric apparent viscosity curves (PPS and PPSG) concerning the reaction time. The apparent viscosity of both samples increased considerably with reaction time. After 2 h of reaction, the apparent viscosity (at 300 s^{-1}) was 7,519 cP, and it increased until reaching 139,316 cP at the end of 24 h. These analyses show how the apparent viscosity of the polyesters (PPSG₂₀) at a given shear rate and fixed temperature increased with reaction time.

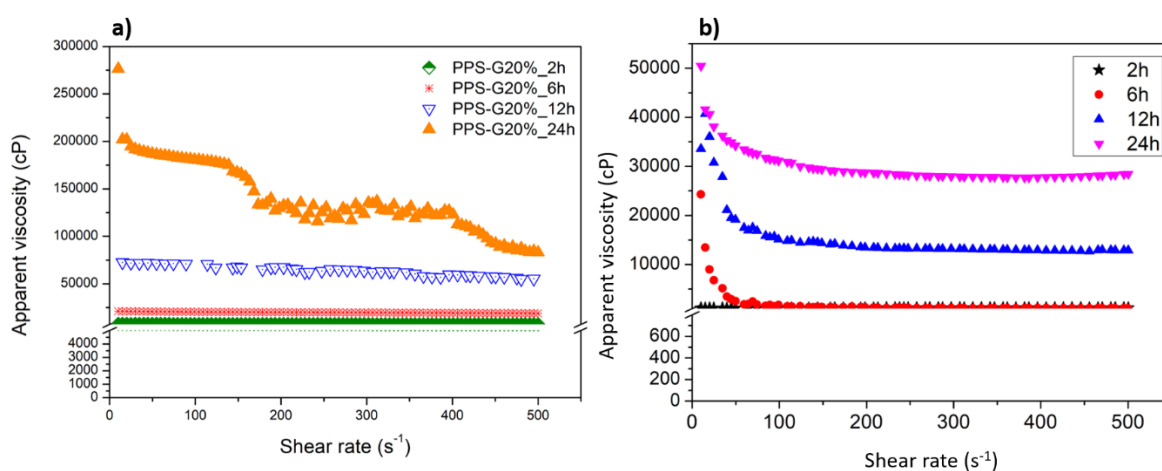


Figure 9. Curves of the apparent viscosity at different reaction times: (a) PPSG₂₀ and (b) PPS. Reproduced from¹⁵⁹.

Moreover, one can see that PPSG₂₀ presented higher viscosities than PPS: at the entire range of shear rate: at 52 s^{-1} (corresponding to a stirring rate of 500 rpm) and 24h, for example, PPSG₂₀ has apparent viscosity of 384,295 cP, substantially higher than PPS with apparent viscosity of 34,274 cP. Therefore, the higher viscosities of polyesters with addition of glycerol explain the lower amounts of collected by-product. Concomitantly, these results are in agreement with the data presented in Figures 6, where the average molar mass increases with the reaction time. This same behaviour has already been demonstrated in different studies where the reaction medium viscosity increases with the polymer molar mass¹⁷⁸. However, despite the increase in viscosity, Mw did not show significant changes after 12 h of reaction in the case of PPSG₂₀. This behaviour suggests that crosslinking and branching control the kinetic mechanism from this reaction time, which is corroborated by the pronounced increase in apparent viscosity

after 12h of reaction with the higher crosslinking and branching degree. Higher viscosity associated with branching and crosslinking occurs for polyesters synthesized with multifunctional monomers such as glycerol, subject to form insoluble gel fractions when reaction conditions are not strictly controlled (i.e. temperature, reaction time, and proportions between the monomers). Therefore, when using glycerol in the polycondensation reactions, an increase in the viscosity of the reaction mixture is observed, which causes a reduction in the mobility of the growing macrochains ¹⁷⁹.

However, as this is a reaction catalyzed by the immobilized enzyme N435, the enzymatic activity may be limited due to viscosity interfering with the diffusion rate of oligomers, both externally and within the pores of the support. Thus, when the viscosity increases, the substrate diffusion rate is lower than the reaction rate in the enzyme site, resulting in an apparent reduction in activity, since not all catalyst molecules will be in contact with the substrate ¹⁸⁰. Given the known sensitivity of the enzymatic activity as a function of the substrate structure, the situation described above becomes quite complicated for crosslinked polymers with high molar masses when using multifunctional monomers, since the increase in viscosity implies an apparent reduction in enzymatic activity ¹⁸⁰. Therefore, despite the findings obtained in these studies, questions about the diffusional effects, and the interaction between enzyme and substrate in bulk reactions requires deeper investigations to control such problems.

3.3.4 Hydrophilicity

The wettability was analyzed on glass slides coated with the polymers to measure the contact angle (θ) and evaluate the hydrophilicity characteristics of the copolymer (Figure 10). Particularly, PPSG demonstrated remarkable flexibility and homogeneity, which facilitated the preparation of a stable film for the contact angle analysis. Furthermore, the addition of glycerol to the polymer chain led to a decrease in the water contact angle. This can result in increased wetting, where the liquid spreads more over the polymer surface, reducing the contact angle. Contact angle tests indicate a decay from $71^\circ \pm 4$ to $37^\circ \pm 1$ when glycerol was added (PPSG₂₀). This observation corroborates the results of H-NMR and Fourier transform infrared which show that the addition of glycerol increases the pendant hydroxyl groups available in the polymer chain. The hydroxyl groups pendant in the copolymer chain were enough to increase the hydrophilicity of the material, showing results superior to those found in the literature. Under

similar conditions, previous studies achieved more hydrophobic samples with a contact angle between $124^{\circ} \pm 2^{\circ}$ for the copolymer P(AO-GA) (adipic acid:1,8-octanediol:glycerol of 1:0.8:0.2)⁶. In contrast, PBS (Polybutylene Succinate) homopolymers with simpler structures presented water contact angles around 58° to 79° , as reported in literature^{181,182}. Therefore, the conditions of low operational temperature, absence of solvents and the selectivity of the enzyme for the primary OH, proved to be an efficient method for the synthesis of hydroxy-functionalized copolymers with glycerol. Thus, enzymatic catalysis has selectivity advantages over thermal polycondensations such as poly(butylene-co-propylene succinate) which use glycerol (0.02 mol%) as a crosslinking agent to create partially cross-linked networks in polycondensation at 230°C with high polydispersity ranging from 6.39 to 5.44¹⁸⁰.

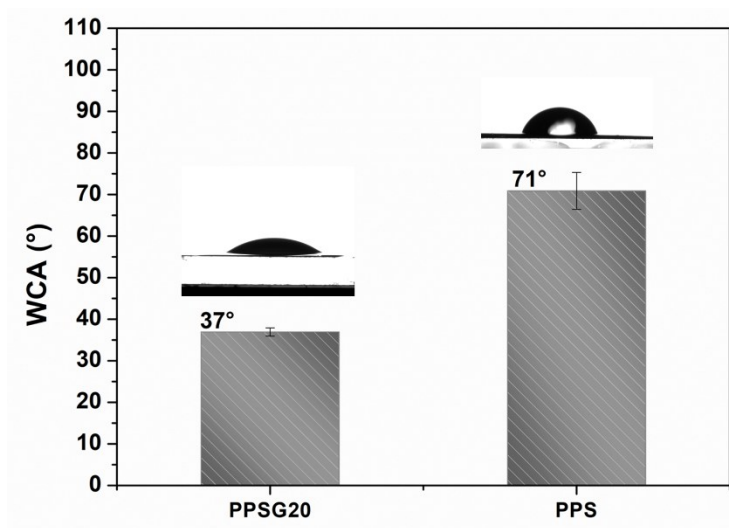


Figure 10. Contact angle of PPS and PPSG20 synthesized at 10% of N435, and 90°C . Reproduced from¹⁵⁹.

3.3.5 Enzyme reuse

In recent years, the search for environmentally friendly routes for the synthesis of polymers has awakened the interest in the use of enzymes as promising catalysts. One of the benefits of this alternative, when using immobilized enzymes, is the possibility of enzyme reuse, which involves recovering the enzyme to return it to new polymerization cycles. However, to date, no studies have been found that investigate the recyclability potential of N435 in polyesters synthesized with glycerol as a comonomer. Above 100°C , the degradation of the biocatalyst occurs due to the denaturation of the enzyme, limiting the selectivity and possibility of its reuse¹⁷⁴.

In this work, the recyclability potential of N435 was investigated to determine the enzymatic stability in bulk copolymerizations (formulation of PPSG₂₀ at 90°C) during several cycles (Figure 11). It was observed that N435 presented a reduction in the activity of about 58%, decreasing from 32.3 U/g to 13.5 U/g right after the first use. At the end of the third cycle, the enzyme activity had already reduced by 86 % (Figure 11a). Concomitant with the catalytic activity, the average molar masses were also progressively reduced (10,000 Da in 1st cycle and 1,800 Da in 3rd cycle) while the dispersity of the chains increased considerably, reaching $\bar{D}$ equal to 12.8 in the 3rd cycle (Figure 11a). This behavior corroborates previous studies that evaluated the recyclability of N435 in the synthesis of glycerol carbonate and the enzymatic activity dropped after the first cycle¹⁸³.

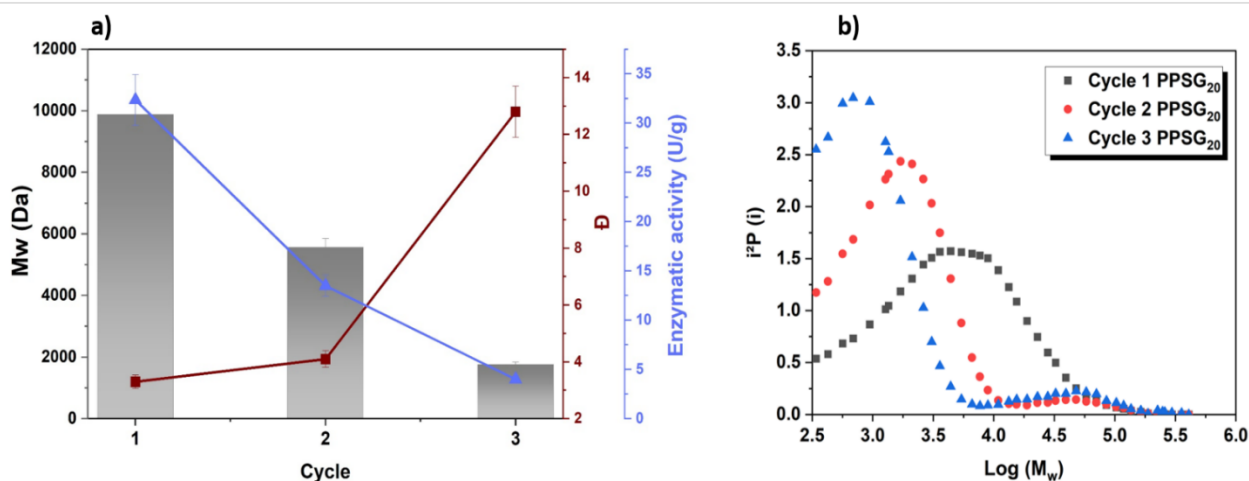


Figure 11. Analysis of: (a) average molar mass correlated with the enzymatic activity of N435 during the reuse of the biocatalyst for the synthesis of PPSG₂₀ and the relationship of the enzyme activity with the polydispersity $\bar{D}$, and (b) Molar mass distributions. Reproduced from¹⁵⁹.

To investigate the possible causes of enzyme activity loss, scanning electron microscopy (SEM) analyses were performed (Figure 12). The images illustrate the interaction of the copolymer synthesized from glycerol with immobilized lipases (N435).

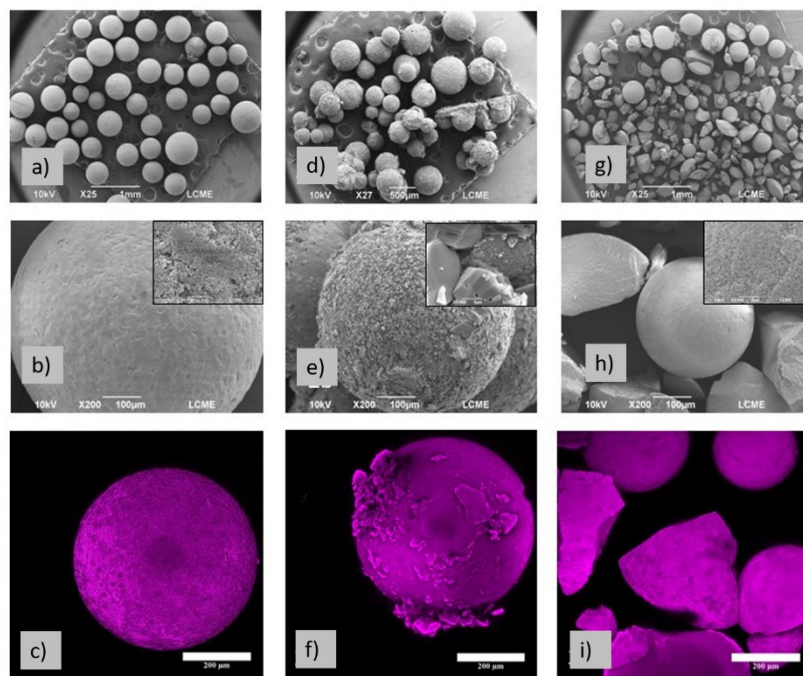


Figure 12. SEM images of the surface of immobilized lipase N435; before the reaction (a;b;c); after the first cycle (d;e;f) at the end of the third cycle (g;h;i). Reproduced from¹⁵⁹.

SEM analysis showed that catalyst deactivation is associated with aggregation of the viscous polymer on the catalyst surface (Figure 12e). However, additional observations support back possible mechanisms that explain the drop in N435 activity. First, substrate diffusion to the pores of the lipase support is a limiting factor that promotes the reduction of catalytic activity. A second explanation is the affinity of the polymer with the PMMA support, since lipase immobilization makes the support less hydrophobic, possibly due to polar groups present in the enzyme. Both hypotheses corroborate proposals that have already been discussed for similar transesterification systems¹³⁸.

Therefore, the polydispersity increases, as the support pores are blocked by chains of high molar mass. As a result, the reaction medium becomes rich in low molar mass oligomers whose chains stop growing due to the impediment to the active sites of the enzyme. The shear effect was also observed at the end of the third cycle of enzyme reuse when compared to the

enzyme at the beginning of the first cycle (Figures 12g,h,i and 12a,b,c respectively). It is important to remember that each reaction cycle lasted 24 h and these were performed at 90°C, which is close to the enzyme denaturation temperature and under constant stirring, contributing to the reduction of the catalytic activity caused by the support shear, leading to a drop in the Mn reached.

3.4 Conclusion

Copolymers of poly(propylene succinate-co-glycerol succinate) were synthesized with different initial comonomer ratios via enzymatic catalysis (Lipase N435). The kinetic behavior, physicochemical characteristics, and potential recyclability of the immobilized biocatalyst were investigated. With 10% and 20% glycerol, the copolymers presented low gel content (around 10%) and Mw of 5,754 and 11 kDa respectively, in transesterification reactions with 10% by weight of N435, at 90°C, during 24h. The main results indicated that the molar masses can be controlled by the reaction time, however, the high viscosity leads to diffusional effects that impair the mass transfer between the oligomers and the catalytic site, the by-product removal, as well as the reuse of the enzymes.

The contact angle data confirmed that the hydrophilic character of polyesters is increased by the addition of glycerol, however, this approach is still little explored in scientific studies. Furthermore, the high regioselectivity of lipase to primary alcohol was maintained for copolymers PPSG₁₀ and PPSG₂₀, while the copolymers PPSG₅₀ presented gel content of 89%, indicating the reaction of all three hydroxyl groups of glycerol and making only to analyze the soluble fraction by NMR. Therefore, the results of this work suggest that the enzymatic polycondensation of diester and short-chain diols such as 1,3-PDO and glycerol depends on comonomer ratios, catalyst selectivity, and diffusional effects caused by high viscosity. Furthermore, the copolymers with pending hydroxyls can be used in functionalization strategies that can add value to these materials through their use in biomedical applications such as controlled drug delivery and adhesive materials.

Chapter 4

Incorporation of Poly(Propylene Succinate-Co-Glycerol Succinate) PPSG as a Renewable Additive In Electrospun PCL Fibers With Bioactive Glass Particles For Soft Tissue Engineering

This chapter contains results on the investigation of the use of PPSG as an additive in PCL fibers incorporation of 45S5 bioactive glass particles. The results obtained were presented at the 6th Franco-Brazilian Meeting on Polymers (2023) and were submitted to ACS Applied Bio Materials, with the candidate as first author. The methodology guidelines and discussion of results are detailed below.

4.1 Introduction

Over the years, the aging of the global population has brought with it a series of medical challenges, particularly with the increase in age-related diseases such as tissue degeneration and congenital disabilities¹⁸⁴. Advances in tissue engineering should make it possible to replace diseased tissues with grafts and biodegradable scaffolds, restoring biological functions that mimic native tissues and aiding in regeneration^{87,185}.

Among the various strategies for manufacturing scaffolds that promote tissue regeneration, electrospinning has proven to be one of the most effective. It generates nanofibers from polymer solutions with structures that replicate the native extracellular matrix^{186,187,188}. The process allows customization by adjusting parameters such as solution properties, voltage, and environmental conditions¹⁸⁹. Electrospun scaffolds must ensure biocompatibility, biodegradability, and mechanical suitability.^{189,190,191} The selection of materials is therefore crucial, and renewable-source polyesters are a major advance.

Among biodegradable polymers, PGS is notable for its mechanical behavior and controlled degradation^{28,192}. However, the synthesis of PGS involves harsh polymerization conditions, such as high temperatures that lead to a high degree of crosslinking due to the reaction with the secondary hydroxyl of glycerol, in addition to the use of toxic catalysts, which can result in loss of glycerol and affect the final properties of the polymer^{99,193,194}. Challenges such as crosslinking and gelation hinder property control in PGS synthesis, motivating the search for alternatives⁹⁹.

Promising substitutes are enzymatically synthesized polyesters, which avoid toxic reagents and offer better structural modulation^{164,195}. One such copolymer is PPSG, a candidate to replace PGS and PBS due to its processability, hydrophilicity, and tunable degradation^{95,96,196}. Unlike PGS, PPSG can be electrospun directly, without the need for thermal or chemical post-treatment^{197–199}, simplifying processing and maintaining material integrity.

PBS, although widely used, exhibits high crystallinity and lower hydrophilicity, limiting cell interaction^{13,149}. PPSG, in contrast, introduces asymmetry via propylene units, increasing hydrophilicity and bioactivity. Synthesized through the enzymatic transesterification of diethyl succinate, 1,3-propanediol, and glycerol with lipase N435^{160,200}. PPSG provides high yield (94%) and controlled branching, which improves flexibility and degradation²⁰¹. The branched structure of PPSG also contributes to hydrophilicity, cell adhesion, and tunable mechanical behavior, which are essential for applications in skin and muscle regeneration^{151,202}.

Bioactive glasses like 45S5 BG are often incorporated into polyesters to improve scaffold performance²⁰³. These glasses release ions that promote osteogenesis, support cell adhesion, and counteract the acidity from polyester degradation^{28,192}. Additionally, BG particles added to polyesters improve mechanical strength without compromising flexibility, enhancing scaffold stability during regeneration.

As mentioned above, electrospinning is a very common technique in tissue engineering²⁰³. Recently, benign solvents have been explored for greener fiber production^{204,205}. In this work, a novel electrospun composite is developed using PCL, PPSG, and bioactive glass particles using acetic acid as a green solvent^{116,206–208}. This combination aims to produce biomechanically and biologically enhanced scaffolds for soft tissue repair, including skin regeneration and diabetic ulcer treatment.

Furthermore, this work addresses a significant gap in the literature. To our knowledge, no previous study has combined PCL, PPSG, and bioactive glass to produce electrospun fibers for soft tissue regeneration. By addressing the mechanical and biological limitations of existing tissue scaffolds, this new composite has the prospective to become a valuable biomaterial for biomedical applications.

4.2 Materials and Methods

4.2.1 PPSG Samples

PPSG was synthesized by enzymatic transesterification according to the protocol reported by Fernandes et al.¹⁹⁵. Briefly, the monomers glycerol, 1,3-propanediol, and succinic acid (all from Sigma Aldrich), were homogenized under N₂ flow for 30 min. 10% (w/w) enzyme (N435) was added with an initial activity of 32.3 U.g⁻¹. The reaction proceeded for 24 hours at 90°C and constant stirring under reduced pressure. PPSG has an average molecular mass (M_w) of 6 kDa, a dispersity (Đ) of ±3, and a mean degree of polymerization (DP_n) of ±9. Based on the ¹C NMR spectra, an enzymatic preference for the primary hydroxyls of glycerol was 94%. The presence of the unreacted tertiary hydroxyl group is used to improve hydrophilicity²⁰¹. The detailed protocol of enzymatic synthesis has been reported elsewhere¹⁹⁵.

4.2.2 Polymer Solution Preparation and Electrospinning Process

The polymer blend solution for electrospinning was prepared using PCL (80 kDa, Sigma Aldrich, Munich, Germany) with different concentrations of PPSG dissolved in 20% (w/v) glacial acetic acid (AA, VWR, Darmstadt, Germany). PCL refers to the blank sample without PPSG, while PCL/5PPSG, PCL/10PPSG, and PCL/15PPSG mean 5% (w/w), 10% (w/w), and 20% (w/w) PPSG, respectively. The preliminary tests were operated at the flow rates of 0.2 mL/h, 0.4 mL/h, and 0.8 mL/h. This variation was conducted to assess its effect on fiber morphology and determine the most suitable condition for generating homogeneous, bead-free fibers capable of incorporating bioactive glass nanoparticles. BG particles of 45S5 composition (in wt% 45% SiO₂, 24,5% Na₂O, 24,5% CaO, 6% P₂O₅) were used (particle size ~4 μm). Samples were labeled as: PCL/10PPSG/5BG, PCL/10PPSG/15BG, and PCL/10PPSG/30BG and represent 5 wt%, 15 wt%, and 30 wt% BG content relative to the polymer phase respectively. To aid in the dispersion of the glass particles, the solution was left in an ultrasonic bath for 10 minutes and then electrospun. All fibers were produced using an electrospinning device at (EC-CLI, IME Technologies, Netherlands) at 15 kV, relative humidity of 25 ± 0.5% and temperature of 24 ± 2 °C, with a 21G needle with the fixed collector at 11 cm distance.

4.2.3 Characterization

4.2.3.1 Wettability

The degree of wettability of electrospun mats was obtained by measuring the angle of water drops on the fibers with 5s of stability using the DSA 2.0 instrument from Krüss GmbH. Five replicates of each composition were observed.

4.2.3.2 Mechanical Characterization

Tensile tests were performed with dry fibers on an Instron® 5960 Dual Column Table Test System. Electrospun mats (thickness 0.3 ± 0.4 mm $\times$ width 5 mm $\times$ length 20 mm) were fixed on paper frames and subjected to a tensile load of 50 N at a $1 \text{ mm} \cdot \text{min}^{-1}$ speed. The ultimate tensile strength (σ), Young's modulus (E), and stress-strain curves were determined for all samples.

4.2.3.3 Hydrolytic Degradation

The fiber mats were immersed in phosphate-buffered saline (PBS) (Gibco™ Thermo Scientific, Schwerte, Germany) for 40 days to evaluate their degradation behavior. The samples were incubated at 37°C and 90 rpm (KS 4000 I Control, IKA Werke GmbH & Co. KG, Staufen, Germany). Every 5 days, samples were collected, washed gently with ultrapure water, and dried at room temperature. When constant weight was reached, degradation could be measured following Eq. (5):

$$\text{Weight loss (\%)} = \left(\frac{m_1 - m_2}{m_1} \right) \cdot 100 \quad (5)$$

Where m_1 is the initial mass of the fiber mat, and m_2 is the mass of the dry fiber mat after degradation.

Complementary SEM and FTIR analyses were performed to investigate changes in the morphology and chemical structure of the samples.

4.2.3.4 In vitro Acellular Bioactivity

Electrospun mats containing of BG particles were evaluated for their acellular bioactivity by immersion tests in simulated body fluid (SBF)²⁰⁹. Triplicate samples were fixed on suitable scaffold supports and submerged in 3 mL SBF prepared according to the protocol of Kokubo et al.²¹⁰. The samples were incubated at 37°C under constant agitation (90 rpm in a table shake; KS 4000i control, IKA, Germany). After 1, 3, 7, and 14 days, samples were washed in ultrapure water and dried in a fume hood at room temperature for subsequent SEM (morphology), XRD, and FTIR (chemical composition changes) analysis.

4.2.3.5 Cell Culture

Cell viability studies were performed using NHDF (normal human dermal fibroblasts; Promocell, Germany). Fiber mats were attached to PCL coronal cell inserts, which were made in-house by 3D printer (Ultimaker S5 3D printer). Fibers were previously disinfected in a UV chamber and stored in sterile 24-well plates. Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium from Gibco™ Thermo Scientific, Schwerte, Germany) supplemented with 1% penicillin (Thermo Scientific, Schwerte, Germany) and 10% FBS (fetal bovine serum from Corning GmbH, Wiesbaden, Germany) in 175 cm² cell flasks until reaching 90% confluence within 48 h. To perform direct assays, cells were seeded at a concentration of 50,000 per 100 µL on each scaffold. After a few minutes of absorption, each well was carefully filled with 900 µL of growth medium until covering the film. The samples were kept in a sterile incubator at 37 °C with CO₂ and humidity control, with renewal of the culture medium every 2 days. After the incubation period, the samples were stained with the WST-8 reagent kit (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt; CCK-8 kit, Sigma-Aldrich) at 5 vol%. The reaction occurred for 3 h with the samples incubated at 37 °C. Then, the viability was measured by absorbance at a wavelength of 450 nm through the spectrophotometric multiplate (FLUOstar Omega, BMG Labtech, Germany).

To investigate the morphology, the electrospun mats with grown cells were analyzed by fluorescence using an Axio Scope A1 microscope (Carl-Zeiss, Jena, Germany) following the protocol reported previously ⁹⁴. Briefly, the samples were washed in DPBS (Dulbecco's phosphate buffered saline from Thermo Scientific, Schwerte, Germany) and maintained under permeabilization buffer for fixation (Triton X-100, sucrose and PBS from Sigma Aldrich). Then, the mats were stained with rhodamine-phalloidin (8 µL/mL solution) and DAPI (4',6-diamidino-2-phenylindole, Thermo Scientific, Schwerte, Germany) (1 µL/mL solution) (Thermo Fisher Scientific).

4.2.4 Statistics

The data were presented as mean values with corresponding standard deviations. Statistical analyses were conducted using Student's t-test and one-way ANOVA (Origin 8.5), with significance determined for p-values less than 0.05.

4.3 Results And Discussion

4.3.1 Electrospinning of PPSG-PCL

To determine the correlation of electrospinning parameters and fiber mat structure, Figure 13 represents the interference analysis of the PPSG and BG concentration parameters and the flow rate to clarify the influence of the polymers component and BG particles on the mechanical and biological performance of the fiber mats.

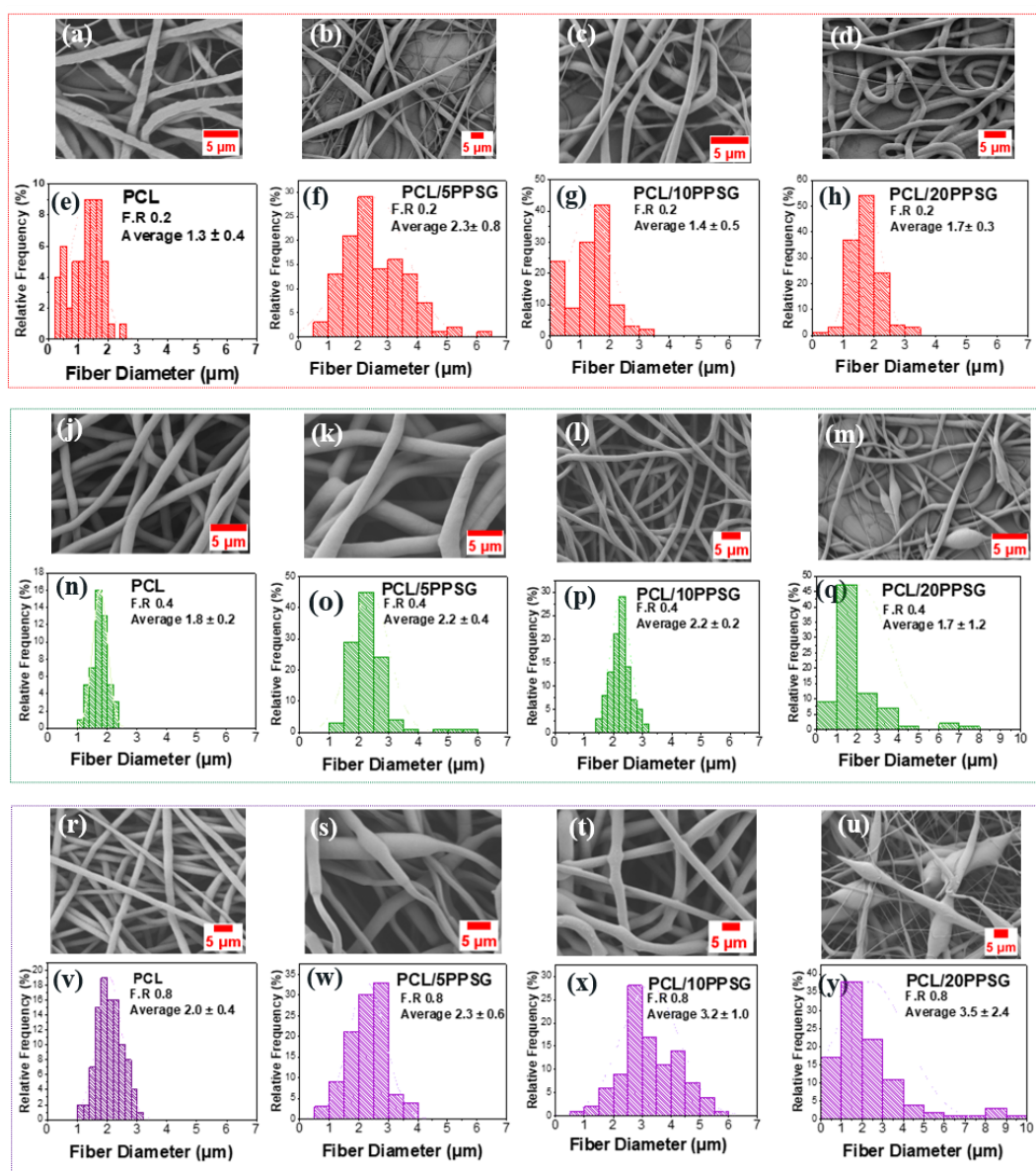


Figure 13. SEM microscopy of the electrospun fibers and their respective average diameter distribution: (a-h) PCL/PPSG flow rate (F.R) 0.2 mL/h; (j-q) PCL/PPSG F.R 0.4 mL/h; (r-y) PCL/PPSG F.R 0.8 mL/h. Reproduced from²¹¹ licensed under CC BY 4.0.

The analysis indicated that with the addition of PPSG, the distribution of the average fiber diameter widens. This phenomenon has been previously reported for PCL/PGS blends and is related to the modification of the polymer solution's properties, particularly viscosity and conductivity, which influence fiber formation during electrospinning. It was observed that at higher PPSG concentrations (20% w/v), the surface tension and viscosity of the solution tend to decrease due to the lower molecular weight of PPSG compared to PCL^{212,213}. According to Fong²¹², the alteration of these parameters causes instability in the jet caused by fluctuations in

the density of electric charges. Therefore, the reduction in surface tension combined with lower viscosity and higher conductivity leads to a tendency for jet breaking, which caused the formation of beads in the PCL/20PPSG samples.

The fiber diameter distribution was more uniform when an intermedium flow rate of 0.4 mL/h was used, achieving narrow distribution diameters for PCL fibers ($1.8 \pm 0.2 \mu\text{m}$), corroborating previous studies of ($0.11\text{--}3.85 \mu\text{m}$) that also used the benign solvent acetic acid^{28,100,214}. Furthermore, the average diameters of fibers composed of PPSG with 5% BG (PCL/5PPSG) of $2.2 \pm 0.4 \mu\text{m}$ and 10% BG (PCL/5PPSG) with $2.2 \pm 0.2 \mu\text{m}$ are in line with the literature regarding for the application of cell mats in the regeneration of fibrous structures of human intramyocardial tissue (perimysial, endomysial and epimysial fibers), ranging from 0.4 to $4 \mu\text{m}$ ²¹⁵.

The stability of the electrospinning jet and the mechanisms underlying fiber formation were strongly influenced by the composition of the polymeric solution and the processing parameters. The applied voltage of +18 kV at the needle and -2 kV at the collector, resulting in a total potential difference of 20 kV, was chosen based on previous studies that demonstrated optimal jet formation and fiber elongation under similar conditions²⁰⁶. This voltage range ensures a balance between polymer jet stretching and solvent evaporation, minimizing droplet formation and promoting uniform fiber deposition^{206,208,216}.

The addition of PPSG to the PCL matrix altered the solution properties, affecting jet stability and fiber morphology. PPSG, due to its structure and hydrophilicity, increases solution conductivity while decreasing viscosity, which are critical factors in electrospinning performance^{217,218}. At concentrations 20%, a disruption in jet stability was observed, likely caused by charge density fluctuations and insufficient viscosity to maintain a stable polymer jet. This phenomenon led to bead formation and decreased fiber uniformity, as evidenced in the SEM analysis (Figure 13). Similar trends have been reported for other polyester-based electrospun blends, where higher hydrophilic content resulted in increased jet instability^{186,190,219}.

The role of solution viscosity and conductivity in jet formation is well established in the literature²²⁰. While a moderate increase in viscosity enhances fiber stretching and diameter

control, excessive viscosity can hinder electrospinning, leading to interrupted jets and droplet formation. The selected polymer concentrations (5%, 10%, and 20% PPSG, relative to the total polymer content) were determined based on the processability of the solution, ensuring fiber formation while avoiding excessive instability⁴⁰. Additionally, the fixed needle-to-collector distance of 11 cm was maintained to optimize solvent evaporation and prevent fiber fusion on the collector, as reported in previous electrospinning studies⁹⁴

The thermal analysis (Figure S7, Table S2) corroborated the electrospinning results by demonstrating a reduction in crystallinity at higher PPSG concentrations, as evidenced by the decrease in enthalpy of fusion (ΔH_f) from 3.8 J/g (PCL) to 3.1 J/g (PCL/20PPSG). The decrease in crystallinity aligns with the lower molecular weight of PPSG, reducing polymer chain entanglement likely contributed to the instability observed during fiber formation at 20% PPSG, supporting the observed bead formation and jet disruptions.

Future investigations should include a more detailed rheological characterization of the polymer solutions, evaluating viscosity, conductivity, and surface tension to better correlate solution properties with fiber formation mechanisms. Understanding these parameters could further refine the electrospinning conditions for PCL/PPSG blends and optimize scaffold morphology and performance for biomedical applications.

Preliminary tests revealed that concentrations above 20% led to significant processing challenges due to high dilution of the polymer solution. The decrease in viscosity at higher PPSG content was primarily due to the reduction in the average molecular weight of the polymer blend, as PPSG has a lower molecular weight than PCL. It is important to note that the total polymer concentration in the solution remained constant at 20% (w/v), and only the PPSG-to-PCL ratio within the polymer blend was modified. The lower viscosity at higher PPSG fractions hindered sufficient polymer chain entanglement, which is crucial for electrospinning, resulting in unstable jet formation and failure to generate continuous fibers. Furthermore, the hydrophilic nature of PPSG increases the conductivity of the solution, and at high concentrations, excessive charge repulsion disrupted fiber elongation, leading to bead formation and fiber coalescence on the collector. Given these observations, 20% PPSG was established as the upper limit for fiber formation, while 10% PPSG operated at 0.4 mL/h was identified as the optimal concentration, balancing fiber morphology, mechanical stability, and

bioactivity. This selection ensures that the electrospun fibers maintain the bead-free structural integrity while incorporating sufficient PPSG to investigate the changes in fiber properties. Therefore, this condition was considered the best and was selected to be maintained in the following steps. Furthermore, the effect of 45S5 BG particles was evaluated at different concentrations (3%, 15%, and 30%) in PCL polymer solution containing 10% PPSG (samples named PCL/PPSG/3BG; PCL/PPSG/15BG; PCL/PPSG/30BG).

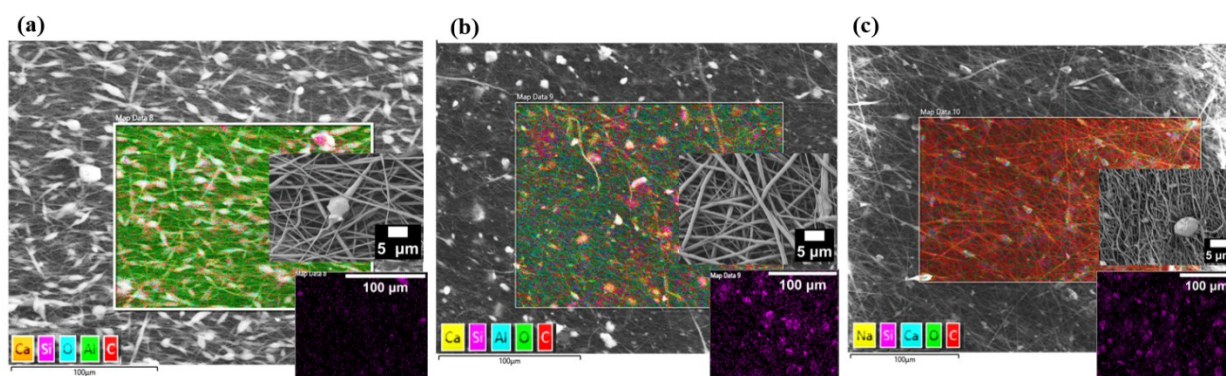


Figure 14. SEM/EDX showing BG particle distribution in the fiber mats: (a) PCL/PPSG/5BG, (b) PCL/PPSG/15BG, (c) PCL/PPSG/30BG. All compositions contain 10% PPSG. Reproduced from ²¹¹ licensed under CC BY 4.0.

The analysis confirmed the presence of silica as a marker for the presence of BG particles in the fiber structures (Figure 14 a–c). The addition of BG particles increased the suspension's conductivity, which resulted in a significant change in the average diameter of the fibers (Figure 13). However, despite the smaller diameter of the fibers of samples containing BG particles ($0.9 \pm 0.4 \mu\text{m}$), they are still within the application range for soft tissues engineering ¹⁹⁴.

4.3.2 Chemical characterization

The chemical composition of the fibers was measured using FTIR spectroscopy. Figure 15 presents the spectra of fibers without any PPSG (PCL), with 10% PPSG (PCL/PPSG), and with different concentrations of BG (PCL/PPSG/BGs). At the same time, the characteristic absorption bands are detailed in Table S3. No absorption bands associated with acetic acid were detected, indicating that the solvent was eliminated.

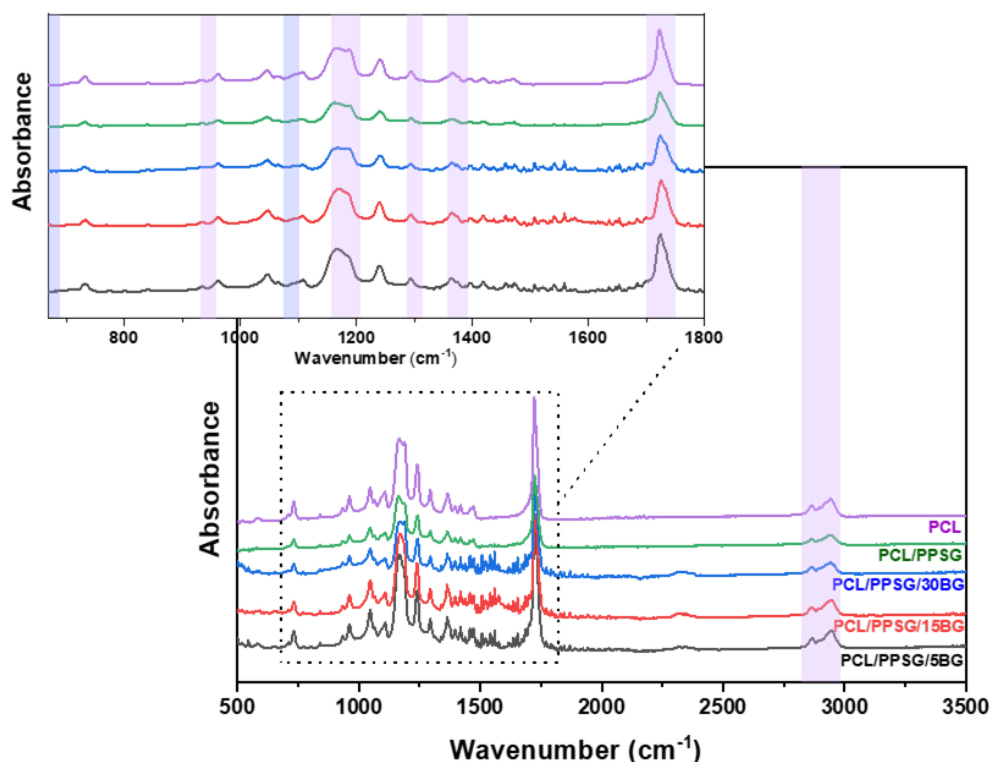


Figure 15. FTIR spectroscopy of fiber mats in the 400 to 3500 cm^{-1} wavenumber range. Highlights are indicated in purple and detailed in Table S3. Reproduced from²¹¹ licensed under CC BY 4.0.

Analysis of the FTIR results confirms the presence of the PCL spectral features as reported in previous studies^{20,219,221}, highlighting the peaks at 2935 cm^{-1} , 2864 cm^{-1} , and 1365 cm^{-1} , related to the CH_2 stretching, as well as peaks at 1296 cm^{-1} and 1166 cm^{-1} , allocated to asymmetric ($\text{C}-\text{O}$) and symmetric ($\text{C}-\text{C}$) stretching. At 1726 cm^{-1} , the peak was referent with carbonyl stretching ($\text{C}=\text{O}$)²²². Furthermore, a 1296 cm^{-1} peak was observed, attributed to stretching the $\text{C}-\text{C}$ and $\text{C}-\text{O}$ backbone²²³.

The overlap of the specific bands related to PPSG and BG 45S5 with the prominent peaks of PCL made their detection difficult in Figure 15. In particular, the polyester exhibits characteristic peaks around 1365 cm^{-1} and 2935 cm^{-1} , corresponding to the bending absorption of CH_2 and asymmetric deformation of the $\text{C}-\text{H}$ bond in order²²³. The axial stretching vibration of the $\text{C}(=\text{O})-\text{O}$ bond, deemed the most distinctive diethyl succinate esters vibrational mode, is observed at approximately 1295 cm^{-1} ²⁰¹. Likewise, axial strain vibrations in the $\text{C}=\text{O}$

functional group of diester compounds were detected at 1726 cm^{-1} , consistent with the observations made for the PCL sample.

Similarly, when considering fibers containing BG45S5 particles (PCL/10PPSG/5BG, PCL/10PPSG/15BG, PCL/10PPSG/30BG), the detection of characteristic absorption bands of the silicate, such as the stretching modes Si–O–Si and Si–O bonds in the range $900\text{--}1,100\text{ cm}^{-1}$, as well as the bending mode of Si–O–Si at 470 cm^{-1} , is difficult due to their relative weakness

4.3.3 Wettability

The hydrophilicity degree of biomaterials represents a crucial parameter in determining their interaction with cells. It is well documented that hydrophobic surfaces with low wettability hinder cellular adhesion and compromise material integration with the surrounding tissue. Conversely, hydrophilic fibers better mimic the natural biological environment, enhancing cellular adhesion and proliferation²²⁴.

The wettability of electrospun blend mats was determined by contact angle assessment and modulated by the PPSG concentration. Fibers containing only PCL exhibited hydrophobic characteristics with a contact angle of $119\pm 8^\circ$. Enzymatically synthesized glycerol-based polyesters such as PGS, PPSG, and PGO exhibit high hydrophilicity due to hydroxyl groups linked to their structure^{7,102,225}. Therefore, mixed fibers containing PPSG (Figure 16b) showed hydrophilic behavior as the PPSG concentration increased ($28\pm 3^\circ$ for PCL/5PPSG and $40\pm 5^\circ$ for PCL/10PPSG). The contact angle for the PCL/20PPSG sample was not detectable, as the water droplet rapidly penetrated and spread on the sample surface.

Conversely, it was observed that fiber mats containing bioactive glass particles, exhibited less hydrophilic behavior as the BG concentration increased, with contact angles of $28\pm 4^\circ$ and $40\pm 5^\circ$ for samples PCL/10PPSG/15BG and PCL/10PPSG/30BG, respectively. Literature reports indicate that electrospun PCL mats exhibit contact angles in the range of $133^\circ\text{--}98^\circ$ ^{15,226,227}, and blends of PCL with PGS (1:0.5) have no measurable contact angles^{28,133,192}. The literature has documented that extreme hydrophobic or hydrophilic properties are unfavorable for promoting cellular adhesion. The ideal wettability for adequate cellular attachment is 40° to 70° ²²⁸. Therefore, the analysis shows that PPSG and BG strongly affect the wettability of PCL/10PPSG/BG blends, and their concentration would be vital to controlling hydrophilicity (Figure 16).

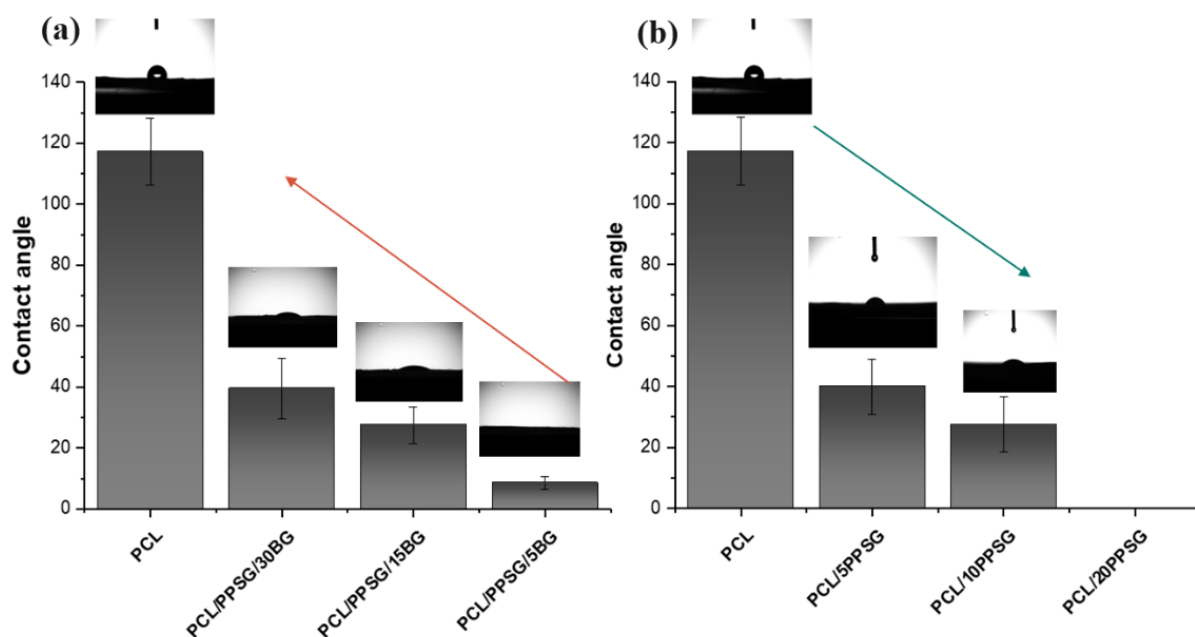


Figure 16. Degree of wettability of electrospun mats, as determined by contact angle measurement. (a) Composite mats containing PCL/PPSG blends and BG 45S5 bioactive glass. (b) Electrospun PCL mats with varying PPSG fractions. Reproduced from ²¹¹ licensed under CC BY 4.0.

4.3.4 Degradation in PBS Solution

When introduced into biological environments, degradable biomaterials undergo surface or bulk degradation, exhibiting progressive alterations in their physicochemical properties over time. In the case of aliphatic polymers like PCL and PPSG, hydrolytic degradation of the ester bonds occurs at varying rates. Typically, glycerol-based polyesters such as PPSG are fully degraded within a few weeks due to their inherent hydrophilicity ^{229,230}. Meanwhile, PCL demonstrates a slower degradation rate due to its semi-crystalline nature and molecular mass ²³¹. From this perspective, PCL/PPSG blends emerge as promising candidates for developing hybrid materials with controlled degradation kinetics. In vitro immersion tests in PBS solution were conducted to explore degradation and weight loss was monitored over 35 days for fibers comprising pure PCL, PCL/PPSG, and PCL/PPSG/BG blends, as shown in Figure 17.

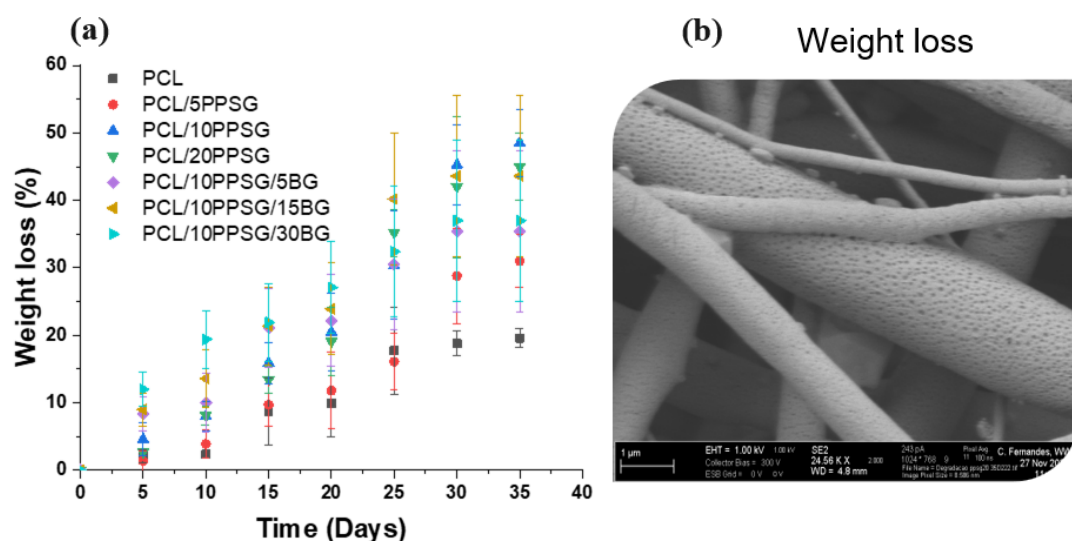


Figure 17. Weight loss during submersion in PBS for 35 days (a) and SEM micrograph of samples after 35 days of immersion PCL/10PPSG fiber (b). Reproduced from²¹¹ licensed under CC BY 4.0.

The weight loss dynamic profiles, depicted in Figure 17a, revealed rapid degradation of PCL/PPSG fiber mats attributed to hydrolytic cleavage of ester bonds in both PCL and PPSG components. Microscopic analysis depicted significant degradation of PCL/20PPSG samples (Figure 17b), evidenced by pore and crack formation likely due to the leaching of PPSG degradation products. In contrast, pure PCL samples exhibited a lower weight loss. Moreover, by the end of 35 days in PBS, the pH of the pure PCL samples hardly changed, while the pH of the PBS degradation medium showed an acidification trend as PPSG concentrations increased by 5%, 10%, and 20%, reaching values of 7.3, 7.0, and 6.9, respectively. Prior studies have also noted common leaching of unreacted glycolic polyesters, such as PGS, in electrospun fibers²³¹.

Incorporating BG particles into PCL/PPSG mats led to small pore formation, indicating bioactive glass particle leaching (blue arrow, figure 18). Furthermore, weight loss measurements confirmed accelerated degradation of PCL/PPSG blends compared to pure PCL mats, with higher PPSG concentrations showing more significant weight loss after 35 days in PBS incubation. Overall, PCL/PPSG blends exhibited a weight loss of approximately $45\% \pm 5$ after 35 days, highlighting the higher degradation rates than PCL/PPSG mats (12%)^{95,146}. Therefore, the measured results of degradation behavior underscore the significance of

understanding polymer interactions and blend compositions for tailored degradation kinetics in biomedical applications.

4.3.5 In vitro Acellular Bioactivity

The ability to form hydroxyapatite on the mat surfaces was evaluated as an indicator of fiber bioactivity²³². Fibers without the presence of bioactive glass were also evaluated as control samples, and no evidence of hydroxyapatite was found after 14 days of analysis (Figure 18b). The other representative SEM micrographs of fibers containing BG particles after 14 days in SBF and PBS solutions are shown in Figure 18.

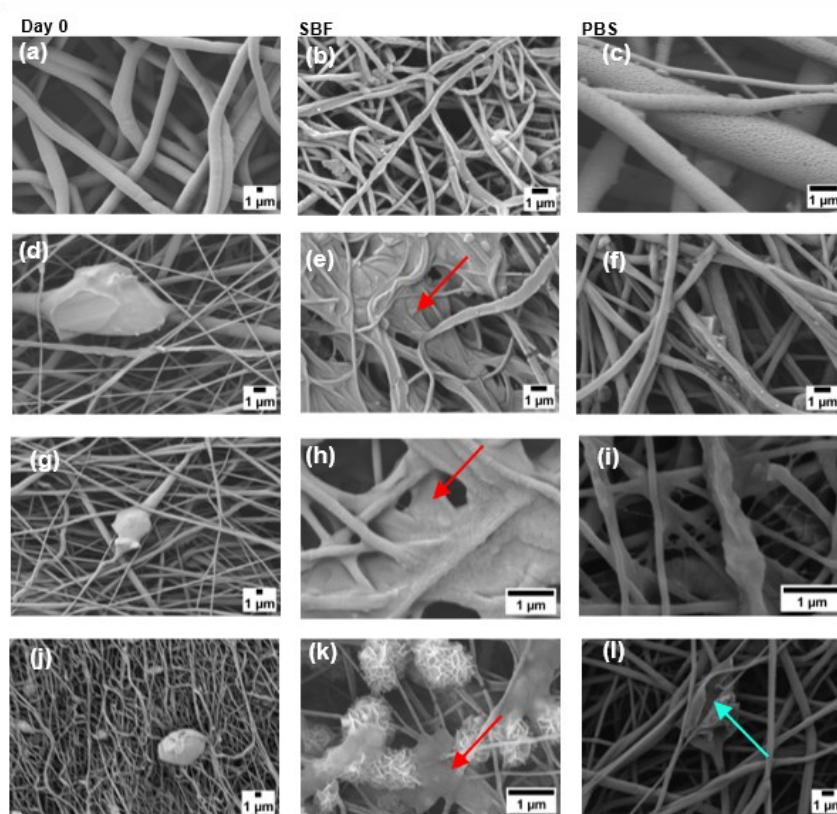


Figure 18. SEM images of fiber with BG under SBF and PBS: (a,b,c) PCL/10PPSG; (d,e,f) CL/10PPSG/5BG; (g,h, i) PCL/10PPSG/15BG; (j,k,l) PCL/10PPSG/30BG. The red arrow indicates Hydroxyapatite. Reproduced from²¹¹ licensed under CC BY 4.0.

Hydroxyapatite deposition was observed in the composite fibers with the addition of BG 45S5 (Figure 18d). Samples PCL/PPSG/5BG, PCL/PPSG/15BG, and PCL/PPSG/30BG

showed biomineralization effects (Figures 18a and 18b). The morphology of the deposited layer exhibited variations observed in the PCL/10PPSG/30BG sample (Figure 18k) at the end of immersion in SBF solution, compared to the samples PCL/PPSG/5BG and PCL/10PPSG/15BG in SBF (Figure 18e and 18h), exhibiting the characteristic morphology of hydroxyapatite, which has been extensively documented in the literature^{200,233,234}. As no morphological change was noted for those samples when immersed in buffered PBS saline solution, it can be

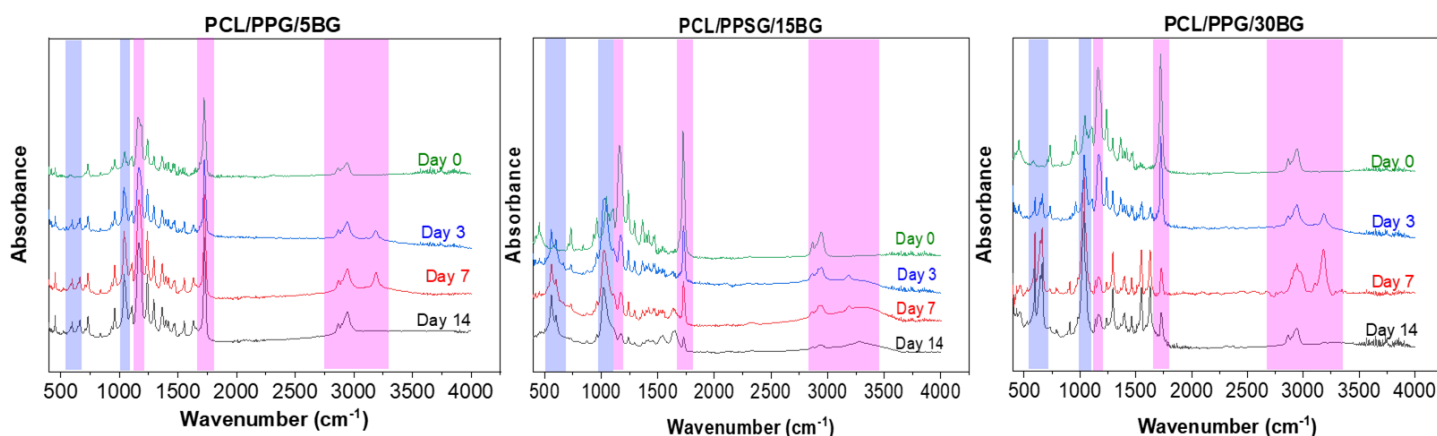


Figure 19. FTIR spectra of samples immersed in SBF over 14 days.

concluded that the change is related to the BG concentration, which caused the mineralization of the samples, as frequently reported in the literature for similar BG containing fibers²⁰⁶. Reproduced from²¹¹ licensed under CC BY 4.0.

The evaluation and investigation of the formation of hydroxyapatite on the fibers was carried out through observations of FTIR spectra, which are presented in Figure 19. Peaks around 560 and 600 cm^{-1} are related to the phosphate group and were detected in all samples after 3 days of immersion in SBF solution, as reported in Table S2. Furthermore, additional peaks attributed to the stretching vibration of silica (Si-OH) were observed at 1050 cm^{-1} in the spectra of all fibers immersed in the SBF solution²³⁵. Moreover, a wide peak at 3402 cm^{-1} and the ester bonds at 1721 cm^{-1} qualitatively decreased in intensity after immersion in SBF solution. This behavior suggests that OH (hydroxyl) and COOH⁻ (carboxyl) groups are present in the PCL/PPSG mats. It is important to note that these changes indicate functional group presence but they do not provide quantitative information about their concentration. Usually, PCL/PPSG undergoes hydrolysis through ester bonds, transforming them into oligomers with hydroxyl and carboxylic terminal groups. Considering SEM micrographs, FTIR spectroscopy

results, and weight loss analysis in PBS medium, leaching or degradation of PGS from PCL/PPSG mixtures in the medium is plausible. Additionally, the samples present peaks related to stretching vibrations at 1030, 810, and 460 cm^{-1} , attributed to Si-O-Si, bonds, indicating the presence of the silica network in the BG particles²³⁶. Previous work also reported the presence of bands in this region due to phosphate groups with stretching vibration, indicating hydroxyapatite growth on the material's surface²³⁶.

XRD analysis confirmed the presence of hydroxyapatite in the fibers containing BG, as illustrated in Figure 20. All samples presented characteristic PCL peaks and evidence of bioactivity was demonstrated after the immersion period. Specifically, the peak at $2\theta = 32^\circ$ and 46.7° can be attributed to the presence of hydroxyapatite^{117,118,236}. This result is significant for tissue engineering applications, as it confirms for the first time the bioactivity of BG 45S5 particles embedded in the new biopolymer matrix.

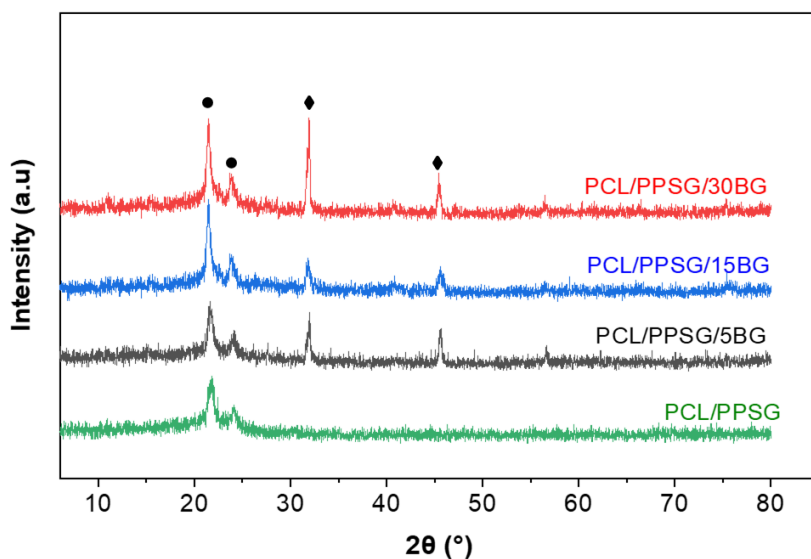


Figure 20. XRD patterns of samples immersed in SBF after 14 days. The presence of hydroxyapatite (◆) is marked by the peaks at $2\theta = 32^\circ$ and 46.7° . The crystallinity of PCL (●) is marked by the peaks at $2\theta = 21.4^\circ$ and 23.7° . Reproduced from²¹¹ licensed under CC BY 4.0.

The evaluation of HA formation in scaffolds designed for soft tissue engineering may offer valuable insights into how the release of bioactive ions affects target cells in this context. The process of HA formation results in the release of ions such as Ca^{2+} , Si^{4+} , and PO_4^{3-} , which promote cell proliferation, angiogenesis, and extracellular matrix synthesis²³⁷. In addition, the HA layer supports cell adhesion through integrin interactions and allows surface functionalization with biomolecules, thus creating a microenvironment that favors regeneration²³⁸. HA also functions as an ion reservoir, ensuring a continuous supply of bioactive stimuli in non-mineralized tissues, such as skin and connective tissue²³⁹. Although the purpose of HA is not to mineralize soft tissues, its presence contributes to the development of a biochemically favorable environment for regeneration and establishes functional integration with the host biological system.

4.3.6 Mechanical Properties

Understanding the mechanical properties of biomaterials is important for developing useful materials for tissue engineering. The mechanical properties of PCL/PPSG fibers and composite fibers incorporating bioactive glasses (PCL/PPSG/BG) were investigated by tensile testing, focusing on the UTS and Young's modulus (Figure 21 and Table 6).

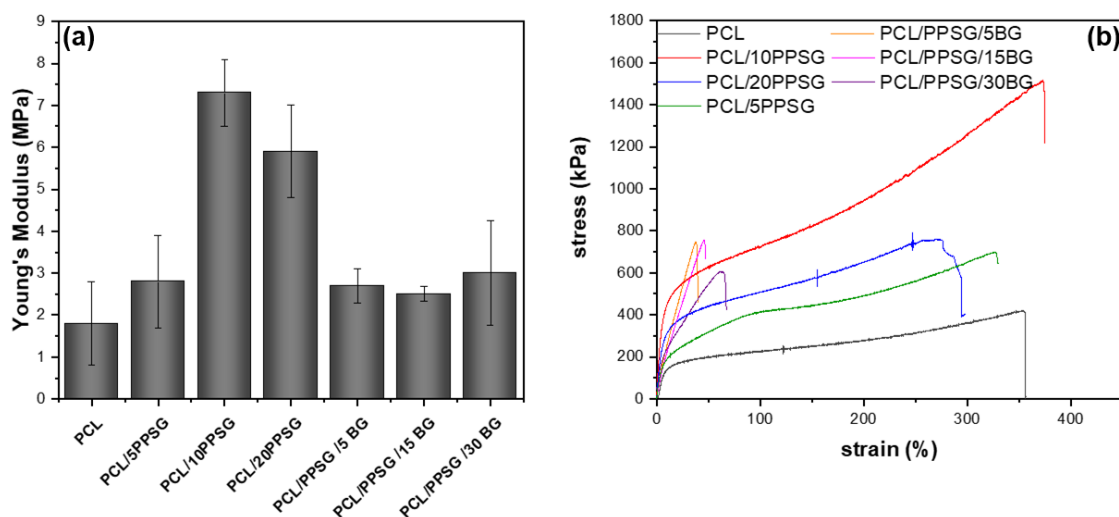


Figure 21. Results of (a) Young's Modulus values and (b) stress-strain curves of PCL/PSG fibers and fibers with bioactive glass PCL/PPSG/BG samples. Reproduced from²¹¹ licensed under CC BY 4.0.

One can observe that the addition of PPSG to the PCL blend resulted in a significant increase in Young's modulus, from 2.0 ± 0.8 MPa to 3.0 ± 1.0 MPa, 7.0 ± 1.0 MPa, and 6.0 ± 0.8

MPa, as the PPSG concentration increased (PCL, PCL/5PPSG; PCL/10PPSG; PCL/20PPSG, respectively). This increase indicates greater stiffness of the material, attributed to the improved mechanical properties conferred by the presence of PPSG. Similar behavior was reported in the literature where the addition of PGS to PCL increased the stiffness of the fibers¹⁰⁰. These results may be related to the interaction between PPSG and PCL creating a more cohesive polymer blend with a larger average fiber diameter and, therefore, with greater stress transfer between the chains in relation to pure PCL. Furthermore, we observed a notable increase in the UTS of the PCL and PPSG blend compared to pure PCL fibers. While PCL fibers presented a UTS of 0.4 MPa, the PCL/PPSG blends achieved a UTS of 1.5 MPa. This increase suggests a higher ability of the material to withstand maximum tensile loads before failure. On the other hand, although the UTS analysis showed that PPSG improved the tensile strength of PCL/PPSG blends, the failure strain and Young's modulus values decreased with the addition of bioactive glasses (PCL/PPSG/BG). These results may be connected to the distribution of fiber morphology and BG particles in the polymeric solution, which causes weak interface points between the BG particles and the polymer. Similar results have been reported in the literature for particle addition in PCL and chitosan fibers. The reduction in Young's modulus and UTS values occurs due to the presence of inorganic particles in high concentration (30 wt%)^{100,206}. Thus, adding BG particles aims to introduce biological functionalities for tissue regeneration. However, BG particle addition also affects the ultimate mechanical behavior of the fibers.

A key factor affecting the mechanical stability of PCL/PPSG/BG scaffolds is the absence of strong covalent interactions between the BG particles and the polymer matrix. Unlike chemically bonded systems, where molecular interactions enhance load transfer, the BG-polymer interface in this study relies on weak physical interactions such as hydrogen bonding and van der Waals forces. This weak interfacial adhesion can result in localized stress accumulation, leading to early mechanical failure under load. As a consequence, the presence of BG, despite its bioactivity benefits, introduces potential points of structural weakness.

Furthermore, the distribution of BG particles within the polymer matrix plays a crucial role in determining scaffold performance. As observed in Figure 14 (SEM/EDX analysis), mapping of the BG dispersion reveals regions of particle aggregation, which likely act as stress concentration points, reducing the overall mechanical strength. These clustered regions may disrupt the homogeneous transfer of load through the scaffold, increasing susceptibility to the

initiation and propagation of mechanical stress points. A more uniform dispersion of BG particles would favor stress dissipation, improving mechanical integrity.

Moving forward, investigating the effects of controlled reduction of BG particle size and optimizing BG concentration within the polymer matrix should be the focus of future studies. This would allow for a better balance between increased bioactivity and mechanical stability, ensuring that the scaffold maintains its structural integrity while still promoting cellular interaction and mineralization.

Table 6. Correlation of tensile test results with fiber mat diameter. Reproduced from²¹¹ licensed under CC BY 4.0.

Sample	Fiber Diameter (μm)	Young's Modulus (MPa)	UTS (MPa)	Elongation at Break (%)
PCL	1.8 $\pm$ 0.2	1.7 $\pm$ 1	0.4 $\pm$ 0.1	355 $\pm$ 45
PCL/5PPSG	2.2 $\pm$ 0.4	2.1 $\pm$ 1.1	0.7 $\pm$ 0.1	330 $\pm$ 27
PCL/10PPSG	2.2 $\pm$ 0.2	6.0 $\pm$ 0.8	1.5 $\pm$ 0.1	374 $\pm$ 33
PCL/20PPSG	1.7 $\pm$ 1.2	8.8 $\pm$ 1.5	0.7 $\pm$ 0.1	295 $\pm$ 25
PCL/10PPSG/5BG	0.93 $\pm$ 0.4	2.7 $\pm$ 0.3	0.7 $\pm$ 0.1	43 $\pm$ 4
PCL/10PPSG/15BG	0.55 $\pm$ 0.3	2.5 $\pm$ 0.1	0.8 $\pm$ 0.1	47 $\pm$ 7
PCL/10PPSG/30BG	0.63 $\pm$ 0.3	3 $\pm$ 0.3	0.6 $\pm$ 0.3	69 $\pm$ 3

The analysis shows that the blends presented higher mechanical properties compared to some soft tissues. For example, Young's modulus of myocardial tissue is expected to be in the range of 0.02 MPa to 0.5 MPa, while its tensile strength is in the range 3-15KPa²⁴⁰. However, it is important to consider that higher values of the mechanical proprieties of biomaterials should not harm their necessarily final performance. Theoretical simulations have demonstrated that a stiffer cardiac patches can positively contribute to the mechanical properties of the myocardium, which reduces stress on the cardiovascular wall, an essential factor in the propensity for infarctions^{240,241}. This behavior suggests that the PCL/PPSG blend is particularly promising for applications where mechanical strength is crucial.

4.3.7 Cell viability

Cell viability was investigated through the proliferation of NHDF cells on electrospun mats of PCL/PPSG, as well as on fibers containing bioactive glass particles PCL/PPSG/BGs. It was found that NHDF cells proliferated and spread well on all fibers over the 3-day culture period. Cell metabolic activity increased considerably during this period, as evidenced by the results shown in Figure 22. Initially, a lower number of cells was observed on the PCL and PCL/PPSG samples in the first 24 h of cell growth, possibly due to the rapid degradation of electrospun surfaces, which may reduce cell adhesion capacity due to medium acidification, as reported in the literature ²²⁰.

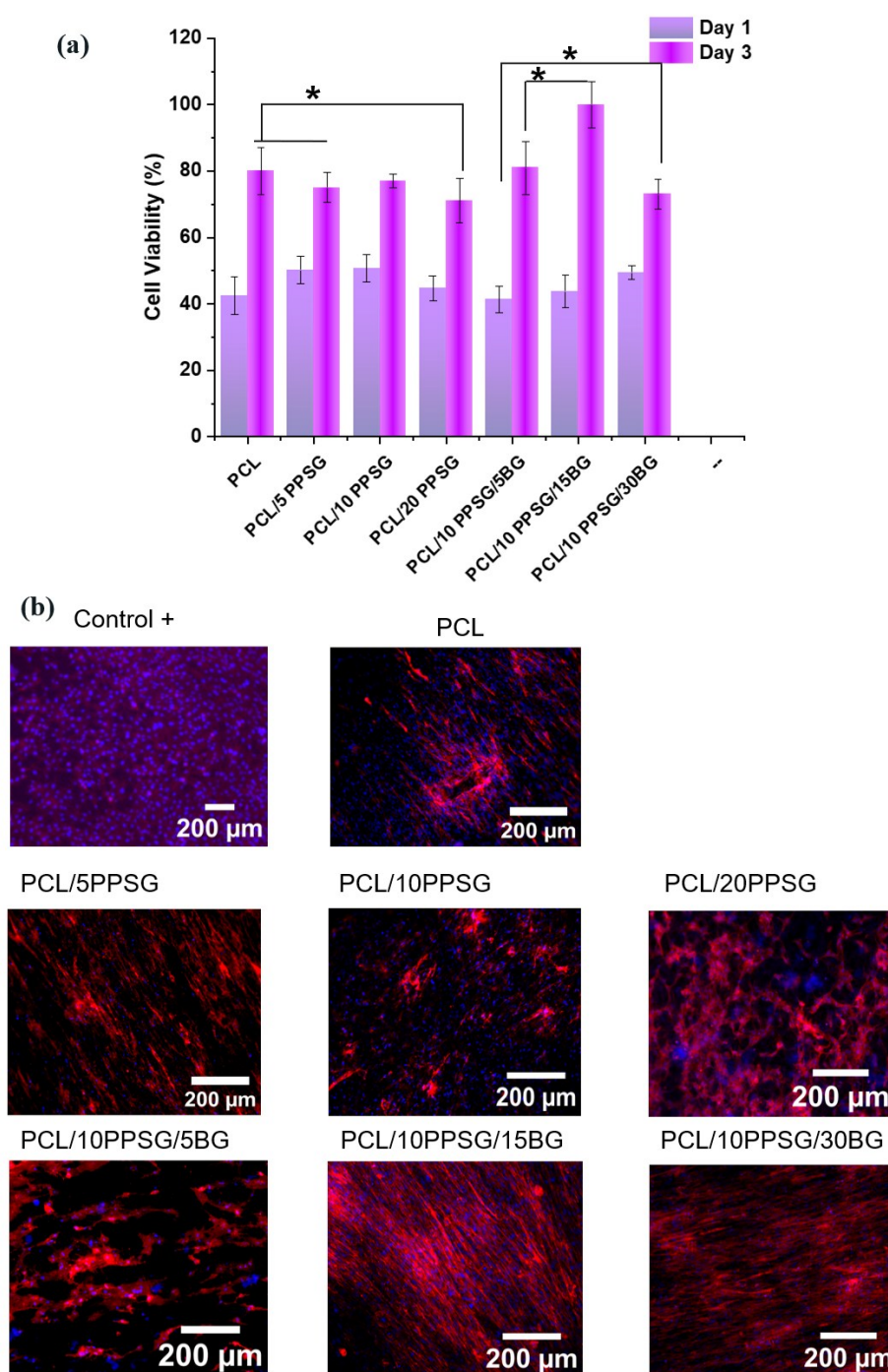


Figure 22. (a) Cell viability analysis was measured by cellular dehydrogenase staining assay at a wavelength of 450 nm (* $p < 0.05$). (b) Fibroblasts cultured on fiber blends were analyzed by fluorescence, DAPI in blue (cell nuclei), and rhodamine-phalloidin in red (actin filaments). Reproduced from ²¹¹ licensed under CC BY 4.0.

However, this effect was mitigated with the addition of BG particles, indicating that ion release from BG stimulates cell growth. Analysis of the samples after 3 days of cell growth

revealed an increase in cell viability, especially in samples containing 15% BG particles in the composition. This result is consistent with previous studies that highlighted the favorable effect of BGs on cell viability, attributing it to ion release that stimulates mRNA-level processes, improving cell adhesion and proliferation^{99,242}. Morphology and differences in mechanical properties of the fibers also play an important role in the biological response. According to Chen et al., a decrease in fiber diameter is associated with increased cell adhesion due to increased specific surface area and consequent adsorption of proteins which is crucial for adhesion on fiber surfaces^{243,244}. Analogous to these observations, it was found that the fiber diameter of PCL/PPSG was greater than that of PCL/PPSG/BG, affecting cell adhesion.

The sudden change in the mechanical properties of the fibers can also be a factor influencing cell adhesion. Generally, greater mechanical strength can provide stability for cell development, while more deformable, less rigid scaffolds tend to present lower cell proliferation rates²⁴⁵. In the case of composites with BG, a decrease in mechanical elasticity can favor proliferation over 3 days. Indeed PCL/PPSG/BG composite fibers showed increased cell viability at the end of the analysis time. Another critical factor is that during this period, ion release from BG occurred, likely overcoming the polymer's physical barrier and assisting cell attachment and proliferation.

Furthermore, analysis of Figure 22b, which shows the nuclei and cell filaments of stained NHDF cells cultured on fibers for 3 days, revealed that the cells spread and interconnected across the surface of the samples. Notably, the red staining indicated that there was elongation of the cell filaments in all samples. This elongation indicates a well connected network, representing effective cell adhesion and proliferation. These results corroborate the WST-8 analysis, in which the PCL/PPSG/15BG sample exhibited the highest cell viability and, consequently, the greatest stretching for interconnection of the filaments. Furthermore, the analysis also agrees with previous investigations in which polymer blends containing glycerol have superior cell adhesion and proliferation^{221,246}.

4.4 Conclusions

Homogeneous and bead-free fiber mats were obtained from PCL/PPSG blends. Bioactive glass 45S5 particles were integrated and homogeneously dispersed into the electrospun fibers. Remarkably, the copolymer PPSG improved the mechanical properties of the fibers, while incorporating BG particles created stress points that reduced the mechanical properties. The presence of PPSG significantly contributed to the hydrophilicity and rapid biodegradability of the fibers, even at low concentrations. Cell viability studies showed that the presence of BG particles contributed to cell attachment and growth. Furthermore, the 30% BG concentration led to hydroxyapatite formation upon immersion in SBF revealing that BG concentration is the key factor for the modulation of mineralization. While PCL was used as a reference for cell viability measurements, a direct comparison with collagen-based scaffolds or other biomaterial controls was not included in this study. Future studies should explore additional biological benchmarks to further validate the biocompatibility and functional performance of these scaffolds in a physiologically relevant context. The results of this work are promising and can serve as a foundation for upcoming comparative studies with similar biopolymer/BG fibrous scaffolds for possible application in soft tissue engineering.

Chapter 5

Boron-Doped Mesoporous Bioactive Glass Nanoparticles (B-MBGNs) in (Poly(E-Caprolactone)/Poly (Propylene Succinate-Co-Glycerol Succinate) Nanofiber Mats For Tissue Engineering

This chapter contains results on the synthesis and use of boron-doped mesoporous glass nanoparticles (MBGNs) in PCL/PPSG fibrous composites. The results obtained have been submitted for publication to ACS Applied Bio Materials in December 2024 with the candidate as first author. The methodology guidelines and discussion of results are detailed below.

5.1 Introduction

The growing demand for advanced biomaterials for tissue repair has stimulated interest in innovative material combinations. Among them, mesoporous bioactive glass nanoparticles (MBGNs) stand out for their ability to promote osteogenesis, induce angiogenesis, and bond with both hard and soft tissues^{233,247}. Their high surface area and nanoporosity enhance apatite formation^{126,248,249}. Recently, metal ion doping has emerged as an effective strategy to enhance the biological performance of bioactive glasses, contributing to the advancement of regenerative medicine³⁰. Among the elements investigated, boron has received particular attention due to its positive effects on cell proliferation, neovascularization, and chronic wound healing^{106,123}.

Incorporating boron into apatite-wollastonite bioceramics has shown no cytotoxic effects on fibroblasts¹⁰⁶. Furthermore, borate-based composite fibers have proven to be non-toxic to endothelial cells, being effective in stimulating angiogenesis, resulting in faster tissue remodeling compared to conventional approaches^{234,250}. Additional studies have demonstrated that boron-containing bioactive glass fibers are compatible with neuronal cells and promote increased vascularization in the chorioallantoic membrane^{27,251,252}. These findings have fueled growing interest in boron-doped mesoporous bioactive glass nanoparticles (B-MBGNs), which exhibit both cytocompatibility and angiogenic activity, making them promising candidates for soft tissue regeneration, particularly in the treatment of chronic wounds such as diabetic ulcers^{233,253,254}.

B-containing bioactive glass fibers have already been approved by the FDA and are commercially available for acute wound dressings^{14,15}. While further studies are

needed to clarify the mechanisms behind boron's regenerative effects, this approval supports the development of boron-containing materials for clinical use.

With increasing research on B-MBGs for soft tissue applications, especially in dermal and neural repair, there is a need to develop controlled-release scaffolds that promote vascular growth¹⁰⁶. New fibrous biomaterials are being designed to offer structural support, moisture retention, and regulation of cell proliferation, especially for diabetic and non-healing wounds^{253,254}. Building upon the electrospun PPSG/PCL scaffolds developed in the previous chapter, this study explores the incorporation of boron-doped mesoporous bioactive glass nanoparticles (B-MBGs) to further enhance the bioactivity and angiogenic potential of the electrospun fibers. The 50SiO₂–40CaO–10B₂O₃ (mol%) composition was selected based on previous studies indicating that boron doping in MBGs reduces inflammatory response without compromising cell viability¹²³. Nanoparticles with this composition exhibited high bioactivity, effective hydroxyapatite formation in SBF, and anti-inflammatory effects, including significant downregulation of IL-1 β and IL-6 in macrophages¹²³. Moreover, the 10 mol% B₂O₃ content proved to be both effective and safe, supporting the application of B-MBGs in inflammatory environments, thus being promising for application in chronic wounds^{100,123,255}.

Thus, this study investigates the incorporation of B-MBGs into PCL/PPSG nanofibers to enhance their physical, chemical, and biological properties, focusing on applications in chronic wound healing. The combination of PPSG with PCL offers a balance of processability, biocompatibility, and tunable degradation, while boron doping is explored for its angiogenic potential. Additionally, acetic and formic acids are used as alternative solvents to minimize the toxicity of the electrospinning process. Thus, we evaluated how the addition of B-MBGs influences the structure and bioactivity of nanofibers developed in a novel PCL/PPSG polymer blend, aiming to develop bioactive scaffolds for soft tissue regeneration.

5.2 Materials And Methods

5.2.1 Synthesis of Boron-doped bioactive glass nanoparticles

Boron-doped mesoporous bioactive glass nanoparticles (B-MBGs) with nominal composition 50SiO₂–40CaO–10B₂O₃ were obtained through the microemulsion-assisted sol-gel technique described previously^{100,123,255}. Initially, 0.79 g of cetyltrimethylammonium bromide (CTAB; $\geq 98\%$, Merck, Germany) was dissolved in 36 mL MilliQ water at 400 rpm for 30 min at 37 °C until a clear solution was obtained.

Then, 11 mL of ethyl acetate ($\geq 99.8\%$, Merck, Germany) was slowly added to the system at room temperature, and the mixture was stirred for an additional 30 min to ensure proper emulsification. Subsequently, an ammonia solution (28%, VWR Chemicals, France) was prepared by mixing 0.85 mL of aqueous ammonia with 19 mL of MilliQ water, adjusting the pH to reach pH~9. This solution was then added dropwise to the reaction mixture, followed by the addition of 4.4 mL of tetraethyl orthosilicate (TEOS, 98%, Sigma-Aldrich, Germany). The reaction was maintained under stirring for 30 min before introducing the calcium and boron precursors.

For calcium incorporation, 3.67 g of calcium nitrate tetrahydrate (99.5%, VWR Chemicals, Belgium) was gradually added to the system, followed by the addition of 0.48 g of boric acid (H_3BO_3 , $\geq 99.5\%$, Sigma-Aldrich, Germany) for boron doping. The resulting solution was continuously stirred at 400 rpm for 4 hours to ensure homogeneous nanoparticle formation. The synthesized nanoparticles were collected through centrifugation at 7000 rpm for 5 min, washed twice with MilliQ water and once with ethanol to remove residual reagents, and then dried overnight at 60°C . Finally, the dried material was calcined at 600°C for 6 hours with a controlled heating rate of $2^\circ\text{C}/\text{min}$ to remove organic templates and stabilize the glass network. The final product was referred to as B-MBGNs.

The characterization of the resulting particles was carried out by nitrogen physisorption using the BET (Brunauer-Emmett-Teller) and BJH (Barrett-Joyner-Halenda) models to obtain the specific surface area (SSA) and the pore size distribution, being conducted using nitrogen sorption analyzer (ASAP2460, Micromeritics Instrument). In addition, with the Zetasizer Nano ZS (Malvern Instruments, United Kingdom), DLS's zeta potential and the average size of the nanoparticles (dynamic light scattering) were obtained by suspending the samples in deionized water.

5.2.2 Electrospinning process

The electrospinning solutions were prepared by dissolving PCL (Sigma Aldrich, 80 kDa) and PPSG (10 wt%) in a 20% (w/v) solvent mixture of formic acid and acetic acid (2:1, v/v). The solution was stirred at room temperature (300 rpm, 12 h) until complete dissolution. Subsequently, B-MBGNs were incorporated at concentrations of 0 wt%, 5 wt%, 10 wt%, and 15 wt% relative to the polymer mass. The resulting dispersions were sonicated for 15 min before electrospinning.

Electrospinning was carried out using the same equipment and basic setup described in Chapter 4 (EC-CLI, IME Technologies, Netherlands), with slight

modifications to accommodate the specific properties of the B-MBGN-loaded solutions. A 21G needle and a fixed distance of 11 cm between the needle and the collector were maintained. However, a nitrogen gas flow of 8 mL/min was introduced, and the process was conducted at 22 °C and 40% controlled humidity. The applied voltage was 18 kV at the needle tip, with an additional -2 kV applied to a rotating drum collector operating at 1000 rpm. Sample names reflect the incorporated B-MBGN content (e.g., PCL/PPSG/5B-MBGNs corresponds to 5 wt% of B-MBGNs).

5.2.3 Characterization

Scanning electron microscopy (SEM) was used to evaluate the morphology of the B-MBGNs, the fiber mats, and the dispersion of the B-MBGNs in fibers. These analyses were performed using Auriga SEM instrument (Zeiss, Oberkochen, Germany). Before analysis, the samples were coated with a thin layer of gold using a coater (Q150T, Quorum Technologies Ltd., Darmstadt, Germany). The diameter of 100 fibers was measured for each study group using Image-J software.

The presence of B-MBGNs in the fiber mats was evaluated using energy dispersive X-ray spectroscopy (EDX) coupled to the SEM using gold coating to avoid sample degradation. EDX spectra were obtained at different points and regions of the samples to ensure a representative elemental composition analysis.

FTIR (Fourier-transform infrared spectroscopy) was performed over the wavenumber range 4000–400 cm^{-1} (Shimadzu Irapinity-1S) using 30 spectrals to evaluate the presence of B-MBGNs in PCL/PPSG/B-MBGNs fiber mats.

To evaluate the formation of hydroxyapatite (HAp) on samples immersed in simulated body fluid (SBF), results were obtained using an X-ray diffractometer (XRD, Miniflex, 600, Rigaku) at 2θ 10–80°. A dwell time of 1 min/degree with a step size of 0.02° was applied.

The degree of hydrophilicity was evaluated by measuring the contact angle between a water droplet and the electrospun fiber surfaces that remained for 3s (DSA30, Kruss GmbH). Each sample was measured 5 times.

5.2.4 Mechanical Characterization

Tensile tests were performed using the same procedure described in Chapter 4. Rectangular samples were tested with a load of 50 N at a crosshead speed of 1 $\text{mm}\cdot\text{min}^{-1}$

using an Instron® 5960 system. For each group, ten replicates were analyzed to determine Young's modulus (MPa), ultimate tensile strength (MPa), and elongation at break (%).

5.2.5 Degradation tests

The hydrolytic degradation protocol followed the same methodology presented in Chapter 4. Briefly, 1 cm² fiber mats were immersed in PBS (Gibco™, Thermo Scientific, Germany) at 37 °C under agitation (90 rpm) for 40 days. Every 5 days, samples were removed, washed in ultrapure water, and dried in a fume hood until constant weight. Degradation was quantified by weight loss (Equation 5). Morphological and chemical changes were assessed via SEM and FTIR, respectively.

5.2.6 Hydroxyapatite (HAp) Formation In Vitro

The formation of hydroxyapatite and ion release from B-MBGNs was evaluated by immersing fiber mats (2 cm²) in 3 mL of simulated body fluid (SBF), prepared as per Kokubo's protocol ²⁰⁹. Samples were incubated at 37 °C and 90 rpm for 15 days. After incubation, samples were washed, dried, and characterized using SEM, XRD, and FTIR to assess mineral deposition and compositional changes ²⁰⁹.

5.2.7 In vitro Cell viability evaluation

Cell viability was assessed via direct contact using the WST-8 assay (CCK-8 kit, Sigma-Aldrich), following the same procedures described in Chapter 4. NHDF cells were seeded onto UV-sterilized fiber mats fixed in PCL inserts and cultured in supplemented DMEM. After 48 h of incubation at 37 °C in 5% CO₂, viability was measured at 450 nm absorbance. Cell viability (*Cv*) was determined according to equation (6), where *A_bSample* is the absorbance measured from the sample *A_bBlank* is the absorbance from wells without cells, and *A_bControl* corresponds to the absorbance detected from wells without samples but with cells only:

$$Cv(\%) = \left(\frac{A_b \text{ Sample} - A_b \text{ Blank}}{A_b \text{ Control} - A_b \text{ Blank}} \right) \times 100 \quad (6)$$

Cytoskeletal and nuclear morphology were assessed by fluorescence microscopy (DAPI and rhodamine-phalloidin staining), following the previously established protocol ⁹⁴.

5.2.8 Statistics

All data are expressed as mean $\pm$ standard deviation. Statistical tests were used in ORIGIN, applying one-way ANOVA for a p-value < 0.05 .

5.3 Results and Discussion

5.3.1 Characterization of B-MBGNs

The synthesized B-MBGNs were characterized before fibers' production. B-MBGNs were synthesized through the sol-gel process assisted by the microemulsion technique an effective technique for synthesizing mesoporous nanoparticles¹²³. Analyses of the B-MBGNs (Figure 23) revealed the formation of spherical and uniform particles, with a well-marked presence of Si and Ca. However, boron could not be detected due to its low emission energy and concentration in the sample.

Table 7 presents the average particle size (nm) measured by DLS, which is 245 nm. Figure 23a shows SEM images of B-MBGNs, where the predominance of spherical particles and a relatively narrow size distribution can be observed. The average particle diameter was slightly larger than the average size of B-containing MBGNs produced by Zheng et al., which was 194 nm¹²³. However, B-MBGNs in the present work were significantly smaller than BG particles produced by the conventional melt-quenching method, with approximately 3.15 μm of average size¹⁵.

Additionally, the adsorption results reveal that obtained B-MBGNs exhibited a surface area of 280 m^2g^{-1} , a pore volume of 0.40 cm^3g^{-1} , and an average pore diameter of 2.4 nm. These characteristics can be attributed to the role of ethyl acetate in the synthesis of B-MBGNs, which creates microemulsion droplets at the oil-in-water interface, favoring the formation of spherical particles. In addition, CTAB acts as a directing agent, promoting the formation of mesopores^{255,256}.

The XRD results presented in Figure 23(b) confirm the amorphous nature of B-MBGNs, evidenced by the lack of crystalline peak characteristic peak at 23°¹²⁹. The absence of diffraction peaks associated with boric acid crystals indicates a uniform integration of boron into the B-MBGNs. This result confirms that B-MBGNs can be synthesized via the microemulsion method, with boric acid serving as a suitable forerunner for boron incorporation into the silicate structure.

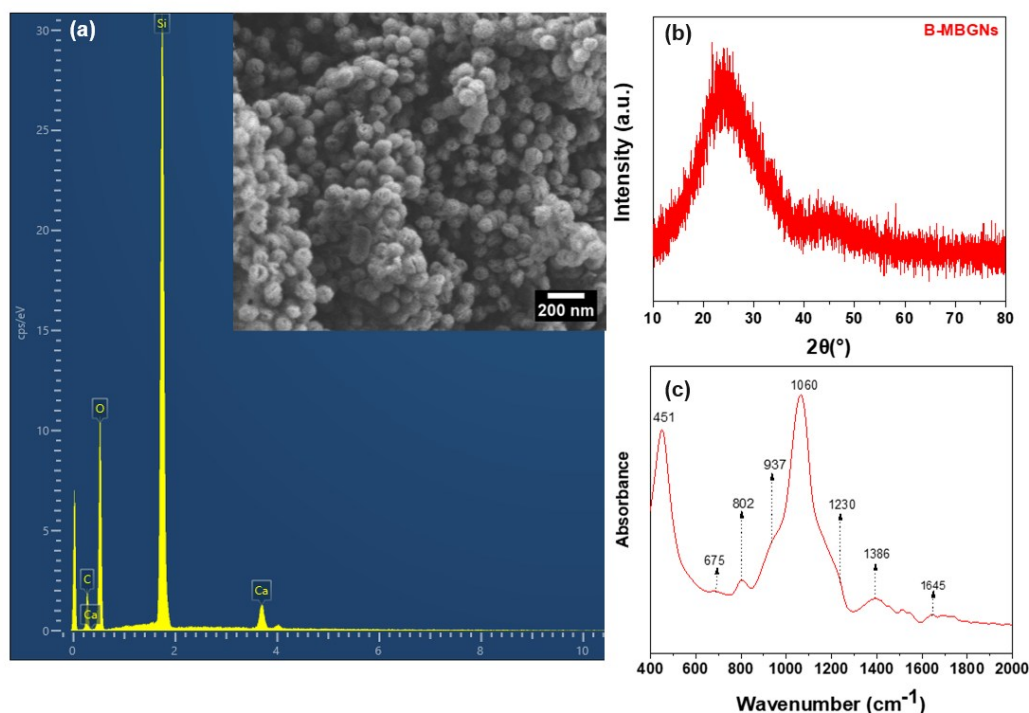


Figure 23. SEM image and corresponding EDX analysis of B-MBGNs (a), XRD pattern (b), and FTIR (c) spectra of the B-MBGNs. Reproduced from ²⁵⁷ licensed under CC BY 4.0.

FTIR results of nanoparticles can be seen in Figure 23(c), in which the resonances around 451 and 802 cm^{-1} refer to the symmetric bending and stretching vibrations of Si-O-Si ²⁵⁸. The vibration around 1060 cm^{-1} reflects the asymmetric stretching of Si-O-Si tetrahedra, while the shoulder observed at 1230 cm^{-1} refers to the stretching of SiO₄ ¹²⁹. The presence of molecular water induced the peak at 1645 cm^{-1} ¹²⁹, which reflects silica's presence in the nanoparticles' composition ²⁵⁹.

Table 7. Physical characterization of B-MBGNs. Reproduced from ²⁵⁷ licensed under CC BY 4.0.

BET Model	BJH Model	DLS
Surface Area	Cumulative pore	Particle Size
(m^2g^{-1})	volume (cm^3g^{-1})	(nm)
280	0.40	245± 13

B-MBGNs show a shoulder at 937 cm^{-1} attributed to the B-O stretching vibration of BO_4 units^{258,260}. Additionally, bands at 1386 and 675 cm^{-1} represent B-O-B vibrations of $[\text{BO}_3]$ units in stretching and B-O-Si bond, respectively^{261,262}. Thus, the FTIR spectrum confirms the presence of boron and the formation of bonds (B-O-Si)¹²³. The B_2O_3 present in silicate BG can cause the three-dimensional structure of Si-O-Si to break down and form tridentate chain triangles (BO_3). However, the FTIR results indicate that boron in B-MBGNs exists mainly as B-O-B bonds. At the same time, B-O-Si bonds are not present in high concentrations, possibly due to the instability of Si-O-B bonds^{129,261}.

5.3.2 Chemical characterization of PCL/PPSG/B-MBGNs electrospun mats

After preparation and characterization, the B-MBGNs were incorporated into PCL/PPSG solutions at concentrations of 5 wt%, 10 wt%, and 15 wt% of the polymer to produce electrospun fibers. After measuring the diameter of 100 fibers for each condition, the average diameter distribution is expressed in Figure 24.

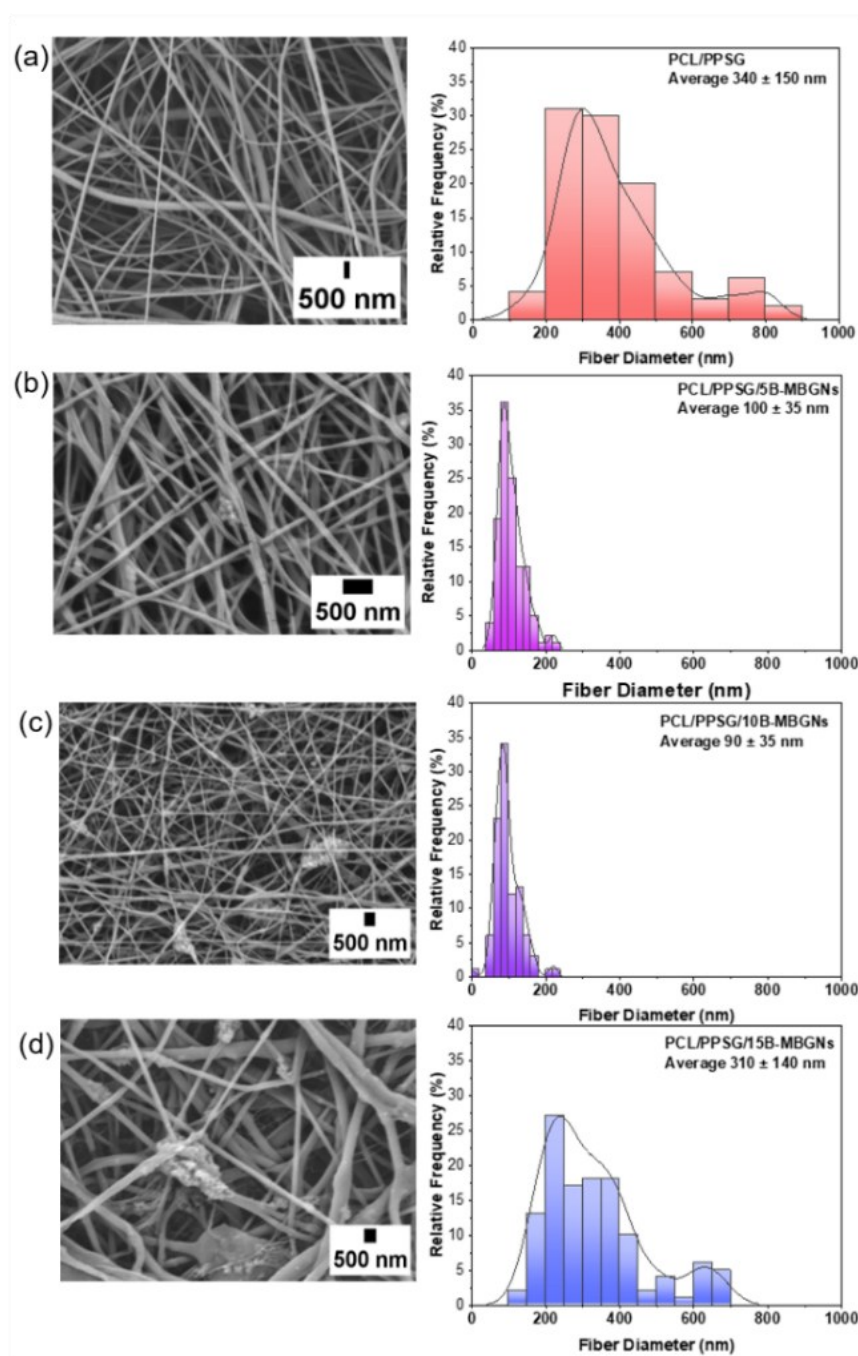


Figure 24. SEM micrographs of the electrospun samples and their respective distribution of average fiber diameter, without B-MBGNs (a) PCL/PPSG, and with 5 wt% (b) PCL/PPSG/5B-MBGNs; with 10 wt% (c) PCL/PPSG/10B-MBGNs; and 15 wt% (d) PCL/PPSG/15B-MBGNs. Reproduced from²⁵⁷ licensed under CC BY 4.0.

It was demonstrated that glass particles were more evenly distributed in the less viscous solutions created by combining the benign solvents formic acid and acetic acid (2:1)^{95,207}. Thus, the applied voltage of 20kV at a distance of 11cm proved to be suitable for the polymer solution with low viscosity. The concentration of B-MBGNs was varied

to investigate its influence on the bioactivity and physical properties of the electrospun mats (Figure 24a–d).

Figure 24 also shows that nanofiber structures were formed and that the B-MBGNs influenced the fiber morphology regarding average fiber diameter. The variation (decrease) in average fiber diameter (PCL/PPSG/5B-MBGNs and PCL/PPSG/10B-MBGNs compared to PCL/PPSG) is possibly due to the increase in conductivity caused by the addition of the nanoparticles^{263,264}. Increased conductivity increases the electrostatic force acting on the polymer jet, leading to greater elongation and smaller fiber diameters. Furthermore, although a moderate increase in conductivity generally reduces fiber diameter, excess nanoparticle loading can lead to jet instability, resulting in particle aggregation. This can cause the fiber diameter distribution to broaden, as observed when there is a significant increase in particle concentration in the solution (PCL/PPSG/15B-MBGNs).

Additionally, the BG nanoparticles should be evenly distributed throughout the electrospun mats, as this affects the scaffolds' bioactive and mechanical properties. To verify the presence of B-MBGNs, the elemental map obtained by EDS (Figure 25) shows the homogeneous presence of Si and Ca in all the PCL/PPSG/B-MBGNs polymer matrices.

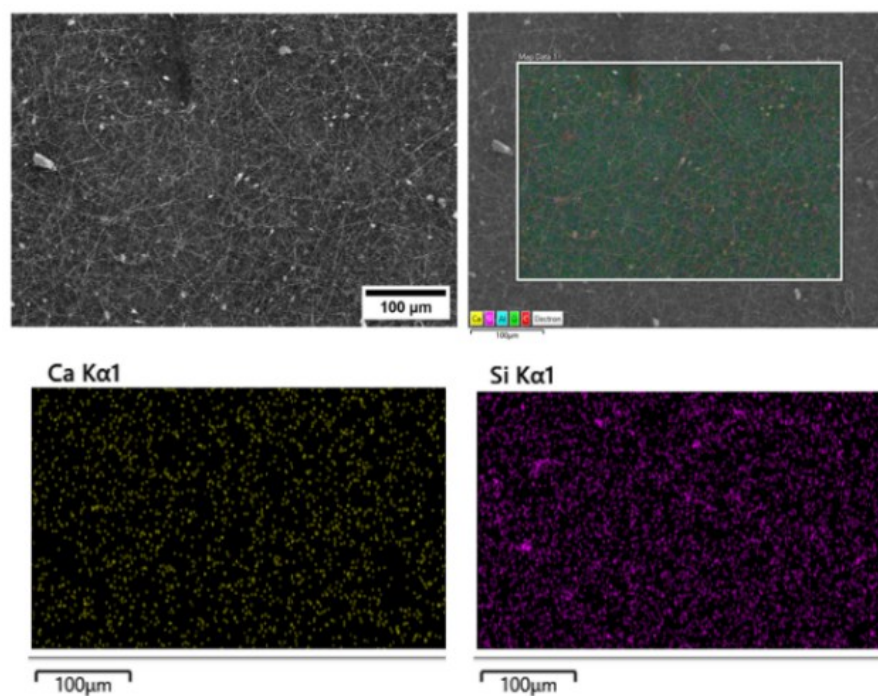


Figure 25. EDS mapping of electrospun nanocomposite scaffold PCL/PPSG/5B-MBGNs showing distribution of Ca and Si elements on the surface. Reproduced from²⁵⁷ licensed under CC BY 4.0.

The FTIR spectral analysis reveals the accentuation of characteristic bands that confirm the presence of the B-MBGNs (Figure 26). The observed bands for the PCL/PPSG polymer blend are consistent with the literature on aliphatic polyesters. The prominent identified bands include the wavelengths of 2937 cm^{-1} and 2860 cm^{-1} . These peaks are attributed to the asymmetric and symmetric stretching of the C–H bonds in the CH_2 ²⁶³. The peak at 1363 cm^{-1} corresponds to the symmetric deformation of the C–H group characteristic of aliphatic polyesters such as PCL and PSG^{20,201,221}. The carbonyl stretching (C=O) present in PCL/PPSG indicates the presence of the ester group in the band at 1726 cm^{-1} ²¹⁹. Similarly, the symmetric and asymmetric stretching of the C–O–C bonds in both PCL and PSG is identified at 1240 cm^{-1} and 1165 cm^{-1} ²²¹.

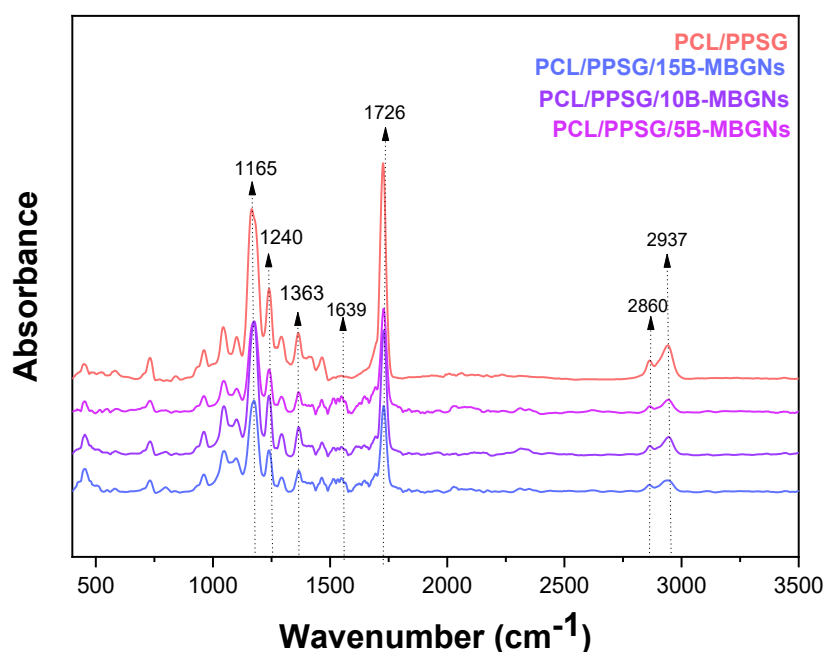


Figure 26. FTIR spectra of fiber mats in the 400 to 3500 cm^{-1} wavenumber range. The peaks are discussed in the text. . Reproduced from ²⁵⁷ licensed under CC BY 4.0.

In samples with B-MBGNs (PCL/PPSG/B-MBGNs), in addition to the aforementioned ones, bands with deformations indicating the presence of bioactive glass were found. The band at 1639 cm^{-1} can be attributed to the presence of adsorbed water, indicating the hygroscopic nature of the bioactive glass nanoparticles ^{265,266}. Similarly, the band's broadening at 450 cm^{-1} can be associated with the bending vibrations of the Si–O–Si bonds in BG, indicating the amorphous silicate structure of the material ²⁰. Conversely, the characteristic peak of the B–O stretching vibrations in BO_4 units, which could confirm the incorporation of boron into the glass structure at 930 cm^{-1} , is overlapped by the stretching vibrations of the C–C and C–O bonds of the PCL/PPSG polyesters.

5.3.3 Mechanical Properties

Tensile tests indicate that incorporating B-MBGNs into electrospun PCL/PPSG fibers significantly impacted their mechanical properties, which can be assigned to the distribution and interaction of BG nanoparticles with the polymer matrix.

The Young's modulus increased simultaneously with the concentration of B-MBGs, indicating, as expected, that the presence of nanoparticles exerts a stiffening effect. On the other hand, although the UTS (MPa) also increased initially (0.8 ± 0.1 MPa and 1.2 ± 0.1 MPa in samples PCL/PPSG, PCL/PPSG/5B-MBGN, respectively), there was a slight decrease with higher MBGNs concentrations (PCL/PPSG/10B-MBGs, PCL/PPSG/15B-MBGs) possibly due to nanoparticle agglomeration, which can create stress concentration points. The elongation at break consistently decreased with increasing B-MBGs concentration, reflecting the reduction in deformation capacity of the fibers before fracture (Table 8).

These results are in agreement with previous studies, where adding rigid particles to a polymer matrix generally results in a more rigid and resistant composite but reduces ductility. For example, Luginina et al.¹⁰⁰ reported an increase in Young's modulus from 3.8 MPa to 4.5 MPa when glass particles were added to the PCL/PGS polymeric blend. At the same time, elongation at break decreased (219% to 117%), in agreement with our findings. Similarly, Liverani et al.²⁶⁷ observed that BG particles also reduced the deformability of PCL fibers blended with chitosan (CS) (PCLCS/mBG) when compared to samples in the absence of particles, going from 37 MPa to 17 MPa.

Therefore, a B-MBGs concentration of around 10% may be ideal for applications that require a combination of stiffness and strength with some residual flexibility. Higher concentrations may be reserved for applications where stiffness is the main priority, but with the awareness that ductility will be significantly reduced.

Table 8. Mechanical properties of composite fibers with B-MBG. Reproduced from²⁵⁷ licensed under CC BY 4.0.

Sample	Young's Modulus (MPa)	UTS (MPa)	Elongation at Break (%)
PCL/PPSG	3.8 ± 0.3	0.8 ± 0.1	55.2 ± 1.9
PCL/PPSG /5B-MBGs	4.7 ± 0.3	1.2 ± 0.1	49.5 ± 4.3
PCL/PPSG /10B-MBGs	8.3 ± 0.5	1.1 ± 0.1	47.3 ± 7.1
PCL/PPSG /15B-MBGs	11 ± 1.2	1.0 ± 0.1	37.0 ± 6.3

5.3.4 Wettability and Degradation in PBS Solution

Hydrophilicity is a critical factor for materials applied in tissue regeneration since more hydrophilic scaffolds favor cell adhesion, protein adsorption, and cell morphology. In this analysis, it was observed that PPSG improved the hydrophilicity of the fiber, which caused the reduction of the contact angle from 117° for pure PCL fibers to around 26° for PCL/PPSG fibers, as can be observed in Figure 27. Previous studies have already reported that blends made with hydrophilic polymers (gelatin, PMMA, PGS, and collagen) can cause modulation of the hydrophobicity of the predominant polymer (PCL, PLA)^{176,268}. Furthermore, the presence of B-MBGNs did not induce a statistically significant difference in the hydrophilicity of the mats.

Despite the significant reduction in hydrophobicity, contact angle measurements did not show a clear trend with increasing nanoparticle concentration. This behavior is likely attributed to variations in surface roughness and the non-uniform distribution of B-MBGNs on the fiber surface, as evidenced by SEM and EDS analyses (Figures 24 and 25). Although no statistically significant trend was observed in the contact angle results, all conditions tested demonstrated a substantial increase in wettability compared to pure PCL fibers. This increase was sufficient to influence the degradation behavior of the electrospun mats in PBS, as reflected in the mass loss analysis (Figure 27a). The topographic interface generated by B-MBGNs is relevant for biodegradation²²⁴. Previous studies have reported that biomaterials undergo progressive changes in their properties over time. In the case of the PCL/PPSG polymer blend, hydrolysis of the ester bonds is expected to result in mass loss^{96,229}.

The mass loss of the fibers immersed in PBS (Figure 27a) points to the strong influence of the hydrophilicity of PPSG. PCL fibers presented a lower degradation rate, while the rapid degradation of PCL/PPSG/B-MBGNs fiber mats is more pronounced due to the hydrolytic breakdown of ester linkages in the material components²³¹. Microscopic analyses show increased fiber roughness, evidenced by pore and crack formation likely due to polymer and B-MBGNs leaching (Figure 27). For instance, the leaching of agglomerated inorganic particles in the mats led to the formation of tiny pores in the sample PCL/PPSG/15B-MBGNs.

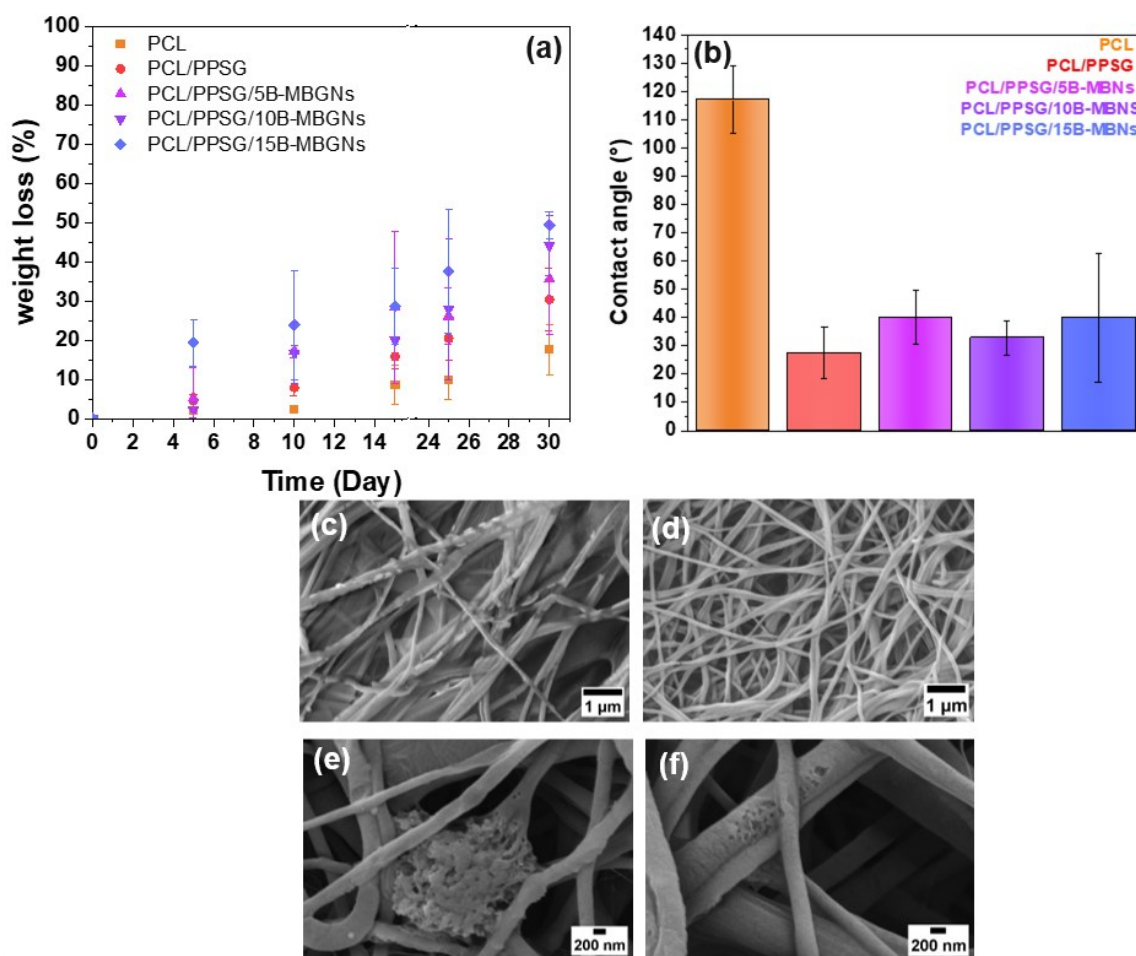


Figure 27. Weight loss during immersion of the fiber mats in PBS at 37 °C for 30 days (a), Contact angle of electrospun mats with varying amounts of B-MBG (b), and SEM microscopy image of samples after 30 days of immersion of the fiber mats PCL/PPSG (c); PCL/PPSG/5B-MBGNs (d); PCL/PPSG/10B-MBGNs (e); PCL/PPSG/15B-MBGNs (f). . Reproduced from ²⁵⁷ licensed under CC BY 4.0.

Additionally, weight loss measurements confirmed the accelerated degradation of PCL/PPSG/B-MBGNs blends compared to pure PCL/PPSG. Overall, the PCL/PPSG blends exhibited a mass loss of approximately 40% after 30 days, highlighting their rapid degradation compared to other natural polymer blends ¹⁴⁶. It is important to note that all samples showed high standard deviations in the results, indicating significant variability in the measurements. This variability can be attributed to intrinsic factors; thus, the results should be interpreted cautiously.

Moreover, the results indicate that PCL/PPSG/B-MBGNs fibers have potential for biomedical applications where controlled degradation and hydrophilicity are desirable. The rapid degradation and increased hydrophilicity can favor controlled drug release and cell interaction, making these fibers promising candidates for tissue

engineering and other biomedical devices. Therefore, the incorporation of B-MBGNs in PCL/PPSG fibers modulates both physical properties and degradation rates, offering a versatile platform for the development of advanced biomaterials.

5.3.5 In vitro Acellular Bioactivity

PCL/PPSG fibers containing B-MBGNs were characterized after 14 days of immersion in a simulated body fluid (SBF) solution. XRD analysis identified hydroxyapatite formation in all samples regardless of the B-MBGNs concentration (Figure 28), while FTIR analysis (Figure 29) revealed the presence of peaks characteristic of interactions between the components. SEM images (Figure 29) confirmed hydroxyapatite formation, corroborating the FTIR and XRD data.

The XRD patterns of PCL/PPSG/B-MBGNs fibers with different concentrations of B-MBGNs (5%, 10%, 15%) showed peaks at 2θ of 21.3° , 23.8° , 31.7° , and 32.2° , at the end of the immersion time (Figure 28). The peaks at 31.7° and 32.2° are characteristic of hydroxyapatite. They may be associated with different phases of calcium phosphate, including β -TCP (calcium triphosphate), indicating the formation of a bioactive phase in the B-MBGNs samples^{15,269}. These results confirm that the B-MBGNs produced are capable of inducing HAp formation.

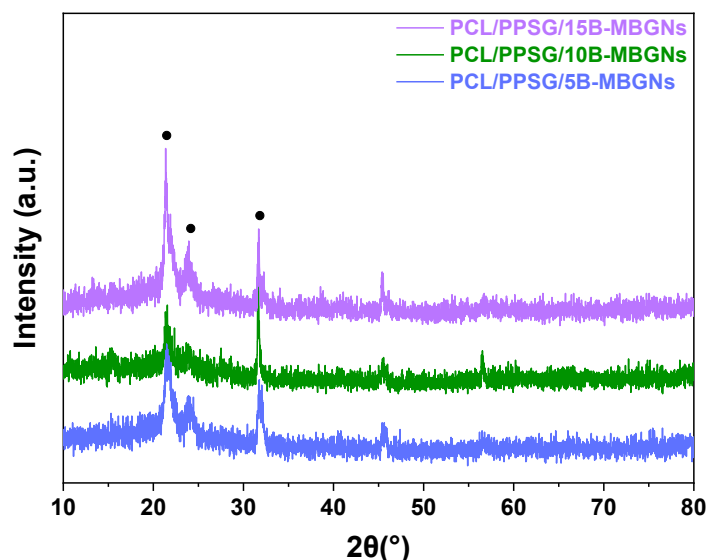


Figure 28. XRD spectra of B-MBGNs electrospun fibers incubated in SBF solution over 14 days. The peaks 21.4° and 23.8° indicate the crystal structure of PCL (●), and the peak 32.2° indicates the presence of hydroxyapatite (◆). Reproduced from²⁵⁷ licensed under CC BY 4.0.

Concerning the FTIR results of the PCL/PPSG/B-MBGNs samples, additional peaks were observed at 1639 cm^{-1} , 930 cm^{-1} , and 450 cm^{-1} after immersion in SBF. The peak at 930 cm^{-1} , attributed to the B-O stretching vibration, and the peak at 450 cm^{-1} , associated with Si-O-Si bending vibrations, indicate the incorporation of B-MBGNs into the polymeric matrix ¹²³. The decrease in the peak at 1726 cm^{-1} suggests interactions between the bioactive glass nanoparticles and the PCL/PPSG, possibly due to the formation of new chemical bonds.

SEM images confirmed the presence of HAp on the fiber surfaces after immersion in SBF (Figure 29). All PCL/PPSG/B-MBGNs samples showed crystalline structures typical of HAp, corroborating the XRD results. HAp formation was observed regardless of boron concentration in the bioactive glass, suggesting that even low B-MBGNs concentrations effectively promote bioactivity.

The results indicate that PCL/PPSG fibers doped with B-MBGNs can induce HAp formation after immersion in SBF, confirming the material's bioactivity. FTIR analysis showed that the characteristic peaks of PCL/PPSG were maintained, while new peaks related to B-MBGNs were identified, indicating interactions between the components. The formation of HAp was confirmed by XRD and SEM, demonstrating that adding B-MBGNs does not compromise its ability to form bioactive phases.

The additional peaks at 930 cm^{-1} and 450 cm^{-1} in the samples containing B-MBGNs suggest the effective integration of the nanoparticles into the PCL/PPSG matrix and the potential creation of new active sites for HAp nucleation ¹⁰⁰. The decrease in the peak at 1726 cm^{-1} can be attributed to the interaction between the PCL/PPSG and the bioactive glass particles, indicating a modification in the polymeric matrix that favors bioactivity ²²⁰. The formation of HA, confirmed by the peaks at 31.7° , and 32.2° , is a clear indication of Surface bioactivity provided by B-MBGs—^{269–271}. Thus, PCL/PPSG/B-MBGNs fibers are promising for bone tissue engineering, providing a matrix that supports HAp formation and possibly improves tissue regeneration.

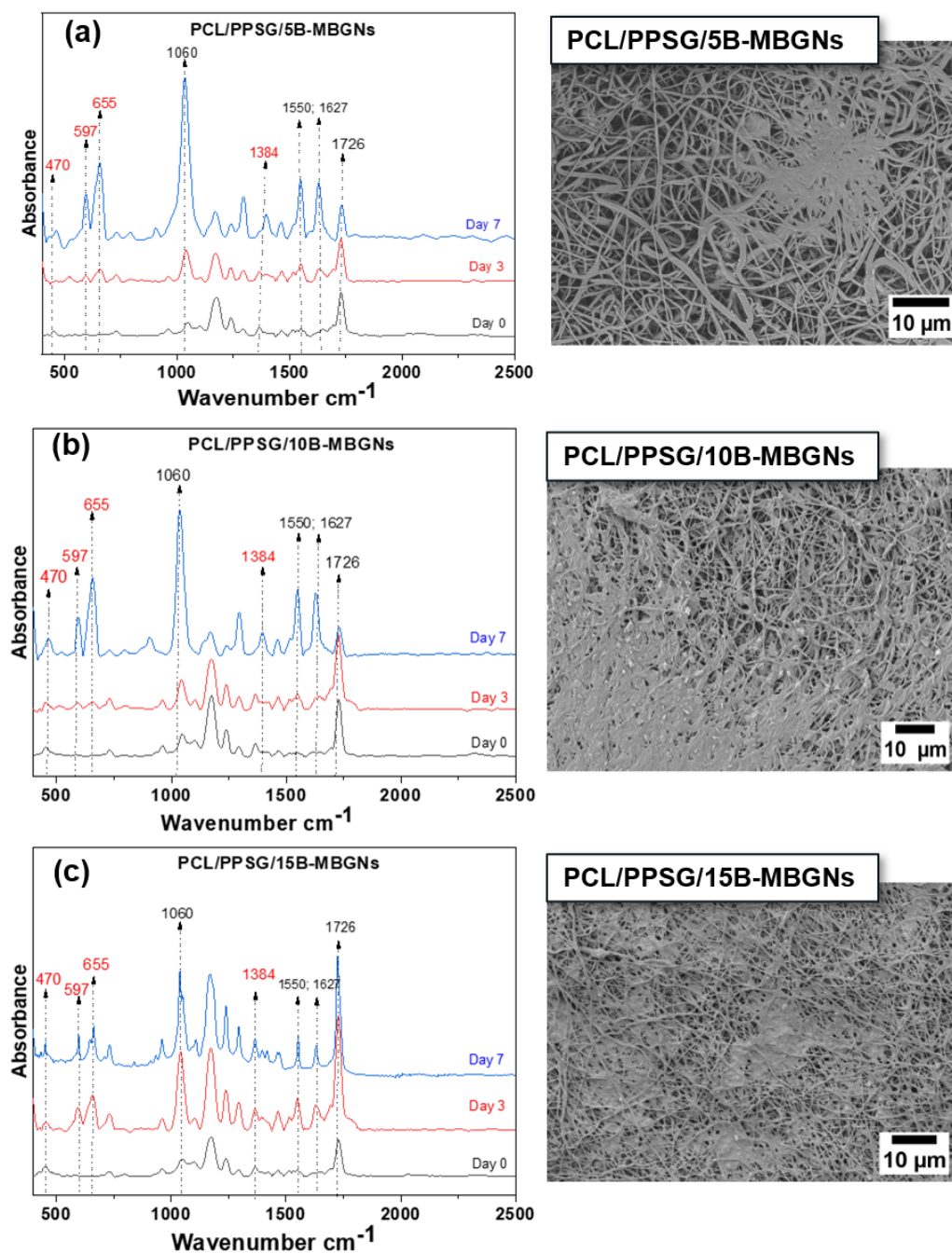


Figure 29. FTIR analysis of electrospun fiber mats containing B-MBGNs immersed in SBF solution over 7 days and their respective SEM images. Reproduced from²⁵⁷ licensed under CC BY 4.0.

5.3.6 Cell viability

Cell viability of PCL/PPSG/B-MBGNs electrospun scaffolds was evaluated *in vitro* for 7 days using NHDF fibroblast cells. WST-8 analysis demonstrated cell viability through metabolic activity. Figure 30 shows cell proliferation in all samples, indicating that the material is not apparent cytotoxic. Although the samples with low concentrations

of B-MBGNs (PCL/PPSG/5B-MBGNs and PCL/PPSG/10B-MBGNs) did not present a statistically significant difference between them, it was observed that all fibers composed of PCL/PPSG/B-MBGNs had greater cell proliferation than PCL fibers

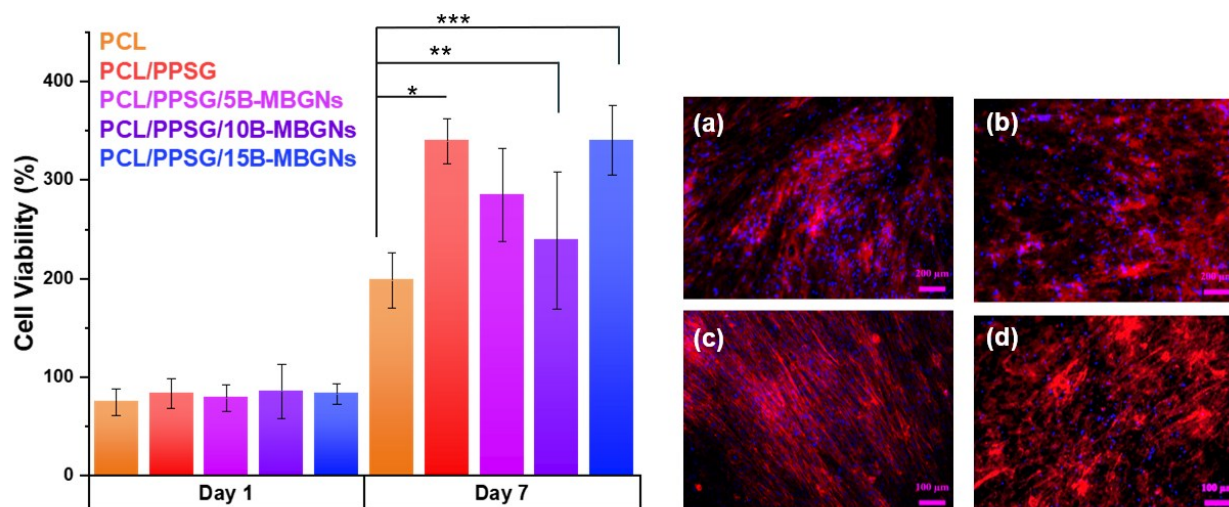


Figure 30. Metabolic activity measured by WST-8 analysis at 450 nm for all seedings (* $p < 0.05$). Fluorescence images of fibroblast cells on fibers after 1 day. PCL/PPSG/5B-MBGNs (a); PCL/PPSG/10B-MBGNs (b); PCL/PPSG/15B-MBGNs (c); PCL/PPSG (d). . Reproduced from²⁵⁷ licensed under CC BY 4.0.

The results are consistent with data reported by Ege et al.²⁷² who observed that high concentration of bioactive glass doped with Boron interferes with the viability of NHDF cells. Under these findings, other studies have observed a recovery in cell viability over time^{124,213}, which aligns with trends observed in our PCL/PPSG/B-MBGNs, particularly at 15% concentration. This behavior is further supported by findings from Zheng et al.¹²³, who showed no reduction in ST2 cell viability at B-MBGN concentrations up to 10 mg/mL using indirect WST assays. Likewise, Luginina et al.¹⁰⁰ observed no cytotoxicity in electrospun mats with 30 wt% borosilicate bioactive glass particles using direct contact assays. Considering that the maximum concentration used in our scaffolds was 15 wt%, the data suggest that despite leaching during degradation tests, the NPs do not present any cytotoxic effect and our system operates within a biocompatibility range

Fluorescent images support the metabolic assays obtained with WST-8. The cell nucleus is pigmented blue (DAPI), and the actin filaments are stained red (phalloidin) (Figure 30). All samples presented cells with well-spread morphology and a robust cytoskeletal organization, indicating an environment conducive to long-term cell proliferation. Release of ions should be investigated in future work by ICP (Inductively

Coupled Plasma), in order to establish a correlation between B-MBGNs concentration and fiber surface area with cell viability. The preliminary results presented in this work confirm that B-MBGNs are suitable for bioactive PCL/PPSG fiber.

5.4 Conclusions

The study showed the development of boron-doped mesoporous bioactive glass nanoparticles (B-MBGNs) incorporated into PCL/PPSG fibers. PCL/PPSG fibers showed a high rate of degradability, hydrophilicity, and biocompatibility. With the incorporation of 5 wt% and 10 wt% by weight of B-MBGNs in the PCL/PPSG solution, the average diameter of the fibers was significantly reduced, probably due to the effect of glass nanoparticles on the conductivity of the polymeric solution. Low concentrations of B-MBGNs did not interfere with the mechanical properties of the fibers. Furthermore, B-MBGNs did not show cytotoxicity on fibroblasts. Therefore, B-MBGNs combined with PCL/PPSG nanofibers offer a combination of bioactivity and promising mechanical properties. For future work, fiber alignment should be explored to enhance fiber stretching, which could influence cellular responses and mechanical performance. Additionally, boron release studies should be conducted to better understand the behavior of the scaffolds. Characterization using inductively coupled plasma optical emission spectrometry (ICP-OES) could provide quantitative insights into boron ion release, contributing to a more comprehensive evaluation of the bioactivity of the fibers. Moreover, the direct measurement of ionic conductivity should be considered to better correlate nanoparticle concentration with fiber morphology and jet behavior during electrospinning. This would provide a more quantitative understanding of the role of B-MBGNs in modifying solution properties.

Chapter 6

General Conclusions And Suggestions For Future Work

6.1 General Conclusions

This research project investigated the development of an enzymatic polycondensation route for aliphatic polyesters aimed at tissue engineering applications, focusing on the development and characterization of nanofiber mats incorporating bioactive glass (BG 45S5) and boron-doped mesoporous bioactive glass nanoparticles (B-MBGs). These materials hold potential for applications in wound healing and bone regeneration. In this thesis, the use of the green polyester PPSG was explored as an additive to enhance the properties of PCL nanofibers. Furthermore, PCL/PPSG scaffolds loaded with 45S5 BG and B-MBGs were examined for their suitability in tissue engineering and the treatment of chronic wounds. The general conclusions of this study, along with future research perspectives, are discussed in this chapter, encompassing the synthesis of PPSG as well as the scaffolds developed by electrospinning incorporating BG and B-MBGs.

Synthesis of Poly(Propylene Succinate-co-Glycerol Succinate) (PPSG)

Candida antarctica lipase B (CALB) was evaluated as a biocatalyst in solvent-free polycondensation. The enzyme proved to be an efficient and sustainable alternative for synthesizing functionalized aliphatic polyesters, using renewable monomers such as diethyl succinate (DS), 1,3-propanediol (PDO), and glycerol (GLY). The reaction under mild, solvent-free conditions resulted in the hydrofunctionalization of the polyester with controlled chemical and thermal properties, making it suitable for biomedical applications. PPSG copolymers were obtained with different stoichiometric fractions, and undesirable branching was controlled. The addition of glycerol increased the hydrophilicity of the polyesters, as indicated by contact angle data. However, an excess of glycerol induced crosslinking of the polymer chain. It was observed that the molar mass of the copolymers could be controlled by the reaction time, but high viscosity led to diffusion limitations that hindered mass transfer between oligomers and the catalytic site, as well as the removal of by-products and enzyme recycling.

Application of PPSG as a Green Additive in Electrospun PCL Fibers Enriched with 45S5 BG

The incorporation of PPSG into PCL fibers added with 45S5 BG particles resulted in scaffolds with enhanced properties, such as improved wettability, biodegradability, and elasticity. Bioactivity assays confirmed the formation of hydroxyapatite in SBF, demonstrating the potential of these fibers for tissue engineering applications. Homogeneous, bead-free fibers were obtained with an average diameter of $1.8 \pm 0.2 \mu\text{m}$ based on the adjustment of PCL/PPSG concentration and electrospinning parameters. The incorporation of 45S5 bioactive glass particles did not affect the homogeneity of the electrospun fibers. The study showed that PPSG improved the mechanical properties of the fibers, while the addition of bioactive glass particles created stress points that reduced Elongation at Break (%). The presence of PPSG also significantly contributed to the fibers' hydrophilicity and rapid biodegradability. Cell viability studies demonstrated that the polymer combination did not cause apparent toxicity, and ion dissociation from the bioactive glass supported cell growth.

Incorporation of Boron-Doped Mesoporous Bioactive Glass Nanoparticles (B-MBGs)

In Chapter 5, boron-doped mesoporous bioactive glass nanoparticles (B-MBGs) were successfully developed and incorporated into PCL/PPSG fibers, enhancing their properties for tissue engineering applications. The incorporation of B-MBGs at concentrations of 5 wt% and 10 wt% significantly reduced the average diameter of the electrospun fibers. This reduction was likely due to the effect of the glass nanoparticles on the conductivity of the polymer solution, which was processed with formic acid and acetic acid (2:1 v/v). Analyses indicated that low concentrations of B-MBGs did not interfere with the mechanical properties of the fibers and showed no cytotoxicity to fibroblast cells (NHDF). This suggests that B-MBGs incorporated in PCL/PPSG nanofibers provide a promising combination of bioactivity and mechanical strength for applications in chronic wound healing.

6.2 Suggestions For Future Work

A promising direction for future studies would be to explore the synthesis of functionalized unsaturated aliphatic polyesters via enzymatic polycondensation, using

bio-based monomers such as dimethyl itaconate (DMI) and diethyl maleate (DEM). These monomers exhibit unique characteristics, such as non-toxicity, biocompatibility, and biodegradability, making them highly attractive for biomedical applications. Furthermore, the unsaturated bonds of these monomers allow for additional functionalization reactions, such as chemical modification through UV-activated thiol-ene reactions, which expand the possibilities for material customization. In this context, thiol-ene reactions enable the functionalization of unsaturated copolymers with biofunctional molecules, such as antimicrobial peptides (AMPs), or their use in nanocarriers for controlled drug delivery systems. This approach not only optimizes biocompatibility but also enhances interaction with specific cellular receptors, further increasing the effectiveness of these materials for biomedical applications.

Complementing this perspective, another relevant proposal would be to investigate the impact of electrospun fiber alignment in hybrid scaffolds of PCL/PPSG (and novel unsaturated polyesters) containing boron-doped bioactive glass nanoparticles (B-MBGs). This study, focusing on both mechanical properties and the controlled release of boron ions, has the potential to significantly enhance scaffold performance. Fiber alignment can be achieved using high-speed rotating collectors during the electrospinning process, resulting in scaffolds with improved structural uniformity and optimized mechanical properties. This would allow for the evaluation of how fiber alignment influences aspects such as elasticity, tensile strength, and structural support, particularly for applications targeting soft tissues that require high flexibility and mechanical adaptation.

Moreover, investigating the kinetics of boron ion release under physiological conditions becomes essential. High-precision techniques, such as inductively coupled plasma optical emission spectrometry (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS), can be employed for this purpose. These analyses are crucial to providing detailed insights into the ion release rate and its relationship with fiber alignment and nanoparticle organization within the scaffold. Complementary biological studies should also be conducted to evaluate the effects of released boron ions on cell viability, proliferation, adhesion, and the formation of angiogenic structures. Such investigations will contribute to a better understanding of the angiogenic and osteogenic potential of these scaffolds.

The expected implications of studying PCL/PPSG fiber alignment and boron ion release include the possibility of designing biomaterials with tailored mechanical properties and controlled bioactive release to meet diverse clinical demands. Combining fiber alignment with precise boron ion release could not only enhance the structural functionality of the scaffolds but also increase their biological efficiency, promoting tissue regeneration in complex applications such as soft tissue repair and chronic wound healing. These results could open new perspectives in the design of advanced scaffolds and in understanding the role of boron ions in regenerative processes.

It can be concluded that the approach presented in this thesis addresses important gaps in the literature by proposing the replacement of traditional routes, based on organometallic catalysts and high temperatures, with sustainable and efficient enzymatic methods. Additionally, the functionalization of polyesters with bioactive molecules and inorganic, bioactive nanoparticles broadens the application potential of these materials in advanced biomedical devices, particularly in drug delivery systems and regenerative therapies

References

- (1) Jiang, Y.; Loos, K. Enzymatic Synthesis of Biobased Polyesters and Polyamides. *Polymers* **2016**, 8 (7). <https://doi.org/10.3390/polym8070243>.
- (2) Fernandes, C. D.; Francisco Oechsler, B.; Sayer, C.; Oliveira, D. de; Araújo, P. H. de. Recent Advances and Challenges on Enzymatic Synthesis of Biobased Polyesters via Polycondensation. *European Polymer Journal* **2022**, 169, 111132. <https://doi.org/10.1016/j.eurpolymj.2022.111132>.
- (3) Malikmammadov, E.; Tanir, T. E.; Kiziltay, A.; Hasirci, V.; Hasirci, N. PCL and PCL-Based Materials in Biomedical Applications. *Journal of Biomaterials Science, Polymer Edition* **2018**, 29 (7–9), 863–893. <https://doi.org/10.1080/09205063.2017.1394711>.
- (4) Gigli, M.; Fabbri, M.; Lotti, N.; Gamberini, R.; Rimini, B.; Munari, A. Poly(Butylene Succinate)-Based Polyesters for Biomedical Applications: A Review. *European Polymer Journal* **2016**, 75, 431–460. <https://doi.org/10.1016/J.EURPOLYMJ.2016.01.016>.
- (5) Gross, R. A.; Ganesh, M.; Lu, W. Enzyme-Catalysis Breathes New Life into Polyester Condensation Polymerizations. *Trends in Biotechnology*. Elsevier Current Trends August 1, 2010, pp 435–443. <https://doi.org/10.1016/j.tibtech.2010.05.004>.
- (6) Yang, X.; Li, D.; Song, C.; Shao, P.; Wang, S.; Wang, Z.; Lv, Y.; Wei, Z. Syntheses of High Molecular Weight Hydroxy Functional Copolymers by Green and Selective Polycondensation Methods. *RSC Advances* **2020**, 10 (11), 6414–6422. <https://doi.org/10.1039/d0ra00120a>.
- (7) Dourado Fernandes, C.; Oechsler, B. F.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H. Enzymatic Synthesis of Hydroxy-Functionalized Glycerol-Based Polyesters: A Study of Functionalization, Regioselectivity Control, and Catalyst Reuse. *Macromolecules* **2023**. https://doi.org/10.1021/ACS.MACROMOL.3C01533/SUPPL_FILE/MA3C01533_SI_001.PDF.
- (8) Elias Guckert, F.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H.; Francisco Oechsler, B. Synthesis of Polybutylene Succinate via Lipase-Catalyzed Transesterification: Enzyme Stability, Reuse and PBS Properties in Bulk

- Polycondensations. *European Polymer Journal* **2022**, *179* (July). <https://doi.org/10.1016/j.eurpolymj.2022.111573>.
- (9) Pellis, A.; Comerford, J. W.; Maneffa, A. J.; Sipponen, M. H.; Clark, J. H.; Farmer, T. J. Elucidating Enzymatic Polymerisations: Chain-Length Selectivity of Candida Antarctica Lipase B towards Various Aliphatic Diols and Dicarboxylic Acid Diesters. *European Polymer Journal* **2018**, *106* (July), 79–84. <https://doi.org/10.1016/j.eurpolymj.2018.07.009>.
 - (10) Jones, J. R. Review of Bioactive Glass: From Hench to Hybrids. *Acta Biomater* **2013**, *9* (1), 4457–4486. <https://doi.org/10.1016/J.ACTBIO.2012.08.023>.
 - (11) Zahir, A.; Mahmood, U.; Nazir, A.; Hussain, T.; Abid, S. Biomaterials for Medical and Healthcare Products. *Medical Textiles from Natural Resources* **2022**, 43–86. <https://doi.org/10.1016/B978-0-323-90479-7.00013-0>.
 - (12) Luginina, M.; Schuhladen, K.; Orrú, R.; Cao, G.; Boccaccini, A. R.; Liverani, L. Electrospun PCL/PGS Composite Fibers Incorporating Bioactive Glass Particles for Soft Tissue Engineering Applications. *Nanomaterials* **2020**, *Vol. 10*, Page 978 **2020**, *10* (5), 978. <https://doi.org/10.3390/NANO10050978>.
 - (13) Elias Guckert, F.; Cesca, K.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H.; Francisco Oechsler, B. Lipase-Catalyzed Solution Polycondensation of 1,4-Butanediol and Diethyl Succinate: Effect of Diphenyl Ether Concentration on Enzyme Stability, Reuse and PBS Molar Mass Distribution. *European Polymer Journal* **2023**, *187*, 111926. <https://doi.org/10.1016/J.EURPOLYMJ.2023.111926>.
 - (14) Hench, L. L. The Story of Bioglass®. *J Mater Sci Mater Med* **2006**, *17* (11), 967–978. <https://doi.org/10.1007/s10856-006-0432-z>.
 - (15) Ege, D.; Pourshahrestani, S.; Iorio, F.; Reinfelder, H.; de Ligny, D.; Boccaccini, A. R. Processing and Characterization of Aligned Electrospun Gelatin/Polycaprolactone Nanofiber Mats Incorporating Borate Glass (13-93B3) Microparticles. *Biomedical Materials* **2023**, *18* (5), 055030. <https://doi.org/10.1088/1748-605X/ACF0AD>.
 - (16) Zheng, K. Synthesis and Modification of Bioactive Glass Nanoparticles for Biomedical Applications Synthese Und Modifizierung von Bioaktiven Glasnanopartikeln Für Biomedizinische Anwendungen Vorgelegt Von. **2017**, 6–8.

- (17) Zheng, K.; Niu, W.; Lei, B.; Boccaccini, A. R. Immunomodulatory Bioactive Glasses for Tissue Regeneration. *Acta Biomaterialia* **2021**, *133*, 168–186. <https://doi.org/10.1016/J.ACTBIO.2021.08.023>.
- (18) Pantulap, U.; Arango-Ospina, M.; Boccaccini, A. R. Bioactive Glasses Incorporating Less-Common Ions to Improve Biological and Physical Properties. *Journal of materials science. Materials in medicine* **2021**, *33* (1). <https://doi.org/10.1007/S10856-021-06626-3>.
- (19) Rai, R.; Tallawi, M.; Grigore, A.; Boccaccini, A. R. Synthesis, Properties and Biomedical Applications of Poly(Glycerol Sebacate) (PGS): A Review. *Progress in Polymer Science* **2012**, *37* (8), 1051–1078. <https://doi.org/10.1016/J.PROGPOLYMSCI.2012.02.001>.
- (20) Masoumi, N.; Larson, B. L.; Annabi, N.; Kharaziha, M.; Zamanian, B.; Shapero, K. S.; Cubberley, A. T.; Camci-Unal, G.; Manning, K. B.; Mayer, J. E.; Khademhosseini, A. Electrospun PGS: PCL Microfibers Align Human Valvular Interstitial Cells and Provide Tunable Scaffold Anisotropy. *Advanced Healthcare Materials* **2014**, *3* (6), 929–939. <https://doi.org/10.1002/adhm.201300505>.
- (21) Sant, S.; Hwang, C. M.; Lee, S.; Khademhosseini, A. Hybrid PGS – PCL Microfibrous Scaffolds with Improved Mechanical and Biological Properties. **2011**, No. July 2010, 283–291. <https://doi.org/10.1002/term>.
- (22) Uyama, H.; Wada, S.; Kobayashi, S. Synthesis of Aliphatic Polyester by Insertion of Glycols into Polyanhydrides Using Lipase Catalyst. *Chemistry Letters*. 1999, pp 893–894. <https://doi.org/10.1246/cl.1999.893>.
- (23) Zeng, F.; Yang, X.; Li, D.; Dai, L.; Zhang, X.; Lv, Y.; Wei, Z. Functionalized Polyesters Derived from Glycerol: Selective Polycondensation Methods toward Glycerol-Based Polyesters by Different Catalysts. *Journal of Applied Polymer Science* **2020**, *137* (16), 48574. <https://doi.org/10.1002/APP.48574>.
- (24) Pellis, A.; Weinberger, S.; Gigli, M.; Guebitz, G. M.; Farmer, T. J. Enzymatic Synthesis of Biobased Polyesters Utilizing Aromatic Diols as the Rigid Component. *European Polymer Journal* **2020**, *130*, 109680. <https://doi.org/10.1016/j.eurpolymj.2020.109680>.
- (25) Rostamian, M.; Kalaei, M. R.; Dehkordi, S. R.; Panahi-Sarmad, M.; Tirgar, M.; Goodarzi, V. Design and Characterization of Poly(Glycerol-Sebacate)-Co-Poly(Caprolactone) (PGS-Co-PCL) and Its Nanocomposites as Novel Biomaterials: The Promising Candidate for Soft Tissue Engineering. *European* **133**

- Polymer Journal* **2020**, *138*, 109985.
<https://doi.org/10.1016/J.EURPOLYMJ.2020.109985>.
- (26) Markiton, M.; Boncel, S.; Janas, D.; Chrobok, A. Highly Active Nanobiocatalyst from Lipase Noncovalently Immobilized on Multiwalled Carbon Nanotubes for Baeyer-Villiger Synthesis of Lactones. *ACS Sustainable Chemistry and Engineering* **2017**, *5* (2), 1685–1691.
<https://doi.org/10.1021/acssuschemeng.6b02433>.
- (27) Gupta, B.; Papke, J. B.; Mohammadkhah, A.; Day, D. E.; Harkins, A. B. Effects of Chemically Doped Bioactive Borate Glass on Neuron Regrowth and Regeneration. *Annals of Biomedical Engineering* **2016**, *44* (12), 3468–3477.
<https://doi.org/10.1007/s10439-016-1689-0>.
- (28) Vogt, L.; Rivera, L. R.; Liverani, L.; Piegat, A.; El Fray, M.; Boccaccini, A. R. Poly(ϵ -Caprolactone)/Poly(Glycerol Sebacate) Electrospun Scaffolds for Cardiac Tissue Engineering Using Benign Solvents. *Materials Science and Engineering C* **2019**, *103* (April), 109712. <https://doi.org/10.1016/j.msec.2019.04.091>.
- (29) Guo, B.; Chen, Y.; Lei, Y.; Zhang, L.; Zhou, W. Y.; Rabie, A. B. M.; Zhao, J. Biobased Poly(Propylene Sebacate) as Shape Memory Polymer with Tunable Switching Temperature for Potential Biomedical Applications. *Biomacromolecules* **2011**, *12* (4), 1312–1321.
https://doi.org/10.1021/BM2000378/SUPPL_FILE/BM2000378_SI_001.PDF.
- (30) Lu, H. H.; Ege, D.; Salehi, S.; Boccaccini, A. R. Ionic Medicine: Exploiting Metallic Ions to Stimulate Skeletal Muscle Tissue Regeneration. *Acta Biomaterialia* **2024**, No. June. <https://doi.org/10.1016/j.actbio.2024.10.033>.
- (31) Yildirim, E. D.; Ayan, H.; Vasilets, V. N.; Fridman, A.; Gucer, S.; Sun, W. Effect of Dielectric Barrier Discharge Plasma on the Attachment and Proliferation of Osteoblasts Cultured over Poly(ϵ -Caprolactone) Scaffolds. *Plasma Processes and Polymers* **2008**, *5* (1), 58–66. <https://doi.org/10.1002/PPAP.200700041>.
- (32) Ghassemi, A. H.; van Steenberg, M. J.; Talsma, H.; van Nostrum, C. F.; Jiskoot, W.; Crommelin, D. J. A.; Hennink, W. E. Preparation and Characterization of Protein Loaded Microspheres Based on a Hydroxylated Aliphatic Polyester, Poly(Lactic-Co-Hydroxymethyl Glycolic Acid). *Journal of Controlled Release* **2009**, *138* (1), 57–63. <https://doi.org/10.1016/J.JCONREL.2009.04.025>.
- (33) Webb, K.; Hlady, V.; Tresco, P. A. Relative Importance of Surface Wettability and Charged Functional Groups on NIH 3T3 Fibroblast Attachment, Spreading, and

- Cytoskeletal Organization. **1998**. [https://doi.org/10.1002/\(SICI\)1097-4636\(19980905\)41:3](https://doi.org/10.1002/(SICI)1097-4636(19980905)41:3).
- (34) Van Dijk, M.; Rijkers, D. T. S.; Liskamp, R. M. J.; Van Nostrum, C. F.; Hennink, W. E. Synthesis and Applications of Biomedical and Pharmaceutical Polymers via Click Chemistry Methodologies. *Bioconjugate Chemistry* **2009**, *20* (11), 2001–2016. https://doi.org/10.1021/BC900087A/ASSET/IMAGES/LARGE/BC-2009-00087A_0001.JPEG.
- (35) Brown, A. H.; Sheares, V. V. Amorphous Unsaturated Aliphatic Polyesters Derived from Dicarboxylic Monomers Synthesized by Diels-Alder Chemistry. *Macromolecules* **2007**, *40* (14), 4848–4853. https://doi.org/10.1021/MA070185V/SUPPL_FILE/MA070185VSI20070510_045704.PDF.
- (36) Seyednejad, H.; Ghassemi, A. H.; Van Nostrum, C. F.; Vermonden, T.; Hennink, W. E. Functional Aliphatic Polyesters for Biomedical and Pharmaceutical Applications. *Journal of Controlled Release* **2011**, *152* (1), 168–176. <https://doi.org/10.1016/J.JCONREL.2010.12.016>.
- (37) Zhao, H.; Toe, C. “Water-like” Ammonium-Based Ionic Liquids for Lipase Activation and Enzymatic Polymerization. *Process Biochemistry* **2020**, *98*, 59–64. <https://doi.org/10.1016/J.PROCBIO.2020.07.016>.
- (38) Zhao, H. Enzymatic Ring-Opening Polymerization (ROP) of Polylactones: Roles of Non-Aqueous Solvents. *Journal of Chemical Technology and Biotechnology*. John Wiley and Sons Ltd January 1, 2018, pp 9–19. <https://doi.org/10.1002/jctb.5444>.
- (39) Bonduelle, C.; Martin-Vaca, B.; Bourissou, D. Lipase-Catalyzed Ring-Opening Polymerization of the O-Carboxylic Anhydride Derived from Lactic Acid. *Biomacromolecules* **2009**, *10* (11), 3069–3073. <https://doi.org/10.1021/bm9007343>.
- (40) Prasad, A.; Kandasubramanian, B. Fused Deposition Processing Polycaprolactone of Composites for Biomedical Applications. *Polymer-Plastics Technology and Materials* **2019**, *58* (13), 1365–1398. <https://doi.org/10.1080/25740881.2018.1563117>.
- (41) Yang, X.; Li, D.; Song, C.; Shao, P.; Wang, S.; Wang, Z.; Lv, Y.; Wei, Z. Syntheses of High Molecular Weight Hydroxy Functional Copolymers by Green

- and Selective Polycondensation Methods. *RSC Advances* **2020**, *10* (11), 6414–6422. <https://doi.org/10.1039/d0ra00120a>.
- (42) Pang, K.; Kotek, R.; Tonelli, A. Review of Conventional and Novel Polymerization Processes for Polyesters. *Progress in Polymer Science (Oxford)*. Pergamon November 1, 2006, pp 1009–1037. <https://doi.org/10.1016/j.progpolymsci.2006.08.008>.
- (43) Dourado Fernandes, C.; Francisco Oechsler, B.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H. Recent Advances and Challenges on Enzymatic Synthesis of Biobased Polyesters via Polycondensation. *Eur Polym J* **2022**, *169*, 111132. <https://doi.org/10.1016/J.EURPOLYMJ.2022.111132>.
- (44) Ning, Z.; Lang, K.; Xia, K.; Linhardt, R. J.; Gross, R. A. Lipase-Catalyzed Synthesis and Characterization of Poly(Glycerol Sebacate). *Biomacromolecules* **2022**, *23* (1), 398–408. <https://doi.org/10.1021/ACS.BIOMAC.1C01351>.
- (45) Chongcharoenchaikul, T.; Thamyongkit, P.; Poompradub, S. Synthesis, Characterization and Properties of a Bio-Based Poly(Glycerol Azelate) Polyester. *Materials Chemistry and Physics* **2016**, *177*, 485–495. <https://doi.org/10.1016/j.matchemphys.2016.04.059>.
- (46) Debuissy, T.; Sangwan, P.; Pollet, E.; Avérous, L. Study on the Structure-Properties Relationship of Biodegradable and Biobased Aliphatic Copolyesters Based on 1,3-Propanediol, 1,4-Butanediol, Succinic and Adipic Acids. *Polymer* **2017**, *122*, 105–116. <https://doi.org/10.1016/j.polymer.2017.06.045>.
- (47) Unal, S.; Ozturk, G. I.; Long, T. E. Synthesis of Hyperbranched Polymers via Polymerization of Functionally Symmetric Monomer Pairs. In *Hyperbranched Polymers*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2011; pp 79–106. <https://doi.org/10.1002/9780470929001.ch3>.
- (48) Umare, S. S.; Chandure, A. S.; Pandey, R. A. Synthesis, Characterization and Biodegradable Studies of 1,3-Propanediol Based Polyesters. *Polymer Degradation and Stability* **2007**, *92* (3), 464–479. <https://doi.org/10.1016/j.polymdegradstab.2006.10.007>.
- (49) Okada, M. Chemical Syntheses of Biodegradable Polymers. *Progress in Polymer Science (Oxford)* **2002**, *27* (1), 87–133. [https://doi.org/10.1016/S0079-6700\(01\)00039-9](https://doi.org/10.1016/S0079-6700(01)00039-9).
- (50) Yang, Y.; Yu, Y.; Wu, D.; Liu, C.; Zhao, Z.; Li, Q. Lipase/Esterase-Catalyzed Synthesis of Aliphatic Polyesters via Polycondensation: A Review. *Process*

- Biochemistry* **2012**, *47* (7), 1027–1036.
<https://doi.org/10.1016/j.procbio.2012.04.006>.
- (51) Linko, Y. Y.; Lämsä, M.; Wu, X.; Uosukainen, E.; Seppälä, J.; Linko, P. Biodegradable Products by Lipase Biocatalysis. *Journal of Biotechnology* **1998**, *66* (1), 41–50. [https://doi.org/10.1016/S0168-1656\(98\)00155-2](https://doi.org/10.1016/S0168-1656(98)00155-2).
- (52) Kundys, A.; Białecka-Florjańczyk, E.; Fabiszewska, A.; Małajowicz, J. Candida Antarctica Lipase B as Catalyst for Cyclic Esters Synthesis, Their Polymerization and Degradation of Aliphatic Polyesters. *Journal of Polymers and the Environment*. Springer New York LLC January 1, 2018, pp 396–407. <https://doi.org/10.1007/s10924-017-0945-1>.
- (53) Martinelle, M.; Holmquist, M.; Hult, K. On the Interfacial Activation of Candida Antarctica Lipase A and B as Compared with Humicola Lanuginosa Lipase. *Biochimica et Biophysica Acta (BBA)/Lipids and Lipid Metabolism* **1995**, *1258* (3), 272–276. [https://doi.org/10.1016/0005-2760\(95\)00131-U](https://doi.org/10.1016/0005-2760(95)00131-U).
- (54) Yang, Y.; Lu, W.; Zhang, X.; Xie, W.; Cai, M.; Gross, R. A. Two-Step Biocatalytic Route to Biobased Functional Polyesters from ω -Carboxy Fatty Acids and Diols. *Biomacromolecules* **2010**, *11* (1), 259–268. <https://doi.org/10.1021/bm901112m>.
- (55) Zeng, F.; Yang, X.; Li, D.; Dai, L.; Zhang, X.; Lv, Y.; Wei, Z. Functionalized Polyesters Derived from Glycerol: Selective Polycondensation Methods toward Glycerol-Based Polyesters by Different Catalysts. *Journal of Applied Polymer Science* **2020**, *137* (16), 48574. <https://doi.org/10.1002/APP.48574>.
- (56) Kartal, F.; Janssen, M. H. A.; Hollmann, F.; Sheldon, R. A.; Kilinc, A. Improved Esterification Activity of Candida Rugosa Lipase in Organic Solvent by Immobilization as Cross-Linked Enzyme Aggregates (CLEAs). *Journal of Molecular Catalysis B: Enzymatic* **2011**, *71* (3–4), 85–89. <https://doi.org/10.1016/j.molcatb.2011.04.002>.
- (57) Warwel, S.; Demes, C.; Steinke, G. Polyesters by Lipase-Catalyzed Polycondensation of Unsaturated and Epoxidized Long-Chain α,ω -Dicarboxylic Acid Methyl Esters with Diols. *Journal of Polymer Science Part A: Polymer Chemistry* **2001**, *39* (10), 1601–1609. <https://doi.org/10.1002/pola.1137>.
- (58) Binns, F.; Harffey, P.; Roberts, S. M.; Taylor, A. Studies of Lipase-catalyzed Polyesterification of an Unactivated Diacid/Diol System. *Journal of Polymer Science Part A: Polymer Chemistry* **1998**, *36* (12), 2069–2080.

- [https://doi.org/10.1002/\(sici\)1099-0518\(19980915\)36:12<2069::aid-pola13>3.3.co;2-q](https://doi.org/10.1002/(sici)1099-0518(19980915)36:12<2069::aid-pola13>3.3.co;2-q).
- (59) Lee, H. J.; Kang, Y. S.; Kim, C. Y.; Seo, E. J.; Pyo, S. H.; Park, J. B. Multi-Step Enzymatic Synthesis of 1,9-Nonanedioic Acid from a Renewable Fatty Acid and Its Application for the Enzymatic Production of Biopolyesters. *Polymers* **2019**, *11* (10), 1–12. <https://doi.org/10.3390/polym11101690>.
 - (60) Simon, L. M.; László, K.; Vértési, A.; Bagi, K.; Szajáni, B. Stability of Hydrolytic Enzymes in Water-Organic Solvent Systems. *Journal of Molecular Catalysis B: Enzymatic* **1998**, *4* (1–2), 41–45.
 - (61) Mahapatro, A.; Kalra, B.; Kumar, A.; Gross, R. A. Lipase-Catalyzed Polycondensations: Effect of Substrates and Solvent on Chain Formation, Dispersity, and End-Group Structure. *Biomacromolecules* **2003**, *4* (3), 544–551. <https://doi.org/10.1021/bm0257208>.
 - (62) Azim, H.; Dekhterman, A.; Jiang, Z.; Gross, R. A. Candida Antarctica Lipase B-Catalyzed Synthesis of Poly(Butylene Succinate): Shorter Chain Building Blocks Also Work. *Biomacromolecules* **2006**, *7* (11), 3093–3097. <https://doi.org/10.1021/bm060574h>.
 - (63) Cruz-Izquierdo, Á.; van den Broek, L. A. M.; Serra, J. L.; Llama, M. J.; Boeriu, C. G. Lipase-Catalyzed Synthesis of Oligoesters of 2,5-Furandicarboxylic Acid with Aliphatic Diols. *Pure and Applied Chemistry* **2015**, *87* (1), 59–69. <https://doi.org/10.1515/pac-2014-1003>.
 - (64) Steffen-Munsberg, F.; Vickers, C.; Thontowi, A.; Schätzle, S.; Meinhardt, T.; Svedendahl Humble, M.; Land, H.; Berglund, P.; Bornscheuer, U. T.; Höhne, M. Revealing the Structural Basis of Promiscuous Amine Transaminase Activity. *ChemCatChem* **2013**, *5* (1), 154–157. <https://doi.org/10.1002/cctc.201200545>.
 - (65) Jiang, Y.; Woortman, A. J. J.; Alberda Van Ekenstein, G. O. R.; Petrović, D. M.; Loos, K. Enzymatic Synthesis of Biobased Polyesters Using 2,5-Bis(Hydroxymethyl) Furan as the Building Block. *Biomacromolecules* **2014**, *15* (7), 2482–2493. <https://doi.org/10.1021/bm500340w>.
 - (66) Inoue, A.; Horikoshi, K. Estimation of Solvent-Tolerance of Bacteria by the Solvent Parameter Log P. *Journal of Fermentation and Bioengineering* **1991**, *71* (3), 194–196. [https://doi.org/10.1016/0922-338X\(91\)90109-T](https://doi.org/10.1016/0922-338X(91)90109-T).
 - (67) Iemhoff, A.; Sherwood, J.; McElroy, C. R.; Hunt, A. J. Towards Sustainable Kinetic Resolution, a Combination of Bio-Catalysis, Flow Chemistry and Bio-

- Based Solvents. *Green Chemistry* **2018**, *20* (1), 136–140. <https://doi.org/10.1039/C7GC03177G>.
- (68) Sprunger, L. M.; Proctor, A.; Acree, W. E.; Abraham, M. H.; Benjelloun-Dakhama, N. Correlation and Prediction of Partition Coefficient between the Gas Phase and Water, and the Solvents Dry Methyl Acetate, Dry and Wet Ethyl Acetate, and Dry and Wet Butyl Acetate. *Fluid Phase Equilibria* **2008**, *270* (1–2), 30–44. <https://doi.org/10.1016/J.FLUID.2008.06.001>.
- (69) Combes, J.; Clavijo Rivera, E.; Clément, T.; Fojeik, C.; Athès, V.; Moussa, M.; Allais, F. Solvent Selection Strategy for an ISPR (In Situ/In Stream Product Recovery) Process: The Case of Microbial Production of p-Coumaric Acid Coupled with a Liquid-Liquid Extraction. *Separation and Purification Technology* **2021**, *259*, 118170. <https://doi.org/10.1016/J.SEPPUR.2020.118170>.
- (70) TECH TIP #56. **2007**.
- (71) Jacob, P. L.; Ruiz Cantu, L. A.; Pearce, A. K.; He, Y.; Lentz, J. C.; Moore, J. C.; Machado, F.; Rivers, G.; Apebende, E.; Fernandez, M. R.; Francolini, I.; Wildman, R.; Howdle, S. M.; Taresco, V. Poly (Glycerol Adipate) (PGA) Backbone Modifications with a Library of Functional Diols: Chemical and Physical Effects. *Polymer* **2021**, *228*, 123912. <https://doi.org/10.1016/J.POLYMER.2021.123912>.
- (72) Pellis, A.; Byrne, F. P.; Sherwood, J.; Vastano, M.; Comerford, J. W.; Farmer, T. J. Safer Bio-Based Solvents to Replace Toluene and Tetrahydrofuran for the Biocatalyzed Synthesis of Polyesters. *Green Chemistry* **2019**, *21* (7), 1686–1694. <https://doi.org/10.1039/c8gc03567a>.
- (73) Sun, S.; Chen, X. Kinetics of Enzymatic Synthesis of Monoferuloyl Glycerol and Diferuloyl Glycerol by Transesterification in [BMIM]PF₆. *Biochemical Engineering Journal* **2015**, *97*, 25–31. <https://doi.org/10.1016/J.BEJ.2015.02.002>.
- (74) Takamoto, T.; Uyama, H.; Kobayashi, S. Lipase-Catalyzed Synthesis of Aliphatic Polyesters in Supercritical Carbon Dioxide. *E-Polymers* **2002**, *1* (1). <https://doi.org/10.1515/EPOLY.2001.1.1.19/MACHINEREADABLECITATION/RIS>.
- (75) Baheti, P.; Gimello, O.; Bouilhac, C.; Lacroix-Desmazes, P.; Howdle, S. M. Sustainable Synthesis and Precise Characterisation of Bio-Based Star Polycaprolactone Synthesised with a Metal Catalyst and with Lipase. *Polymer Chemistry* **2018**, *9* (47), 5594–5607. <https://doi.org/10.1039/C8PY01266K>.

- (76) Curia, S.; Barclay, A. F.; Torron, S.; Johansson, M.; Howdle, S. M. Green Process for Green Materials: Viable Low-Temperature Lipase-Catalysed Synthesis of Renewable Telechelics in Supercritical CO₂. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **2015**, 373 (2057). <https://doi.org/10.1098/RSTA.2015.0073>.
- (77) Tomietto, P.; Russo, F.; Galiano, F.; Loulergue, P.; Salerno, S.; Paugam, L.; Audic, J. L.; De Bartolo, L.; Figoli, A. Sustainable Fabrication and Pervaporation Application of Bio-Based Membranes: Combining a Polyhydroxyalkanoate (PHA) as Biopolymer and CyreneTM as Green Solvent. *Journal of Membrane Science* **2022**, 643, 120061. <https://doi.org/10.1016/J.MEMSCI.2021.120061>.
- (78) Deniz, P.; Guler, S.; Çelik, E.; Hosseinian, P.; Aydin, H. M. Use of Cyclic Strain Bioreactor for the Upregulation of Key Tenocyte Gene Expression on Poly(Glycerol-Sebacate) (PGS) Sheets. *Materials Science and Engineering C* **2020**, 106, 110293. <https://doi.org/10.1016/j.msec.2019.110293>.
- (79) Veras, S. T. S.; Rojas, P.; Florencio, L.; Kato, M. T.; Sanz, J. L. Production of 1,3-Propanediol from Pure and Crude Glycerol Using a UASB Reactor with Attached Biomass in Silicone Support. *Bioresource Technology* **2019**, 279, 140–148. <https://doi.org/10.1016/j.biortech.2019.01.125>.
- (80) Zhang, T.; Howell, B. A.; Dumitrascu, A.; Martin, S. J.; Smith, P. B. Synthesis and Characterization of Glycerol-Adipic Acid Hyperbranched Polyesters. *Polymer* **2014**, 55 (20), 5065–5072. <https://doi.org/10.1016/j.polymer.2014.08.036>.
- (81) Slavko, E.; Taylor, M. S. Catalyst-Controlled Polycondensation of Glycerol with Diacyl Chlorides: Linear Polyesters from a Trifunctional Monomer. *Chemical Science* **2017**, 8 (10), 7106–7111. <https://doi.org/10.1039/c7sc01886j>.
- (82) Fehling, E.; Bergander, K.; Klein, E.; Weber, N.; Vosmann, K. Thiol-Functionalized Copolymeric Polyesters by Lipase-Catalyzed Esterification and Transesterification of 1,12-Dodecanedioic Acid and Its Diethyl Ester, Respectively, with 1-Thioglycerol. *Biotechnology Letters* **2010**, 32 (10), 1463–1471. <https://doi.org/10.1007/s10529-010-0311-z>.
- (83) Debuissy, T.; Pollet, E.; Avérous, L. Biotic and Abiotic Synthesis of Renewable Aliphatic Polyesters from Short Building Blocks Obtained from Biotechnology. *ChemSusChem* **2018**, 11 (22), 3836–3870. <https://doi.org/10.1002/cssc.201801700>.

- (84) Kluge, M.; Bikiaris, D. N.; Robert, T. Enhancing the Properties of Poly(Propylene Succinate) by the Incorporation of Crystallizable Symmetrical Amido Diols. *European Polymer Journal* **2019**, *120* (August), 109195. <https://doi.org/10.1016/j.eurpolymj.2019.08.022>.
- (85) Stebbins, N. D.; Yu, W.; Uhrich, K. E. Enzymatic Polymerization of an Ibuprofen-Containing Monomer and Subsequent Drug Release. *Macromolecular Bioscience* **2015**, *15* (8), 1115–1124. <https://doi.org/10.1002/mabi.201500030>.
- (86) Yang, Y.; Lu, W.; Cai, J.; Hou, Y.; Ouyang, S.; Xie, W.; Gross, R. A. Poly(Oleic Diacid- Co -Glycerol): Comparison of Polymer Structure Resulting from Chemical and Lipase Catalysis. *Macromolecules* **2011**, *44* (7), 1977–1985. <https://doi.org/10.1021/ma102939k>.
- (87) Hutmacher, D. W. *Scaffolds in Tissue Engineering Bone and Cartilage*; Woodhead Publishing Limited, 2000; Vol. 21. <https://doi.org/10.1016/B978-008045154-1.50021-6>.
- (88) Chen, H.; Truckenmüller, R.; Van Blitterswijk, C.; Moroni, L. Fabrication of Nanofibrous Scaffolds for Tissue Engineering Applications. *Nanomaterials in Tissue Engineering: Fabrication and Applications* **2013**, 158–183. <https://doi.org/10.1533/9780857097231.1.158>.
- (89) Heimann, R. B. Materials for Medical Application. *Materials for Medical Application* **2020**, 1–615. <https://doi.org/10.1515/9783110619249/PDF>.
- (90) Masoudi Rad, M.; Nouri Khorasani, S.; Ghasemi-Mobarakeh, L.; Prabhakaran, M. P.; Foroughi, M. R.; Kharaziha, M.; Saadatkish, N.; Ramakrishna, S. Fabrication and Characterization of Two-Layered Nanofibrous Membrane for Guided Bone and Tissue Regeneration Application. *Materials Science and Engineering: C* **2017**, *80*, 75–87. <https://doi.org/10.1016/J.MSEC.2017.05.125>.
- (91) Irastorza, A.; Zarandona, I.; Andonegi, M.; Guerrero, P.; de la Caba, K. The Versatility of Collagen and Chitosan: From Food to Biomedical Applications. *Food Hydrocolloids* **2021**, *116*, 106633. <https://doi.org/10.1016/J.FOODHYD.2021.106633>.
- (92) Zhao, X.; Hu, D. A.; Wu, D.; He, F.; Wang, H.; Huang, L.; Shi, D.; Liu, Q.; Ni, N.; Pakvasa, M.; Zhang, Y.; Fu, K.; Qin, K. H.; Li, A. J.; Hagag, O.; Wang, E. J.; Sabharwal, M.; Wagstaff, W.; Reid, R. R.; Lee, M. J.; Wolf, J. M.; El Dafrawy, M.; Hynes, K.; Strelzow, J.; Ho, S. H.; He, T. C.; Athiviraham, A. Applications of Biocompatible Scaffold Materials in Stem Cell-Based Cartilage Tissue

- Engineering. *Frontiers in Bioengineering and Biotechnology* **2021**, *9*, 603444. <https://doi.org/10.3389/FBIOE.2021.603444>.
- (93) Zhao, L.; Zhou, Y.; Zhang, J.; Liang, H.; Chen, X.; Tan, H. Natural Polymer-Based Hydrogels: From Polymer to Biomedical Applications. *Pharmaceutics* **2023**, *15* (10), 2514. <https://doi.org/10.3390/PHARMACEUTICS15102514>.
- (94) Luginina, M.; Schuhladen, K.; Orrú, R.; Cao, G.; Boccaccini, A. R.; Liverani, L. Electrospun PCL/PGS Composite Fibers Incorporating Bioactive Glass Particles for Soft Tissue Engineering Applications. *Nanomaterials* **2020**, *10* (5). <https://doi.org/10.3390/nano10050978>.
- (95) Vogt, L.; Boccaccini, A. R. Random and Aligned Electrospun Poly(ϵ -Caprolactone) (PCL)/Poly(1,8-Octanediol-Co-Citrate) (POC) Fiber Mats for Cardiac Tissue Engineering Using Benign Solvents. *European Polymer Journal* **2021**, *160* (September), 110772. <https://doi.org/10.1016/j.eurpolymj.2021.110772>.
- (96) Wang, Y.; Ameer, G. A.; Sheppard, B. J.; Langer, R. A Tough Biodegradable Elastomer. *Nature Biotechnology* **2002**, *20*:6 (6), 602–606. <https://doi.org/10.1038/nbt0602-602>.
- (97) Kargozar, S.; Singh, R. K.; Kim, H. W.; Baino, F. “Hard” Ceramics for “Soft” Tissue Engineering: Paradox or Opportunity? *Acta Biomaterialia* **2020**, *115*, 1–28. <https://doi.org/10.1016/j.actbio.2020.08.014>.
- (98) Yin, G. Z.; Yang, X. M.; Zhou, Z.; Li, Q. F. A Green Pathway to Adjust the Mechanical Properties and Degradation Rate of PCL by Blending Bio-Sourced Poly(Glycerol-Succinate) Oligoesters. *Materials Chemistry Frontiers* **2018**, *2* (3), 544–553. <https://doi.org/10.1039/C7QM00600D>.
- (99) Vogt, L.; Ruther, F.; Salehi, S.; Boccaccini, A. R. Poly(Glycerol Sebacate) in Biomedical Applications—A Review of the Recent Literature. *Advanced Healthcare Materials* **2021**, *10* (9). <https://doi.org/10.1002/adhm.202002026>.
- (100) Luginina, M.; Schuhladen, K.; Orrú, R.; Cao, G.; Boccaccini, A. R.; Liverani, L. Electrospun PCL/PGS Composite Fibers Incorporating Bioactive Glass Particles for Soft Tissue Engineering Applications. *Nanomaterials* **2020**, *10* (5). <https://doi.org/10.3390/nano10050978>.
- (101) Hassani Najafabadi, A.; Tamayol, A.; Annabi, N.; Ochoa, M.; Mostafalu, P.; Akbari, M.; Nikkhah, M.; Rahimi, R.; Dokmeci, M. R.; Sonkusale, S.; Ziaie, B.; Khademhosseini, A.; Dokmeci, M. R.; Khademhosseini, A.; Najafabadi, A. H.

- Tamayol, A.; Annabi, N.; Akbari, M.; Nikkhah, M.; Khademhosseini Harvard-, A.; Ochoa, M.; Rahimi, R.; Ziaie, B.; Mostafalu, P.; Sonkusale, S. Biodegradable Nanofibrous Polymeric Substrates for Generating Elastic and Flexible Electronics. *Advanced Materials* **2014**, *26* (33), 5823–5830. <https://doi.org/10.1002/ADMA.201401537>.
- (102) Lang, K.; Bhattacharya, S.; Ning, Z.; Sánchez-Leija, R. J.; Bramson, M. T. K.; Centore, R.; Corr, D. T.; Linhardt, R. J.; Gross, R. A. Enzymatic Polymerization of Poly(Glycerol-1,8-Octanediol-Sebacate): Versatile Poly(Glycerol Sebacate) Analogues That Form Monocomponent Biodegradable Fiber Scaffolds. *Biomacromolecules* **2020**, *21* (8), 3197–3206. <https://doi.org/10.1021/acs.biomac.0c00641>.
- (103) Boccaccini, A. R. ; BraunerDelia S.; Chen, Q.-Z.; Clare, A. G. ; Comack, A. N.; Et al. *Bio-Glasses: An Introduction* , 1st ed.; Jones, J. R. ; C. G., Ed.; 2012.
- (104) Narayan, R.; Nayak, U. Y.; Raichur, A. M.; Garg, S. Mesoporous Silica Nanoparticles: A Comprehensive Review on Synthesis and Recent Advances. *Pharmaceutics* **2018**, *10* (3), 118. <https://doi.org/10.3390/PHARMACEUTICS10030118>.
- (105) De Oliveira, A. A. R.; De Souza, D. A.; Dias, L. L. S.; De Carvalho, S. M.; Mansur, H. S.; De Magalhães Pereira, M. Synthesis, Characterization and Cytocompatibility of Spherical Bioactive Glass Nanoparticles for Potential Hard Tissue Engineering Applications. *Biomedical materials (Bristol, England)* **2013**, *8* (2). <https://doi.org/10.1088/1748-6041/8/2/025011>.
- (106) Balasubramanian, P.; Büttner, T.; Miguez Pacheco, V.; Boccaccini, A. R. Boron-Containing Bioactive Glasses in Bone and Soft Tissue Engineering. *J Eur Ceram Soc* **2018**, *38* (3), 855–869. <https://doi.org/10.1016/j.jeurceramsoc.2017.11.001>.
- (107) Stöber, W.; Fink, A.; Bohn, E. Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. *Journal of Colloid and Interface Science* **1968**, *26* (1), 62–69. [https://doi.org/10.1016/0021-9797\(68\)90272-5](https://doi.org/10.1016/0021-9797(68)90272-5).
- (108) Simila, H. O.; Boccaccini, A. R. Sol-Gel Bioactive Glass Containing Biomaterials for Restorative Dentistry: A Review. *Dental Materials* **2022**, *38* (5), 725–747. <https://doi.org/10.1016/J.DENTAL.2022.02.011>.
- (109) Fernandes, R. S.; Raimundo, I. M.; Pimentel, M. F. Revising the Synthesis of Stöber Silica Nanoparticles: A Multivariate Assessment Study on the Effects of Reaction Parameters on the Particle Size. *Colloids and Surfaces A:*

- Physicochemical and Engineering Aspects* **2019**, 577, 1–7. <https://doi.org/10.1016/J.COLSURFA.2019.05.053>.
- (110) Liang, Q.; Hu, Q.; Miao, G.; Yuan, B.; Chen, X. A Facile Synthesis of Novel Mesoporous Bioactive Glass Nanoparticles with Various Morphologies and Tunable Mesostructure by Sacrificial Liquid Template Method. *Mater Lett* **2015**, 148, 45–49. <https://doi.org/10.1016/J.MATLET.2015.01.122>.
- (111) Baino, F. Bioactive Glasses – When Glass Science and Technology Meet Regenerative Medicine. *Ceram Int* **2018**, 44 (13), 14953–14966. <https://doi.org/10.1016/J.CERAMINT.2018.05.180>.
- (112) Hosseini Koupaie, E.; Alavi Moghaddam, M. R.; Hashemi, S. H. Evaluation of Integrated Anaerobic/Aerobic Fixed-Bed Sequencing Batch Biofilm Reactor for Decolorization and Biodegradation of Azo Dye Acid Red 18: Comparison of Using Two Types of Packing Media. *Bioresour Technol* **2013**, 127, 415–421. <https://doi.org/10.1016/j.biortech.2012.10.003>.
- (113) Neščáková, Z.; Kaňková, H.; Galusková, D.; Galusek, D.; Boccaccini, A. R.; Liverani, L. Polymer (PCL) Fibers with Zn-Doped Mesoporous Bioactive Glass Nanoparticles for Tissue Regeneration. *International Journal of Applied Glass Science* **2021**, 12 (4), 588–600. <https://doi.org/10.1111/ijag.16292>.
- (114) Ensoylu, M.; Deliormanlı, A. M.; Atmaca, H. Hexagonal Boron Nitride/PCL/PLG Coatings on Borate Bioactive Glass Scaffolds for Bone Regeneration. *Journal of Inorganic and Organometallic Polymers and Materials* **2022**, 32 (5), 1551–1566. <https://doi.org/10.1007/s10904-022-02246-8>.
- (115) Neščáková, Z.; Kaňková, H.; Galusková, D.; Galusek, D.; Boccaccini, A. R.; Liverani, L. Polymer (PCL) Fibers with Zn-Doped Mesoporous Bioactive Glass Nanoparticles for Tissue Regeneration. *International Journal of Applied Glass Science* **2021**, 12 (4), 588–600. <https://doi.org/10.1111/ijag.16292>.
- (116) Balasubramanian, P.; Roether, J. A.; Schubert, D. W.; Beier, J. P.; Boccaccini, A. R. Bi-Layered Porous Constructs of PCL-Coated 45S5 Bioactive Glass and Electrospun Collagen-PCL Fibers. *Journal of Porous Materials* **2015**, 22 (5), 1215–1226. <https://doi.org/10.1007/s10934-015-9998-5>.
- (117) Adams, L. A.; Essien, E. R.; Adesalu, A. T.; Julius, M. L. Bioactive Glass 45S5 from Diatom Biosilica. *Journal of Science: Advanced Materials and Devices* **2017**, 2 (4), 476–482. <https://doi.org/10.1016/J.JSAMD.2017.09.002>.

- (118) Furlan, R. G.; Correr, W. R.; Russi, A. F. C.; da Costa Iemma, M. R.; Trovatti, E.; Pecoraro, É. Preparation and Characterization of Boron-Based Bioglass by Sol–gel Process. *Journal of Sol-Gel Science and Technology* **2018**, 88 (1), 181–191. <https://doi.org/10.1007/S10971-018-4806-8/FIGURES/7>.
- (119) Sharifianjazi, F.; Sharifianjazi, M.; Irandoost, M.; Tavamaishvili, K.; Mohabatkah, M.; Montazerian, M. Advances in Zinc-Containing Bioactive Glasses: A Comprehensive Review. *Journal of Functional Biomaterials* **2024**, 15 (9), 258. <https://doi.org/10.3390/JFB15090258>.
- (120) Yang, Q.; Chen, S.; Shi, H.; Xiao, H.; Ma, Y. In Vitro Study of Improved Wound-Healing Effect of Bioactive Borate-Based Glass Nano-/Micro-Fibers. *Materials Science and Engineering: C* **2015**, 55, 105–117. <https://doi.org/10.1016/J.MSEC.2015.05.049>.
- (121) Uluisik, I.; Karakaya, H. C.; Koc, A. The Importance of Boron in Biological Systems. *Journal of Trace Elements in Medicine and Biology* **2018**, 45, 156–162. <https://doi.org/10.1016/J.JTEMB.2017.10.008>.
- (122) Pizzorno, L. Nothing Boring About Boron. *Integrative Medicine: A Clinician's Journal* **2015**, 14 (4), 35.
- (123) Zheng, K.; Fan, Y.; Torre, E.; Balasubramanian, P.; Taccardi, N.; Cassinelli, C.; Morra, M.; Iviglia, G.; Boccaccini, A. R. Incorporation of Boron in Mesoporous Bioactive Glass Nanoparticles Reduces Inflammatory Response and Delays Osteogenic Differentiation. *Particle and Particle Systems Characterization* **2020**, 37 (7). <https://doi.org/10.1002/ppsc.202000054>.
- (124) Xia, L.; Ma, W.; Zhou, Y.; Gui, Z.; Yao, A.; Wang, D.; Takemura, A.; Uemura, M.; Lin, K.; Xu, Y. Stimulatory Effects of Boron Containing Bioactive Glass on Osteogenesis and Angiogenesis of Polycaprolactone: In Vitro Study. *BioMed Research International* **2019**, 2019. <https://doi.org/10.1155/2019/8961409>.
- (125) Tepedelen, B. E.; Soya, E.; Korkmaz, M. Boric Acid Reduces the Formation of DNA Double Strand Breaks and Accelerates Wound Healing Process. *Biological Trace Element Research* **2016**, 174 (2), 309–318. <https://doi.org/10.1007/S12011-016-0729-9/FIGURES/7>.
- (126) Balasubramanian, P.; Büttner, T.; Miguez Pacheco, V.; Boccaccini, A. R. Boron-Containing Bioactive Glasses in Bone and Soft Tissue Engineering. *Journal of the European Ceramic Society* **2018**, 38 (3), 855–869. <https://doi.org/10.1016/j.jeurceramsoc.2017.11.001>.

- (127) Rico, P.; Rodrigo-Navarro, A.; Salmerón-Sánchez, M. Borax-Loaded PLLA for Promotion of Myogenic Differentiation. *https://home.liebertpub.com/tea* **2015**, *21* (21–22), 2662–2672. <https://doi.org/10.1089/TEN.TEA.2015.0044>.
- (128) Ciriza, J.; Rodríguez-Romano, A.; Noguerolles, I.; Gallego-Ferrer, G.; Cabezuelo, R. M.; Pedraz, J. L.; Rico, P. Borax-Loaded Injectable Alginate Hydrogels Promote Muscle Regeneration in Vivo after an Injury. *Materials Science and Engineering: C* **2021**, *123*, 112003. <https://doi.org/10.1016/J.MSEC.2021.112003>.
- (129) Yang, Q.; Chen, S.; Shi, H.; Xiao, H.; Ma, Y. In Vitro Study of Improved Wound-Healing Effect of Bioactive Borate-Based Glass Nano-/Micro-Fibers. *Materials Science and Engineering C* **2015**, *55*, 105–117. <https://doi.org/10.1016/j.msec.2015.05.049>.
- (130) Gadomska-Gajadur, A.; Wrzecionek, M.; Matyszcak, G.; Pietowski, P.; Wieclaw, M.; Ruśkowski, P. Optimization of Poly(Glycerol Sebacate) Synthesis for Biomedical Purposes with the Design of Experiments. *Organic Process Research and Development* **2018**, *22* (12), 1793–1800. https://doi.org/10.1021/ACS.OPRD.8B00306/ASSET/IMAGES/LARGE/OP-2018-003066_0007.JPEG.
- (131) Shi, M.; Bai, L.; Xu, M.; Li, Z.; Hu, T.; Hu, J.; Zhang, Z.; Yin, Z.; Guo, B. Micropatterned Conductive Elastomer Patch Based on Poly (Glycerol Sebacate)-Graphene for Cardiac Tissue Repair. *Biofabrication* **2022**, *14* (3), 035001. <https://doi.org/10.1088/1758-5090/AC59F2>.
- (132) Sencadas, V.; Sadat, S.; Silva, D. M. Mechanical Performance of Elastomeric PGS Scaffolds under Dynamic Conditions. *Journal of the Mechanical Behavior of Biomedical Materials* **2020**, *102*, 103474. <https://doi.org/10.1016/J.JMBBM.2019.103474>.
- (133) Luginina, M.; Schuhladen, K.; Orrú, R.; Cao, G.; Boccaccini, A. R.; Liverani, L. Electrospun PCL/PGS Composite Fibers Incorporating Bioactive Glass Particles for Soft Tissue Engineering Applications. *Nanomaterials* **2020**, *10* (5), 978. <https://doi.org/10.3390/NANO10050978>.
- (134) Olalla, Á. S.; Talavera, V. H.; García, D. L.; Torres, E. G.; García, M. F. Glycerol-Based Enzymatically Synthesized Renewable Polyesters: Control of Molecular Weight, Degree of Branching and Functional Endgroups. *European Polymer Journal* **2022**, *170*, 111173. <https://doi.org/10.1016/J.EURPOLYMJ.2022.111173>.

- (135) Choi, W. Glycerol-Based Biorefinery for Fuels and Chemicals. *Recent Patents on Biotechnology* **2008**, *2* (3), 173–180. <https://doi.org/10.2174/187220808786241006>.
- (136) Sarma, S.; Dubey, V. K.; Moholkar, V. S. Circular Bioeconomy for Biodiesel Industry: Upgradation of Waste Glycerol to Value-Added Products. *Biomass, Biofuels, Biochemicals: Circular Bioeconomy: Technologies for Biofuels and Biochemicals* **2022**, 419–438. <https://doi.org/10.1016/B978-0-323-89855-3.00017-0>.
- (137) Werpy, T.A, Holladay, J. E. *Top Value Added Chemicals from Biomass Volume I- Results of Screening for Potential Candidates from Sugars and Synthesis Gas Produced by the Staff at Pacific Northwest National Laboratory (PNNL) National Renewable Energy Laboratory (NREL) Office of Biomass*; 2004.
- (138) CFR - Code of Federal Regulations Title 21. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=182.1320&SearchTerm=glycerin> (accessed 2022-05-25).
- (139) Li, X.; Hong, A. T. L.; Naskar, N.; Chung, H. J. Criteria for Quick and Consistent Synthesis of Poly(Glycerol Sebacate) for Tailored Mechanical Properties. *Biomacromolecules* **2015**, *16* (5), 1525–1533. https://doi.org/10.1021/ACS.BIOMAC.5B00018/ASSET/IMAGES/LARGE/BM-2015-00018B_0010.JPEG.
- (140) Zhang, T.; Smith, P. B.; Howell, B. A. Controlled Synthesis of Hyperbranched Poly (Ester)s From Biorenewable Monomers for the Delivery of Therapeutic Agents. *Global Journal of Engineering Sciences* **2019**, 1–9. <https://doi.org/10.33552/GJES.2019.03.000552>.
- (141) Zhang, T.; Howell, B. A.; Dumitrascu, A.; Martin, S. J.; Smith, P. B. Synthesis and Characterization of Glycerol-Adipic Acid Hyperbranched Polyesters. *Polymer* **2014**, *55* (20), 5065–5072. <https://doi.org/10.1016/J.POLYMER.2014.08.036>.
- (142) Zhang, T.; Howell, B. A.; Smith, P. B. Rational Synthesis of Hyperbranched Poly(Ester)s. *Industrial and Engineering Chemistry Research* **2017**, *56* (6), 1661–1670. <https://doi.org/10.1021/acs.iecr.6b04435>.
- (143) Wang, H.; Pu, X. The Structure and Properties of a Novel Hydroxyl-Terminated Hyperbranched Polymer for Inhibiting Shale Hydration. *ChemistrySelect* **2019**, *4* (38), 11239–11246. <https://doi.org/10.1002/SLCT.201902524>.

- (144) Zhao, Z.; Shi, X.; Shen, Z.; Gu, Y.; He, L.; Zhang, M.; Lu, N. Single-Atom Fe Nanozymes Coupling with Atomic Clusters as Superior Oxidase Mimics for Ratiometric Fluorescence Detection. *Chemical Engineering Journal* **2023**, 469 (June). <https://doi.org/10.1016/j.cej.2023.143923>.
- (145) Yi, Y.; Lin, G.; Chen, S.; Liu, J.; Zhang, H.; Mi, P. Polyester Micelles for Drug Delivery and Cancer Theranostics: Current Achievements, Progresses and Future Perspectives. *Materials Science and Engineering: C* **2018**, 83, 218–232. <https://doi.org/10.1016/J.MSEC.2017.10.004>.
- (146) Fakult, D. T.; Vogt, L. Development and Characterization of Biopolymer-Based Fibrous Scaffolds with Enhanced Functionality for Cardiac Tissue Engineering., Friedrich-Alexander-Universitat Erlangen-Nurnbergurnberg, 2021.
- (147) Rai, R.; Tallawi, M.; Barbani, N.; Frati, C.; Madeddu, D.; Cavalli, S.; Graiani, G.; Quaini, F.; Roether, J. A.; Schubert, D. W.; Rosellini, E.; Boccaccini, A. R. Biomimetic Poly(Glycerol Sebacate) (PGS) Membranes for Cardiac Patch Application. *Materials Science and Engineering: C* **2013**, 33 (7), 3677–3687. <https://doi.org/10.1016/J.MSEC.2013.04.058>.
- (148) Peñas, M. I.; Pérez-Camargo, R. A.; Hernández, R.; Müller, A. J. A Review on Current Strategies for the Modulation of Thermomechanical, Barrier, and Biodegradation Properties of Poly (Butylene Succinate) (PBS) and Its Random Copolymers. *Polymers* 2022, Vol. 14, Page 1025 **2022**, 14 (5), 1025. <https://doi.org/10.3390/POLYM14051025>.
- (149) Elias Guckert, F.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H.; Francisco Oechsler, B. Synthesis of Polybutylene Succinate via Lipase-Catalyzed Transesterification: Enzyme Stability, Reuse and PBS Properties in Bulk Polycondensations. *European Polymer Journal* **2022**, 179, 111573. <https://doi.org/10.1016/J.EURPOLYMJ.2022.111573>.
- (150) Adamopoulou, E. Poly(Butylene Succinate): A Promising Biopolymer.
- (151) Dourado Fernandes, C.; Francisco Oechsler, B.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H. Recent Advances and Challenges on Enzymatic Synthesis of Biobased Polyesters via Polycondensation. *European Polymer Journal* **2022**, 169, 111132. <https://doi.org/10.1016/J.EURPOLYMJ.2022.111132>.
- (152) Uyama, H.; Inada, K.; Kobayashi, S. Regioselectivity Control in Lipase-Catalyzed Polymerization of Divinyl Sebacate and Triols. *Macromolecular Bioscience* **2001**, 40–44. <https://doi.org/10.1002/1616-5195>.

- (153) Bilal, M. H.; Prehm, M.; Njau, A. E.; Samiullah, M. H.; Meister, A.; Kressler, J. Enzymatic Synthesis and Characterization of Hydrophilic Sugar Based Polyesters and Their Modification with Stearic Acid. *Polymers* **2016**, Vol. 8, Page 80 **2016**, 8 (3), 80. <https://doi.org/10.3390/POLYM8030080>.
- (154) Kline, B. J.; Beckman, E. J.; Russell, A. J. One-Step Biocatalytic Synthesis of Linear Polyesters with Pendant Hydroxyl Groups. *Journal of the American Chemical Society* **1998**, 120 (37), 9475–9480. <https://doi.org/10.1021/ja9808907>.
- (155) Taresco, V.; Creasey, R. G.; Kennon, J.; Mantovani, G.; Alexander, C.; Burley, J. C.; Garnett, M. C. Variation in Structure and Properties of Poly(Glycerol Adipate) via Control of Chain Branching during Enzymatic Synthesis. *Polymer* **2016**, 89, 41–49. <https://doi.org/10.1016/j.polymer.2016.02.036>.
- (156) Papageorgiou, G. Z.; Bikiaris, D. N. Synthesis, Cocrystallization, and Enzymatic Degradation of Novel Poly(Butylene-Co-Propylene Succinate) Copolymers. *Biomacromolecules* **2007**, 8 (8), 2437–2449. <https://doi.org/10.1021/BM0703113/ASSET/IMAGES/LARGE/BM0703113F00013.JPEG>.
- (157) Debuissy, T.; Pollet, E.; Avérous, L. Lipase-Catalyzed Synthesis of Biobased and Biodegradable Aliphatic Copolyesters from Short Building Blocks. Effect of the Monomer Length. *European Polymer Journal* **2017**, 97, 328–337. <https://doi.org/10.1016/J.EURPOLYMJ.2017.10.028>.
- (158) Oh, B. R.; Lee, S. M.; Heo, S. Y.; Seo, J. W.; Kim, C. H. Efficient Production of 1,3-Propanediol from Crude Glycerol by Repeated Fed-Batch Fermentation Strategy of a Lactate and 2,3-Butanediol Deficient Mutant of *Klebsiella Pneumoniae*. *Microbial Cell Factories* **2018**, 17 (1), 1–9. <https://doi.org/10.1186/S12934-018-0921-Z/FIGURES/5>.
- (159) Dourado Fernandes, C.; Oechsler, B. F.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H. Enzymatic Synthesis of Hydroxy-Functionalized Glycerol-Based Polyesters: A Study of Functionalization, Regioselectivity Control, and Catalyst Reuse. *Macromolecules* **2023**. https://doi.org/10.1021/ACS.MACROMOL.3C01533/SUPPL_FILE/MA3C01533_SI_001.PDF.
- (160) Dutra, L.; Oechsler, B. F.; Brandão, A. L. T.; Lima, R. C.; Nele, M.; Pinto, J. C. Preparation of Polymer Microparticles through Nonaqueous Suspension Polycondensations: Part V—Modeling and Parameter Estimation for

- Poly(Butylene Succinate) Polycondensations. *Macromolecular Reaction Engineering* **2020**, *14* (4), 2000007. <https://doi.org/10.1002/MREN.202000007>.
- (161) Lang, K.; Bhattacharya, S.; Ning, Z.; Sánchez-Leija, R. J.; Bramson, M. T. K.; Centore, R.; Corr, D. T.; Linhardt, R. J.; Gross, R. A. Enzymatic Polymerization of Poly(Glycerol-1,8-Octanediol-Sebacate): Versatile Poly(Glycerol Sebacate) Analogues That Form Monocomponent Biodegradable Fiber Scaffolds. *Biomacromolecules* **2020**, *21* (8), 3197–3206. <https://doi.org/10.1021/acs.biomac.0c00641>.
- (162) Mamajanov, I. Wet-Dry Cycling Delays the Gelation of Hyperbranched Polyesters: Implications to the Origin of Life. *Life* **2019**, *Vol. 9*, Page 56 **2019**, *9* (3), 56. <https://doi.org/10.3390/LIFE9030056>.
- (163) Jiang, Y.; Woortman, A. J. J.; Alberda Van Ekenstein, G. O. R.; Loos, K. Environmentally Benign Synthesis of Saturated and Unsaturated Aliphatic Polyesters via Enzymatic Polymerization of Biobased Monomers Derived from Renewable Resources. *Polymer Chemistry* **2015**, *6* (30), 5451–5463. <https://doi.org/10.1039/C5PY00660K>.
- (164) Perin, G. B.; Felisberti, M. I. Enzymatic Synthesis of Poly(Glycerol Sebacate): Kinetics, Chain Growth, and Branching Behavior. *Macromolecules* **2020**, *53* (18), 7925–7935. <https://doi.org/10.1021/acs.macromol.0c01709>.
- (165) Flory, P. J. Molecular Size Distribution in Three Dimensional Polymers. VI. Branched Polymers Containing A-R-Bf-1 Type Units. *Journal of the American Chemical Society* **1952**, *74* (11), 2718–2723. <https://doi.org/10.1021/ja01131a008>.
- (166) Hevilla, V.; Sonseca, A.; Echeverría, C.; Muñoz-Bonilla, A.; Fernández-García, M. Enzymatic Synthesis of Polyesters and Their Bioapplications: Recent Advances and Perspectives. *Macromolecular Bioscience* **2021**, *21* (10), 1–28. <https://doi.org/10.1002/mabi.202100156>.
- (167) Greco-Duarte, J.; Collaço, A. C. A.; Costa, A. M. M.; Silva, L. O.; Da Silva, J. A. C.; Torres, A. G.; Fernandez-Lafuente, R.; Freire, D. M. G. Understanding the Degree of Estolide Enzymatic Polymerization and the Effects on Its Lubricant Properties. *Fuel* **2019**, *245* (November 2018), 286–293. <https://doi.org/10.1016/j.fuel.2019.02.004>.
- (168) Fu, H.; Kulshrestha, A. S.; Gao, W.; Gross, R. A.; Baiardo, M.; Scandola, M. Physical Characterization of Sorbitol or Glycerol Containing Aliphatic

- Copolyesters Synthesized by Lipase-Catalyzed Polymerization. *Macromolecules* **2003**, *36*, 9804–9808. <https://doi.org/10.1021/ma035129i>.
- (169) Yang, Y.; Lu, W.; Cai, J.; Hou, Y.; Ouyang, S.; Xie, W.; Gross, R. A. Poly(Oleic Diacid-Co-Glycerol): Comparison of Polymer Structure Resulting from Chemical and Lipase Catalysis. *Macromolecules* **2011**, *44* (7), 1977–1985. <https://doi.org/10.1021/ma102939k>.
- (170) Kulshrestha, A. S.; Gao, W.; Gross, R. A. Glycerol Copolyesters: Control of Branching and Molecular Weight Using a Lipase Catalyst. *Macromolecules* **2005**, *38* (8), 3193–3204. <https://doi.org/10.1021/ma0480190>.
- (171) Mao, Y.; Lee, Y. Y.; Xie, X.; Wang, Y.; Zhang, Z. Preparation, Acyl Migration and Applications of the Acylglycerols and Their Isomers: A Review. *Journal of Functional Foods* **2023**, *106* (June), 105616. <https://doi.org/10.1016/j.jff.2023.105616>.
- (172) Taresco, V.; Suksiriworapong, J.; Creasey, R.; Burley, J. C.; Mantovani, G.; Alexander, C.; Treacher, K.; Booth, J.; Garnett, M. C. Properties of Acyl Modified Poly(Glycerol-Adipate) Comb-like Polymers and Their Self-Assembly into Nanoparticles. *Journal of Polymer Science Part A: Polymer Chemistry* **2016**, *54* (20), 3267–3278. <https://doi.org/10.1002/pola.28215>.
- (173) Perin, G. B.; Felisberti, M. I. Mechanism and Kinetics of Lipase-Catalyzed Polycondensation of Glycerol and Sebacic Acid: Influence of Solvent and Temperature. *Biomacromolecules* **2022**, *23* (7), 2968–2975. <https://doi.org/10.1021/acs.biomac.2c00458>.
- (174) Liang, S. L.; Cook, W. D.; Thouas, G. A.; Chen, Q. Z. The Mechanical Characteristics and in Vitro Biocompatibility of Poly(Glycerol Sebacate)-Bioglass® Elastomeric Composites. *Biomaterials* **2010**, *31* (33), 8516–8529. <https://doi.org/10.1016/J.BIOMATERIALS.2010.07.105>.
- (175) Conejero-García, Á.; Gimeno, H. R.; Sáez, Y. M.; Vilariño-Feltrer, G.; Ortuño-Lizarán, I.; Vallés-Lluch, A. Correlating Synthesis Parameters with Physicochemical Properties of Poly(Glycerol Sebacate). *European Polymer Journal* **2017**, *87*, 406–419. <https://doi.org/10.1016/J.EURPOLYMJ.2017.01.001>.
- (176) Trotta, G.; Stampone, B.; Fassi, I.; Tricarico, L. Study of Rheological Behaviour of Polymer Melt in Micro Injection Moulding with a Miniaturized Parallel Plate Rheometer. *Polymer Testing* **2021**, *96*, 107068. <https://doi.org/10.1016/J.POLYMERTESTING.2021.107068>.

- (177) Hoddy, B.; Ahmed, N.; Al-lamee, K.; Bullett, N.; Bressloff, N. W. Exploring a Parallel Rheological Framework to Capture the Mechanical Behaviour of a Thin-Strut Polymeric Bioresorbable Coronary Scaffold. *Journal of the Mechanical Behavior of Biomedical Materials* **2022**, *130*, 105154. <https://doi.org/10.1016/J.JMBBM.2022.105154>.
- (178) CHARLESBY, A. *Atomic Radiation and Polymers*; Pinner, J., Ed.; Pergamon Press: New York, 1960.
- (179) Huang, Y. J.; James Lee, L. Optimization of Diffusion-Controlled Free Radical Polymerizations in a Distributed Parameter System. *Chemical Engineering Science* **1989**, *44* (2), 363–376. [https://doi.org/10.1016/0009-2509\(89\)85073-0](https://doi.org/10.1016/0009-2509(89)85073-0).
- (180) Hsu, K. H.; Chen, C. W.; Wang, L. Y.; Chan, H. W.; He, C. L.; Cho, C. J.; Rwei, S. P.; Kuo, C. C. Bio-Based Thermoplastic Poly(Butylene Succinate-: Co - Propylene Succinate) Copolyesters: Effect of Glycerol on Thermal and Mechanical Properties. *Soft Matter* **2019**, *15* (47), 9710–9720. <https://doi.org/10.1039/c9sm01958h>.
- (181) Barrino, F.; De La Rosa-Ramírez, H.; Schiraldi, C.; López-Martínez, J.; Samper, M. D. Preparation and Characterization of New Bioplastics Based on Polybutylene Succinate (PBS). *Polymers* **2023**, *15* (5), 1–15. <https://doi.org/10.3390/polym15051212>.
- (182) Platnieks, O.; Gaidukovs, S.; Barkane, A.; Sereda, A.; Gaidukova, G.; Grase, L.; Thakur, V. K.; Filipova, I.; Fridrihsone, V.; Skute, M.; Laka, M. Bio-Based Poly(Butylene Succinate)/Microcrystalline Cellulose/Nanofibrillated Cellulose-Based Sustainable Polymer Composites: Thermo-Mechanical and Biodegradation Studies. *Polymers* **2020**, *12* (7), 1–20. <https://doi.org/10.3390/polym12071472>.
- (183) Gao, J.; Wang, Y.; Du, Y.; Zhou, L.; He, Y.; Ma, L.; Yin, L.; Kong, W.; Jiang, Y. Construction of Biocatalytic Colloidosome Using Lipase-Containing Dendritic Mesoporous Silica Nanospheres for Enhanced Enzyme Catalysis. *Chemical Engineering Journal* **2017**, *317*, 175–186. <https://doi.org/10.1016/J.CEJ.2017.02.012>.
- (184) Salgado, J.; Coutinho, O. P.; Reis, R. L. Bone Tissue Engineering : State of the Art and Future Trends. **2004**, 743–765. <https://doi.org/10.1002/mabi.200400026>.
- (185) Qu, H. RSC Advances Biomaterials for Bone Tissue Engineering Scaffolds : **2019**, 26252–26262. <https://doi.org/10.1039/c9ra05214c>.

- (186) Ibrahim, H. M.; Klingner, A. A Review on Electrospun Polymeric Nanofibers : Production Parameters and Potential Applications. *Polymer Testing* **2020**, *90* (May), 106647. <https://doi.org/10.1016/j.polymertesting.2020.106647>.
- (187) Jing Yan, Weimin Kang, B. C. *Advanced Nanofibrous Materials Manufacture Technology Based on Electrospinning*, 1st Editio.; Press, C., Ed.; 2019.
- (188) Quirós, J.; Boltes, K.; Rosal, R. Bioactive Applications for Electrospun Fibers. *Polymer Reviews* **2016**, *56* (4), 631–667. <https://doi.org/10.1080/15583724.2015.1136641>.
- (189) Aazmi, A.; Zhang, D.; Mazzaglia, C.; Yu, M.; Wang, Z.; Yang, H.; Yan, Y.; Huang, S.; Ma, L. Bioactive Materials Biofabrication Methods for Reconstructing Extracellular Matrix Mimetics. *Bioactive Materials* **2024**, *31* (September 2023), 475–496. <https://doi.org/10.1016/j.bioactmat.2023.08.018>.
- (190) Vasudevan, J.; Jiang, K.; Fernandez, J. G.; Lim, C. T. Acta Biomaterialia Extracellular Matrix Mechanobiology in Cancer Cell Migration ☆. **2023**, *163*, 351–364. <https://doi.org/10.1016/j.actbio.2022.10.016>.
- (191) Stratton, S.; Shelke, N. B.; Hoshino, K.; Rudraiah, S.; Kumbar, S. G. Bioactive Materials Bioactive Polymeric Scaffolds for Tissue Engineering. *Bioactive Materials* **2016**, *1* (2), 93–108. <https://doi.org/10.1016/j.bioactmat.2016.11.001>.
- (192) Touré, A. B. R.; Mele, E.; Christie, J. K. Multi-Layer Scaffolds of Poly(Caprolactone), Poly(Glycerol Sebacate) and Bioactive Glasses Manufactured by Combined 3D Printing and Electrospinning. *Nanomaterials* **2020**, *10* (4), 626. <https://doi.org/10.3390/NANO10040626>.
- (193) Mazzoni, E.; Iaquina, M. R.; Lanzillotti, C.; Mazziotta, C.; Maritati, M.; Montesi, M.; Sprio, S.; Tampieri, A.; Tognon, M.; Martini, F. Bioactive Materials for Soft Tissue Repair. **2021**, *9* (February), 1–17. <https://doi.org/10.3389/fbioe.2021.613787>.
- (194) Singh, G.; Chanda, A. Mechanical Properties of Whole-Body Soft Human Tissues : A Review. *Biomedical materials* **2021**, *16*, 062004. <https://doi.org/https://doi.org/10.1088/1748-605X/ac2b7a>.
- (195) Fernandes, C. D.; Oechsler, B. F.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H. Enzymatic Synthesis of Hydroxy-Functionalized Glycerol-Based Polyesters: A Study of Functionalization, Regioselectivity Control, and Catalyst Reuse. *Macromolecules* **2024**, *57* (2), 456–469.

- https://doi.org/10.1021/ACS.MACROMOL.3C01533/SUPPL_FILE/MA3C01533_SI_001.PDF.
- (196) Pourhoseyini, T.; Naeimi, F.; Mehrazin, M.; Madadi, M.; Khonakdar, H. A. Biodegradable Microfibrous, Electrospun Hydroxyapatite Nanoparticles/Poly(Glycerol Sebacate)-Co-Poly(ϵ -Caprolactone) Nanocomposite Scaffolds for Tissue Engineering Applications. *Polymer Bulletin* **2024**, *81* (13), 11499–11515. <https://doi.org/10.1007/s00289-024-05235-8>.
- (197) Saudi, A.; Rafienia, M.; Zargar Kharazi, A.; Salehi, H.; Zarrabi, A.; Karevan, M. Design and Fabrication of Poly (Glycerol Sebacate)-Based Fibers for Neural Tissue Engineering: Synthesis, Electrospinning, and Characterization. *Polymers for Advanced Technologies* **2019**, *30* (6), 1427–1440. <https://doi.org/10.1002/PAT.4575>.
- (198) Denis, P.; Wrzecionek, M.; Gadomska-Gajadur, A.; Sajkiewicz, P. Poly(Glycerol Sebacate)–Poly(l-Lactide) Nonwovens. Towards Attractive Electrospun Material for Tissue Engineering. *Polymers* **2019**, *Vol. 11, Page 2113* **2019**, *11* (12), 2113. <https://doi.org/10.3390/POLYM11122113>.
- (199) Wu, Z.; Jin, K.; Wang, L.; Fan, Y. A Review: Optimization for Poly(Glycerol Sebacate) and Fabrication Techniques for Its Centered Scaffolds. *Macromolecular Bioscience* **2021**, *21* (9), 2100022. <https://doi.org/10.1002/MABI.202100022>.
- (200) Tabia, Z.; Akhtach, S.; Bricha, M.; El Mabrouk, K. Tailoring the Biodegradability and Bioactivity of Green-Electrospun Polycaprolactone Fibers by Incorporation of Bioactive Glass Nanoparticles for Guided Bone Regeneration. *European Polymer Journal* **2021**, *161* (August), 110841. <https://doi.org/10.1016/j.eurpolymj.2021.110841>.
- (201) Fernandes, C. D.; Oechsler, B. F.; Sayer, C.; de Oliveira, D.; Hermes de Araújo, P. H. Enzymatic Synthesis of Hydroxy-Functionalized Glycerol-Based Polyesters: A Study of Functionalization, Regioselectivity Control, and Catalyst Reuse. *Macromolecules* **2024**, *57* (2), 456–469. <https://doi.org/10.1021/acs.macromol.3c01533>.
- (202) Varma, I. K.; Albertsson, A. C.; Rajkhowa, R.; Srivastava, R. K. Enzyme Catalyzed Synthesis of Polyesters. *Progress in Polymer Science (Oxford)* **2005**, *30* (10), 949–981. <https://doi.org/10.1016/j.progpolymsci.2005.06.010>.
- (203) Kirillova, A.; Yeazel, T. R.; Asheghali, D.; Petersen, S. R.; Dort, S.; Gall, K.; Becker, M. L. Fabrication of Biomedical Scaffolds Using Biodegradable

- Polymers. *Chemical Reviews* **2021**, *121* (18), 11238–11304. <https://doi.org/10.1021/acs.chemrev.0c01200>.
- (204) Qin, X.; Wu, D. Effect of Different Solvents on Poly(Caprolactone)(PCL) Electrospun Nonwoven Membranes. *Journal of Thermal Analysis and Calorimetry* **2012**, *107* (3), 1007–1013. <https://doi.org/10.1007/s10973-011-1640-4>.
- (205) Van Der Schueren, L.; De Schoenmaker, B.; Kalaoglu, Ö. I.; De Clerck, K. An Alternative Solvent System for the Steady State Electrospinning of Polycaprolactone. *European Polymer Journal* **2011**, *47* (6), 1256–1263. <https://doi.org/10.1016/j.eurpolymj.2011.02.025>.
- (206) Liverani, L.; Lacina, J.; Roether, J. A.; Boccardi, E.; Killian, M. S.; Schmuki, P.; Schubert, D. W.; Boccaccini, A. R. Incorporation of Bioactive Glass Nanoparticles in Electrospun PCL/Chitosan Fibers by Using Benign Solvents. *Bioactive Materials* **2018**, *3* (1), 55–63. <https://doi.org/10.1016/j.bioactmat.2017.05.003>.
- (207) Liverani, L.; Boccaccini, A. R. Versatile Production of Poly(Epsilon-Caprolactone) Fibers by Electrospinning Using Benign Solvents. *Nanomaterials* **2016**, *6* (4). <https://doi.org/10.3390/nano6040075>.
- (208) Sergi, R.; Cannillo, V.; Boccaccini, A. R.; Liverani, L. A New Generation of Electrospun Fibers Containing Bioactive Glass Particles for Wound Healing. *Materials* **2020**, *13* (24), 1–14. <https://doi.org/10.3390/ma13245651>.
- (209) Kokubo, T.; Takadama, H. How Useful Is SBF in Predicting in Vivo Bone Bioactivity? *Biomaterials* **2006**, *27* (15), 2907–2915. <https://doi.org/10.1016/J.BIOMATERIALS.2006.01.017>.
- (210) Kokubo, T.; Takadama, H. How Useful Is SBF in Predicting in Vivo Bone Bioactivity? *Biomaterials* **2006**, *27* (15), 2907–2915. <https://doi.org/10.1016/j.biomaterials.2006.01.017>.
- (211) Dourado Fernandes, C.; Grünewald, A.; Hadzhieva, Z.; Oechsler, B. F.; Sayer, C.; Hermes de Araújo, P. H.; Boccaccini, A. R. Incorporation of Poly(Propylene Succinate-Co-Glycerol Succinate) (PPSG) as a Renewable Additive in Electrospun PCL Fibers with Bioactive Glass Particles for Soft Tissue Engineering. *ACS Appl Bio Mater* **2025**, *8* (6), 4791–4804. <https://doi.org/10.1021/acsabm.5c00176>.
- (212) Fong, H.; Chun, I.; Reneker, D. H. Beaded Nanofibers Formed during Electrospinning. *Polymer* **1999**, *40* (16), 4585–4592. [https://doi.org/10.1016/S0032-3861\(99\)00068-3](https://doi.org/10.1016/S0032-3861(99)00068-3).

- (213) Shahin-Shamsabadi, A.; Hashemi, A.; Tahriri, M.; Bastami, F.; Salehi, M.; Mashhadi Abbas, F. Mechanical, Material, and Biological Study of a PCL/Bioactive Glass Bone Scaffold: Importance of Viscoelasticity. *Materials Science and Engineering C* **2018**, *90* (August 2017), 280–288. <https://doi.org/10.1016/j.msec.2018.04.080>.
- (214) Liverani, L.; Boccaccini, A. R. Versatile Production of Poly(Epsilon-Caprolactone) Fibers by Electrospinning Using Benign Solvents. *Nanomaterials* **2016**, *Vol. 6, Page 75* **2016**, *6* (4), 75. <https://doi.org/10.3390/NANO6040075>.
- (215) Miguez-Pacheco, V.; Hench, L. L.; Boccaccini, A. R. Bioactive Glasses beyond Bone and Teeth: Emerging Applications in Contact with Soft Tissues. *Acta Biomaterialia* **2015**, *13*, 1–15. <https://doi.org/10.1016/j.actbio.2014.11.004>.
- (216) Silva, J. C.; Udangawa, R. N.; Chen, J.; Mancinelli, C. D.; Garrudo, F. F. F.; Mikael, P. E.; Cabral, J. M. S.; Ferreira, F. C.; Linhardt, R. J. Kartogenin-Loaded Coaxial PGS/PCL Aligned Nanofibers for Cartilage Tissue Engineering. *Materials Science and Engineering C* **2020**, *107* (July 2019), 110291. <https://doi.org/10.1016/j.msec.2019.110291>.
- (217) Zhang, J.; Yan, L.; Zhou, M.; Ma, J.; Wang, K.; Zhang, Y.; Drioli, E.; Cheng, X. Recent Progress on Functional Electrospun Polymeric Nanofiber Membranes. *Materials Today Communications* **2024**, *41*, 110530. <https://doi.org/10.1016/J.MTCOMM.2024.110530>.
- (218) Topuz, F.; Uyar, T. Electrospinning of Sustainable Polymers from Biomass for Active Food Packaging. *Sustainable Food Technology* **2024**, *2* (5), 1266–1296. <https://doi.org/10.1039/D4FB00147H>.
- (219) Salehi, S.; Fathi, M.; Javanmard, S. H.; Bahners, T.; Gutmann, J. S.; Ergün, S.; Steuhl, K. P.; Fuchsluger, T. A. Generation of PGS/PCL Blend Nanofibrous Scaffolds Mimicking Corneal Stroma Structure. *Macromolecular Materials and Engineering* **2014**, *299* (4), 455–469. <https://doi.org/10.1002/mame.201300187>.
- (220) Vogt, L.; Rivera, L. R.; Liverani, L.; Piegat, A.; El Fray, M.; Boccaccini, A. R. Poly(ϵ -Caprolactone)/Poly(Glycerol Sebacate) Electrospun Scaffolds for Cardiac Tissue Engineering Using Benign Solvents. *Materials Science and Engineering: C* **2019**, *103*, 109712. <https://doi.org/10.1016/J.MSEC.2019.04.091>.
- (221) Gaharwar, A. K.; Nikkhah, M.; Sant, S.; Khademhosseini, A. Anisotropic Poly (Glycerol Sebacate)-Poly (-Caprolactone) Electrospun Fibers Promote Endothelial

- Cell Guidance. *Biofabrication* **2015**, 7 (1). <https://doi.org/10.1088/1758-5090/7/1/015001>.
- (222) Cheng, S.; Huang, A.; Wang, S.; Zhang, Q. Effect of Different Heat Treatment Temperatures on the Chemical Composition and Structure of Chinese Fir Wood. *BioResources* **2016**, 11 (2), 4006–4016. <https://doi.org/10.15376/biores.11.2.4006-4016>.
- (223) Jiang, Y.; Woortman, A. J. J.; Alberda Van Ekenstein, G. O. R.; Loos, K. Environmentally Benign Synthesis of Saturated and Unsaturated Aliphatic Polyesters via Enzymatic Polymerization of Biobased Monomers Derived from Renewable Resources. *Polymer Chemistry* **2015**, 6 (30), 5451–5463. <https://doi.org/10.1039/C5PY00660K>.
- (224) Hisamatsu, E.; Nagao, M.; Toh, R.; Irino, Y.; Iino, T.; Hara, T.; Tanaka, H.; Satomi-Kobayashi, S.; Ishida, T.; Hirata, K. I. Fibronectin-Containing High-Density Lipoprotein Is Associated with Cancer Cell Adhesion and Proliferation. *Kobe Journal of Medical Sciences* **2020**, 66 (1), E40–E48.
- (225) Ning, Z.; Lang, K.; Xia, K.; Linhardt, R. J.; Gross, R. A. Lipase-Catalyzed Synthesis and Characterization of Poly(Glycerol Sebacate). *Biomacromolecules* **2022**, 23 (1), 398–408. https://doi.org/10.1021/ACS.BIOMAC.1C01351/ASSET/IMAGES/LARGE/BM1C01351_0007.JPEG.
- (226) Saudi, A.; Zebarjad, S. M.; Alipour, H.; Katoueizadeh, E.; Alizadeh, A.; Rafienia, M. A Study on the Role of Multi-Walled Carbon Nanotubes on the Properties of Electrospun Poly(Caprolactone)/Poly(Glycerol Sebacate) Scaffold for Nerve Tissue Applications. *Materials Chemistry and Physics* **2022**, 282, 125868. <https://doi.org/10.1016/J.MATCHEMPHYS.2022.125868>.
- (227) Tallawi, M.; Dippold, D.; Rai, R.; D'Atri, D.; Roether, J. A.; Schubert, D. W.; Rosellini, E.; Engel, F. B.; Boccaccini, A. R. Novel PGS/PCL Electrospun Fiber Mats with Patterned Topographical Features for Cardiac Patch Applications. *Materials Science and Engineering: C* **2016**, 69, 569–576. <https://doi.org/10.1016/J.MSEC.2016.06.083>.
- (228) Xu, L. C.; Siedlecki, C. A. Effects of Surface Wettability and Contact Time on Protein Adhesion to Biomaterial Surfaces. *Biomaterials* **2007**, 28 (22), 3273–3283. <https://doi.org/10.1016/j.biomaterials.2007.03.032>.

- (229) Sundback, C. A.; Shyu, J. Y.; Wang, Y.; Faquin, W. C.; Langer, R. S.; Vacanti, J. P.; Hadlock, T. A. Biocompatibility Analysis of Poly(Glycerol Sebacate) as a Nerve Guide Material. *Biomaterials* **2005**, *26* (27), 5454–5464. <https://doi.org/10.1016/j.biomaterials.2005.02.004>.
- (230) Wang, Y.; Kim, Y. M.; Langer, R.; Truesdell, C. Technical Note In Vivo Degradation Characteristics of Poly (Glycerol Sebacate). *The Journal of Chemical Physics* **2002**, *37* (85), 2336.
- (231) Sun, H.; Mei, L.; Song, C.; Cui, X.; Wang, P. The in Vivo Degradation, Absorption and Excretion of PCL-Based Implant. *Biomaterials* **2006**, *27* (9), 1735–1740. <https://doi.org/10.1016/j.biomaterials.2005.09.019>.
- (232) Kokubo, T.; Takadama, H. How Useful Is SBF in Predicting in Vivo Bone Bioactivity? *Biomaterials* **2006**, *27* (15), 2907–2915. <https://doi.org/10.1016/J.BIOMATERIALS.2006.01.017>.
- (233) Rezwan, K.; Chen, Q. Z.; Blaker, J. J.; Boccaccini, A. R. Biodegradable and Bioactive Porous Polymer/Inorganic Composite Scaffolds for Bone Tissue Engineering. *Biomaterials* **2006**, *27* (18), 3413–3431. <https://doi.org/10.1016/j.biomaterials.2006.01.039>.
- (234) Desimone, D.; Li, W.; Roether, J. A.; Schubert, D. W.; Crovace, M. C.; Rodrigues, A. C. M.; Zanotto, E. D.; Boccaccini, A. R. Biosilicate®-Gelatin Bone Scaffolds by the Foam Replica Technique: Development and Characterization. *Science and Technology of Advanced Materials* **2013**, *14* (4). <https://doi.org/10.1088/1468-6996/14/4/045008>.
- (235) Mawhinney, D. B.; Glass, J. A.; Yates, J. T. FTIR Study of the Oxidation of Porous Silicon. *Journal of Physical Chemistry B* **1997**, *101* (7), 1202–1206. <https://doi.org/10.1021/JP963322R/ASSET/IMAGES/MEDIUM/JP963322RE00003.GIF>.
- (236) Ma, A. N.; Gong, N.; Lu, J. M.; Huang, J. L.; Hao, B.; Guo, Y.; Zhong, J.; Xu, Y.; Chang, J.; Wang, Y. X. Local Protective Effects of Oral 45S5 Bioactive Glass on Gastric Ulcers in Experimental Animals. *Journal of Materials Science: Materials in Medicine* **2013**, *24* (3), 803–809. <https://doi.org/10.1007/s10856-012-4844-7>.
- (237) Hoppe, A.; Gldal, N. S.; Boccaccini, A. R. A Review of the Biological Response to Ionic Dissolution Products from Bioactive Glasses and Glass-Ceramics. *Biomaterials* **2011**, *32* (11), 2757–2774. <https://doi.org/10.1016/j.biomaterials.2011.01.004>.

- (238) Kaur, G.; Pandey, O. P.; Singh, K.; Homa, D.; Scott, B.; Pickrell, G. A Review of Bioactive Glasses: Their Structure, Properties, Fabrication and Apatite Formation. *Journal of Biomedical Materials Research - Part A* **2014**, *102* (1), 254–274. <https://doi.org/10.1002/jbm.a.34690>.
- (239) Ren, Z.; Tang, S.; Wang, J.; Lv, S.; Zheng, K. Bioactive Glasses: Advancing Skin Tissue Repair through Multifunctional Mechanisms and Innovations. *Biomaterials Research* **2025**, *29* (0134), 1–20.
- (240) Wall, S. T.; Walker, J. C.; Healy, K. E.; Ratcliffe, M. B.; Guccione, J. M. Theoretical Impact of the Injection of Material Into the Myocardium A Finite Element Model Simulation. **2006**. <https://doi.org/10.1161/CIRCULATIONAHA.106.657270>.
- (241) Kitsara, M.; Agbulut, O.; Kontziampasis, D.; Chen, Y.; Menasché, P. Acta Biomaterialia Fibers for Hearts : A Critical Review on Electrospinning for Cardiac Tissue Engineering. *Acta Biomaterialia* **2017**, *48*, 20–40. <https://doi.org/10.1016/j.actbio.2016.11.014>.
- (242) Rai, R.; Tallawi, M.; Grigore, A.; Boccaccini, A. R. Synthesis, Properties and Biomedical Applications of Poly(Glycerol Sebacate) (PGS): A Review. *Progress in Polymer Science* **2012**, *37* (8), 1051–1078. <https://doi.org/10.1016/J.PROGPOLYMSCI.2012.02.001>.
- (243) Canales, D. A.; Reyes, F.; Saavedra, M.; Peponi, L.; Leonés, A.; Palza, H.; Boccaccini, A. R.; Grünewald, A.; Zapata, P. A. Electrospun Fibers of Poly (Lactic Acid) Containing Bioactive Glass and Magnesium Oxide Nanoparticles for Bone Tissue Regeneration. *International Journal of Biological Macromolecules* **2022**, *210* (January), 324–336. <https://doi.org/10.1016/j.ijbiomac.2022.05.047>.
- (244) Chen, M.; Patra, P. K.; Warner, S. B.; Bhowmick, S. Role of Fiber Diameter in Adhesion and Proliferation of NIH 3T3 Fibroblast on Electrospun Polycaprolactone Scaffolds. *Tissue Engineering* **2007**, *13* (3), 579–587. <https://doi.org/10.1089/TEN.2006.0205>.
- (245) Pauly, H. M.; Kelly, D. J.; Popat, K. C.; Trujillo, N. A.; Dunne, N. J.; McCarthy, H. O.; Haut Donahue, T. L. Mechanical Properties and Cellular Response of Novel Electrospun Nanofibers for Ligament Tissue Engineering: Effects of Orientation and Geometry. *Journal of the Mechanical Behavior of Biomedical Materials* **2016**, *61*, 258–270. <https://doi.org/10.1016/j.jmbbm.2016.03.022>.

- (246) Sireesha, M.; Babu, J.; Ramakrishna, S. Fi Brinogen Composite Electrospun Scaffolds For. **2015**, 103308–103314. <https://doi.org/10.1039/c5ra20322h>.
- (247) Bosch-Ru  ,   .; D  ez-Tercero, L.; Buitrago, J. O.; Castro, E.; P  rez, R. A. Angiogenic and Immunomodulation Role of Ions for Initial Stages of Bone Tissue Regeneration. *Acta Biomaterialia* **2023**, 166, 14–41. <https://doi.org/10.1016/j.actbio.2023.06.001>.
- (248) Chen, X.; Zhao, Y.; Geng, S.; Miron, R. J.; Zhang, Q.; Wu, C.; Zhang, Y. In Vivo Experimental Study on Bone Regeneration in Critical Bone Defects Using PIB Nanogels/Boron-Containing Mesoporous Bioactive Glass Composite Scaffold. *International Journal of Nanomedicine* **2015**, 10, 839–846. <https://doi.org/10.2147/IJN.S69001>.
- (249) Wu, C.; Miron, R.; Sculean, A.; Kaskel, S.; Doert, T.; Schulze, R.; Zhang, Y. Proliferation, Differentiation and Gene Expression of Osteoblasts in Boron-Containing Associated with Dexamethasone Deliver from Mesoporous Bioactive Glass Scaffolds. *Biomaterials* **2011**, 32 (29), 7068–7078. <https://doi.org/10.1016/j.biomaterials.2011.06.009>.
- (250) Haro Durand, L. A.; Vargas, G. E.; Romero, N. M.; Vera-Mesones, R.; Porto-L  pez, J. M.; Boccaccini, A. R.; Zago, M. P.; Baldi, A.; Gorustovich, A. Angiogenic Effects of Ionic Dissolution Products Released from a Boron-Doped 45S5 Bioactive Glass. *Journal of Materials Chemistry B* **2015**, 3 (6), 1142–1148. <https://doi.org/10.1039/c4tb01840k>.
- (251) Marquardt, L. M.; Day, D.; Sakiyama-Elbert, S. E.; Harkins, A. B. Effects of Borate-Based Bioactive Glass on Neuron Viability and Neurite Extension. *Journal of Biomedical Materials Research - Part A* **2014**, 102 (8), 2767–2775. <https://doi.org/10.1002/jbm.a.34944>.
- (252) Haro Durand, L. A.; G  ngora, A.; Porto L  pez, J. M.; Boccaccini, A. R.; Zago, M. P.; Baldi, A.; Gorustovich, A. In Vitro Endothelial Cell Response to Ionic Dissolution Products from Boron-Doped Bioactive Glass in the SiO₂-CaO-P₂O₅-Na₂O System. *Journal of Materials Chemistry B* **2014**, 2 (43), 7620–7630. <https://doi.org/10.1039/c4tb01043d>.
- (253) Wray, P. Cotton Candy That Heals? Borate Glass Nanofibers Look Promising. *American Ceramic Society Bulletin* **2011**, 90, 25–29.
- (254) WRAY, P. Wound Healing: An Update on Mo-Sci’s Novel Borate Glass Fibers. *American Ceramic Society Bulletin* **2013**, 92 (4), 30–35.

- (255) Neščáková, Z.; Zheng, K.; Liverani, L.; Nawaz, Q.; Galusková, D.; Kaňková, H.; Michálek, M.; Galusek, D.; Boccaccini, A. R. Multifunctional Zinc Ion Doped Sol – Gel Derived Mesoporous Bioactive Glass Nanoparticles for Biomedical Applications. *Bioactive Materials* **2019**, *4* (September 2019), 312–321. <https://doi.org/10.1016/j.bioactmat.2019.10.002>.
- (256) Wang, X.; Feng, Y.; Dong, P.; Huang, J. A Mini Review on Carbon Quantum Dots: Preparation, Properties, and Electrocatalytic Application. *Frontiers in Chemistry* **2019**, *7* (October), 1–9. <https://doi.org/10.3389/fchem.2019.00671>.
- (257) Dourado Fernandes, C.; Harmanci, S.; Grünwald, A.; Hadzhieva, Z.; Oechsler, B. F.; Sayer, C.; Hermes de Araújo, P. H.; Boccaccini, A. R. Boron-Doped Mesoporous Bioactive Glass Nanoparticles (B-MBGs) in Poly(ϵ -Caprolactone)/Poly(Propylene Succinate-Co-Glycerol Succinate) Nanofiber Mats for Tissue Engineering. *ACS Appl Bio Mater* **2025**. <https://doi.org/10.1021/acsabm.4c01871>.
- (258) Moonesi Rad, R.; Alshemary, A. Z.; Evis, Z.; Keskin, D.; Altunbaş, K.; Tezcaner, A. Structural and Biological Assessment of Boron Doped Bioactive Glass Nanoparticles for Dental Tissue Applications. *Ceramics International* **2018**, *44* (8), 9854–9864. <https://doi.org/10.1016/j.ceramint.2018.02.230>.
- (259) Aguiar, H.; Serra, J.; González, P.; León, B. Structural Study of Sol-Gel Silicate Glasses by IR and Raman Spectroscopies. *Journal of Non-Crystalline Solids* **2009**, *355* (8), 475–480. <https://doi.org/10.1016/j.jnoncrysol.2009.01.010>.
- (260) Lucacel Ciceo, R.; Trandafir, D. L.; Radu, T.; Ponta, O.; Simon, V. Synthesis, Characterisation and in Vitro Evaluation of Sol-Gel Derived SiO₂-P₂O₅-CaO-B₂O₃ Bioactive System. *Ceramics International* **2014**, *40* (7 PART A), 9517–9524. <https://doi.org/10.1016/j.ceramint.2014.02.025>.
- (261) Parashar, V. K.; Orhan, J. B.; Sayah, A.; Cantoni, M.; Gijs, M. A. M. Borosilicate Nanoparticles Prepared by Exothermic Phase Separation. *Nature Nanotechnology* **2008**, *3* (10), 589–594. <https://doi.org/10.1038/nnano.2008.262>.
- (262) Li, Y.; Chen, X.; Ning, C.; Yuan, B.; Hu, Q. Facile Synthesis of Mesoporous Bioactive Glasses with Controlled Shapes. *Materials Letters* **2015**, *161*, 605–608. <https://doi.org/10.1016/j.matlet.2015.09.057>.
- (263) Nagrath, M.; Alhalawani, A.; Rahimnejad Yazdi, A.; Towler, M. R. Bioactive Glass Fiber Fabrication via a Combination of Sol-Gel Process with Electro-

- Spinning Technique. *Materials Science and Engineering C* **2019**, *101* (March), 521–538. <https://doi.org/10.1016/j.msec.2019.04.003>.
- (264) Xia, W.; Zhang, D.; Chang, J. Fabrication and in Vitro Biomineralization of Bioactive Glass (BG) Nanofibres. *Nanotechnology* **2007**, *18* (13). <https://doi.org/10.1088/0957-4484/18/13/135601>.
- (265) Kumar, A.; Han, S. S. Enhanced Mechanical, Biomineralization, and Cellular Response of Nanocomposite Hydrogels by Bioactive Glass and Halloysite Nanotubes for Bone Tissue Regeneration. *Materials Science and Engineering C* **2021**, *128* (June), 112236. <https://doi.org/10.1016/j.msec.2021.112236>.
- (266) Goh, Y. F.; Alshemary, A. Z.; Akram, M.; Abdul Kadir, M. R.; Hussain, R. In-Vitro Characterization of Antibacterial Bioactive Glass Containing Ceria. *Ceramics International* **2014**, *40* (1 PART A), 729–737. <https://doi.org/10.1016/j.ceramint.2013.06.062>.
- (267) Liverani, L.; Piegat, A.; Niemczyk, A.; El Fray, M.; Boccaccini, A. R. Electrospun Fibers of Poly(Butylene Succinate–Co–Dilinoleic Succinate) and Its Blend with Poly(Glycerol Sebacate) for Soft Tissue Engineering Applications. *European Polymer Journal* **2016**, *81*, 295–306. <https://doi.org/10.1016/j.eurpolymj.2016.06.009>.
- (268) Hu, J.; Kai, D.; Ye, H.; Tian, L.; Ding, X.; Ramakrishna, S.; Loh, X. J. Electrospinning of Poly(Glycerol Sebacate)-Based Nanofibers for Nerve Tissue Engineering. *Materials Science and Engineering: C* **2017**, *70*, 1089–1094. <https://doi.org/10.1016/J.MSEC.2016.03.035>.
- (269) Mansour, S. F.; El-Dek, S. I.; Ahmed, M. K. Physico-Mechanical and Morphological Features of Zirconia Substituted Hydroxyapatite Nano Crystals. *Scientific Reports* **2017**, *7* (January), 1–21. <https://doi.org/10.1038/srep43202>.
- (270) Li, Y.; Liu, C.; Zhai, H.; Zhu, G.; Pan, H.; Xu, X.; Tang, R. Biomimetic Graphene Oxide-Hydroxyapatite Composites via in Situ Mineralization and Hierarchical Assembly. *RSC Advances* **2014**, *4* (48), 25398–25403. <https://doi.org/10.1039/c4ra02821j>.
- (271) Madupalli, H.; Pavan, B.; Tecklenburg, M. M. J. Carbonate Substitution in the Mineral Component of Bone: Discriminating the Structural Changes, Simultaneously Imposed by Carbonate in A and B Sites of Apatite. *Journal of Solid State Chemistry* **2017**, *255* (July), 27–35. <https://doi.org/10.1016/j.jssc.2017.07.025>.

- (272) Ege, D.; Zheng, K.; Boccaccini, A. R. Borate Bioactive Glasses (BBG): Bone Regeneration, Wound Healing Applications, and Future Directions. *ACS Applied Bio Materials* **2022**, 5 (8), 3608–3622. <https://doi.org/10.1021/acsabm.2c00384>.

SUPPLEMENTARY MATERIAL

¹H-NMR spectroscopy was performed at 400 MHz with samples concentrated at 40 mg/mL in CDCl₃-d. Integrals of protons (e'), (e''), (b), (o), and (q) were directly obtained from the ¹H NMR spectrum, as presented in Figures S1-S4. Integral values for (h',i'), (h,i), (m',n'), (m,n), (j,k), and (e) protons were obtained indirectly taking into account the following equalities:

$$I_{f,g} = I_{h,i} \quad \text{(Equation 1)}$$

$$I_{m,n} = I_{j,k} \quad \text{(Equation 2)}$$

$$I_{h,i,m',n'} - I_{f,g} = I_{m',n'} \quad \text{(Equation 3)}$$

$$4 \cdot I_{e''} = I_{h',i'} \quad \text{(Equation 4)}$$

$$I_{h',i',c,d,e,j,k} - I_{h',i'} - I_{j,k} = I_{c,d,e} \quad \text{(Equation 5)}$$

$$I_{c,d} = \left(\frac{I_{c,d,e}}{5} \right) \cdot 4 \quad \text{(Equation 6)}$$

Total glycerol Integral was calculated as follows:

$$I_{Total\ GLY} = \frac{8 \cdot (I_{e''} + I_{e'} + I_e) + 4 \cdot (I_{(m,n)}) + 2 \cdot (I_{(m',n')})}{8} \quad \text{(Equation 7)}$$

Therefore, % of terminal glycerol, linear 1,3 and 1,2 substituted as well as dendritic % of glycerol in the polymers can be calculated using the following equations:

$$\%TG\ (1\ \text{ou}\ 3 - \text{sub}) = \frac{\frac{I_{m,n}}{2}}{I_{Total\ GLY}} \cdot 100 \quad \text{(Equation 8)}$$

$$\%TG2(2 - \text{sub}) = \frac{\frac{I_{m',n'}}{2}}{I_{Total\ GLY}} \cdot 100 \quad \text{(Equation 9)}$$

$$\%L\ 1,3(1,3 - \text{sub}) = \frac{I_e}{I_{Total\ GLY}} \cdot 100 \quad \text{(Equation 10)}$$

$$\%L\ 1,2(1,2 - \text{sub}) = \frac{I_{e'}}{I_{Total\ GLY}} \cdot 100 \quad \text{(Equation 11)}$$

$$\%Den(1,2,3 - \text{sub}) = \frac{I_{e''}}{I_{Total\ GLY}} \cdot 100 \quad \text{(Equation 12)}$$

where, %TG refers to terminal glycerol 1 or 3 substituted units; %TG2 refers to terminal glycerol 2 substituted units; %L 1,3 refers to 1,3 substituted linear glycerol units; %L 1,2 refers to 1,2 linear glycerol units; %D refers to dendritic or 1,2,3 substituted glycerol units.

The % regioselectivity of acylation at the primary sites of glycerol units was calculated from the normalized intensity using the following equation:

$$\%_{REGI} = \frac{2 \cdot \frac{I_{c,d}}{4} + 2 \cdot I_{e''} + I_{e'} + I_l}{2 \cdot \frac{I_{c,d}}{4} + 3 \cdot I_{e''} + 2 \cdot I_{e'} + I_l + \frac{I_{m',n'}}{2}} \cdot 100 \quad (\text{Equation 13})$$

The relative abundance of G, 1,3-PDO and DS end groups were calculated as follows:

$$I_{END\ GROUPS} = \frac{I_{m,n} + I_{m',n'} + 2I_p}{2} \quad (\text{Equation 14})$$

$$\%_G = \frac{\frac{I_{m,n} + I_{m',n'}}{2}}{I_{END\ GROUPS}} \cdot 100 \quad (\text{Equation 14})$$

$$\%_{DS} = \frac{I_o}{I_{END\ GROUPS}} \cdot 100 \quad (\text{Equation 15})$$

$$\%_{PDO} = \frac{I_a}{I_{END\ GROUPS}} \cdot 100 \quad (\text{Equation 16})$$

The degree of branching can be calculated using the following equation:

$$\text{Degree of Branching (DB)} = \frac{2D}{2D + L_{1,3\ sub}} \cdot 100 \quad (\text{Equation 18})$$

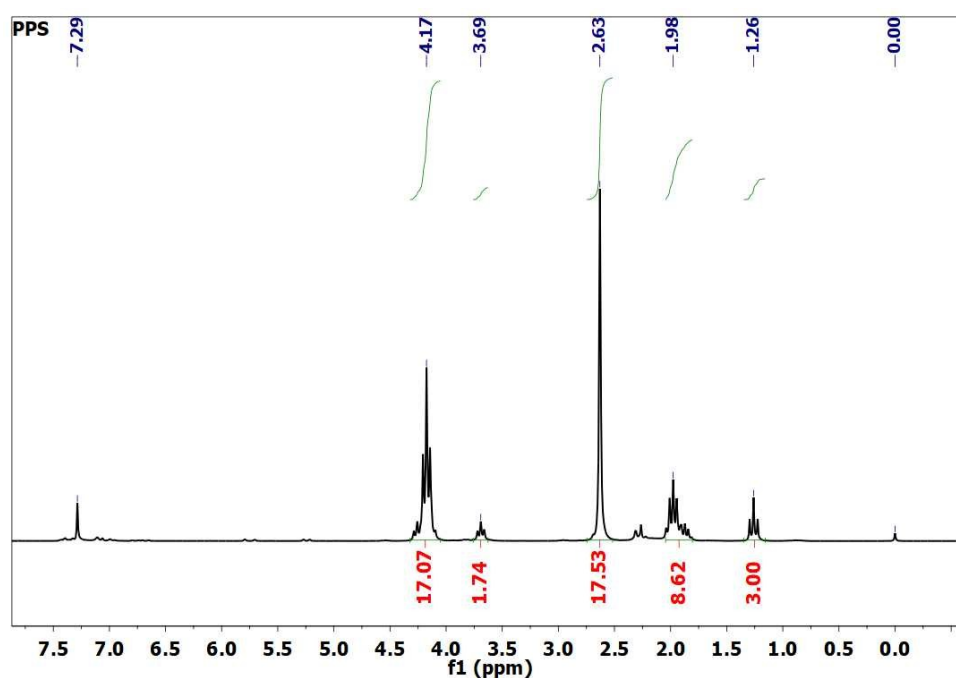


Figure S 1. ¹H-NMR spectra of PPS synthesized without glycerol and 10 wt% of N435 in relation to the monomers at 90°C for 24h.

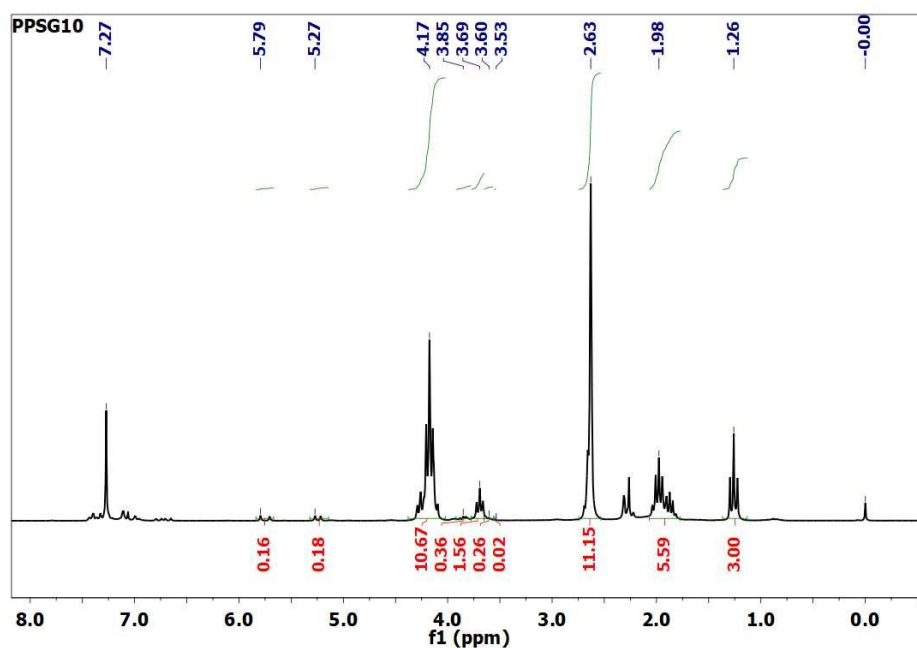


Figure S 2. ^1H -NMR spectra of PPSG10 synthesized with 10% of the glycerol and 10 wt% of N435 in relation to the monomers at 90 °C for 24 h.

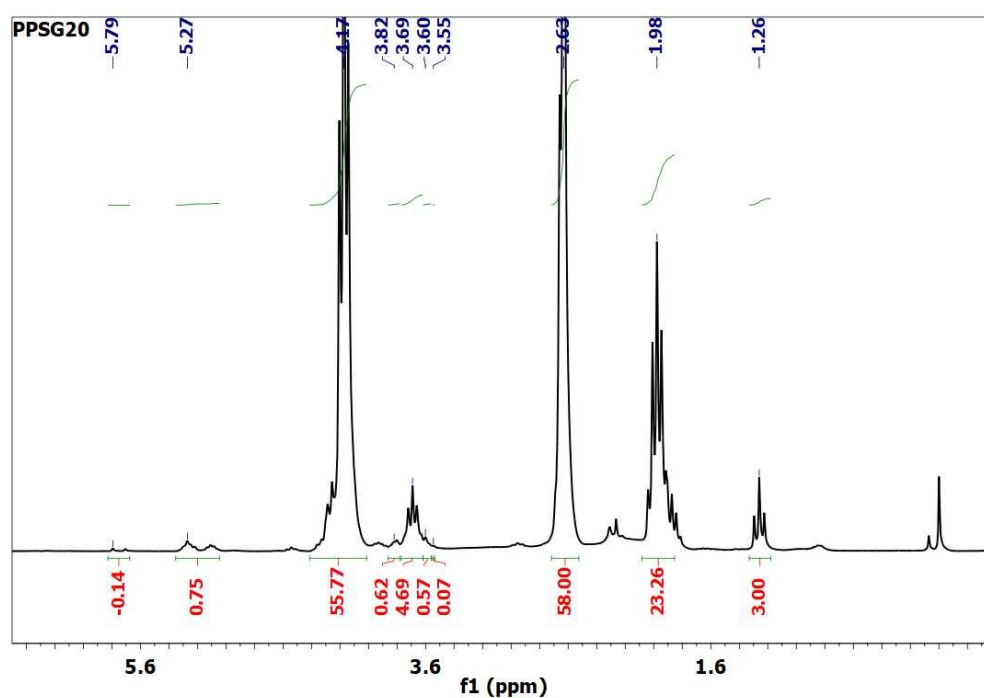


Figure S 3. ^1H -NMR spectra of PPSG20 synthesized with 10% of the glycerol and 10 wt% of N435 in relation to the monomers at 90 °C for 24 h.

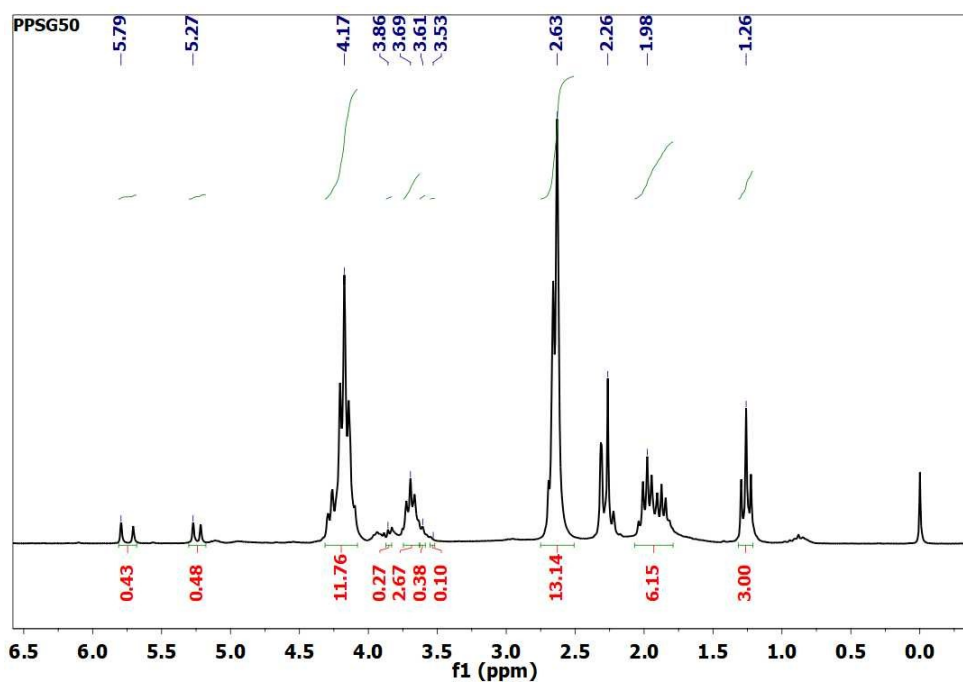


Figure S 4. ^1H -NMR spectra of the soluble fraction of PPSG50 synthesized with 10% of the glycerol and 10 wt% of N435 in relation to the monomers at 90 °C for 24 h.

^{13}C -NMR spectroscopy was performed at 700 MHz with samples concentrated at 80 mg/mL in $\text{CDCl}_3\text{-d}$. ^{13}C -NMR spectrum are presented in Figure S5 and Table S2. The % regioselectivity in the primary sites of the glycerol units was calculated from the intensity normalized in relation to the tertiary carbons using the following equation:

$$\%_{\text{REGI}} = 100 - \frac{E'' + E' + E + L}{I_{\text{gly total}}} \cdot 100$$

Where $I_{\text{gly total}}$ is the sum of all carbon signals referring to glycerol:

$$I_{\text{gly total}} = \frac{14 \cdot \sum I_{\text{gly}}}{11}$$

Table S 1. Representation of peaks in ppm of carbons and their respective integrals of PPS, PPSG₁₀ and PPSG₂₀ found in ¹³C NMR.

		Protons	ppm	Description	PPS	PPSG ₁₀	PPSG ₂₀
1,2,3-sub	Dendritic; ROCH ₂ - CH (OR')-CH ₂ OR	1	63.1	E''		0.09	0.01
	Dendritic; ROCH ₂ - CH (OR')-CH ₂ OR	1	79.1	E''		0.05	0.01
	Linear 1,2; ROCH ₂ - CH (OR')-CH ₂ OH	1	72	E'		0.11	0.02
	Linear 1,3; ROCH ₂ - CH (OH)-CH ₂ OR	1	67.6	E		0.15	0.03
	Linear 1,2; ROCH ₂ - CH (OR')-CH ₂ OH	1	61.5	H		0.73	0.72
	Linear 1,2; ROCH ₂ - CH (OR')-CH ₂ OH	1	62.5	F		0.11	0.01
1,3-sub	Linear 1,3 ROCH ₂ -CHOH-CH ₂ OR	2	65	J		0.18	0.01
Terminal Gly	Terminal 1,3 ROCH ₂ -CHOH-CH ₂ OH	<u>1</u>	<u>65</u>	G		0.18	0.01
	Terminal 2 HOCH ₂ - CH (OR)CH ₂ OH	1	75.6	L'		0.07	0.01
	Terminal 2 HOCH ₂ -CH(OR) CH ₂ OH	2	61.3	M',N'		0.73	0.72
	Terminal 1,3 ROCH ₂ -CHOH-CH ₂ OH	1	70.9	L		0.12	0.02
	Terminal 1,3 ROCH ₂ -CHOH- CH ₂ OH	1	63.7	N		0.09	0.01
Terminal DS	Terminal DS ROCOCH ₂ -CH ₂ -COOCH ₂ CH ₃	1	14.1	O	0.07	0.08	0.02
	Terminal DS ROCOCH ₂ -CH ₂ -COOCH ₂ CH ₃	1	61.3	O'	0.86	0.73	0.72
Terminal PDO	Terminal PDO ROCH ₂ -CH ₂ -CH ₂ OH ₃	1	59	A	0.12	0.11	0.04
	Terminal PDO ROCH ₂ - CH ₂ -CH ₂ OH ₃	1	30.9	B'	0.09	0.12	0.04
DS	ROCOCH ₂ -CH ₂ -COOR	2	173.1	R	0.44	0.41	0.38
	ROCOCH ₂ -CH ₂ -COOR	2	29.5	Q	1	1	1
PDO	ROCH ₂ -CH ₂ -CH ₂ OR	2	61	P	0.86	0.73	0.72
	ROCH ₂ -CH ₂ -CH ₂ OR	1	27.6	B	0.42	0.44	0.40

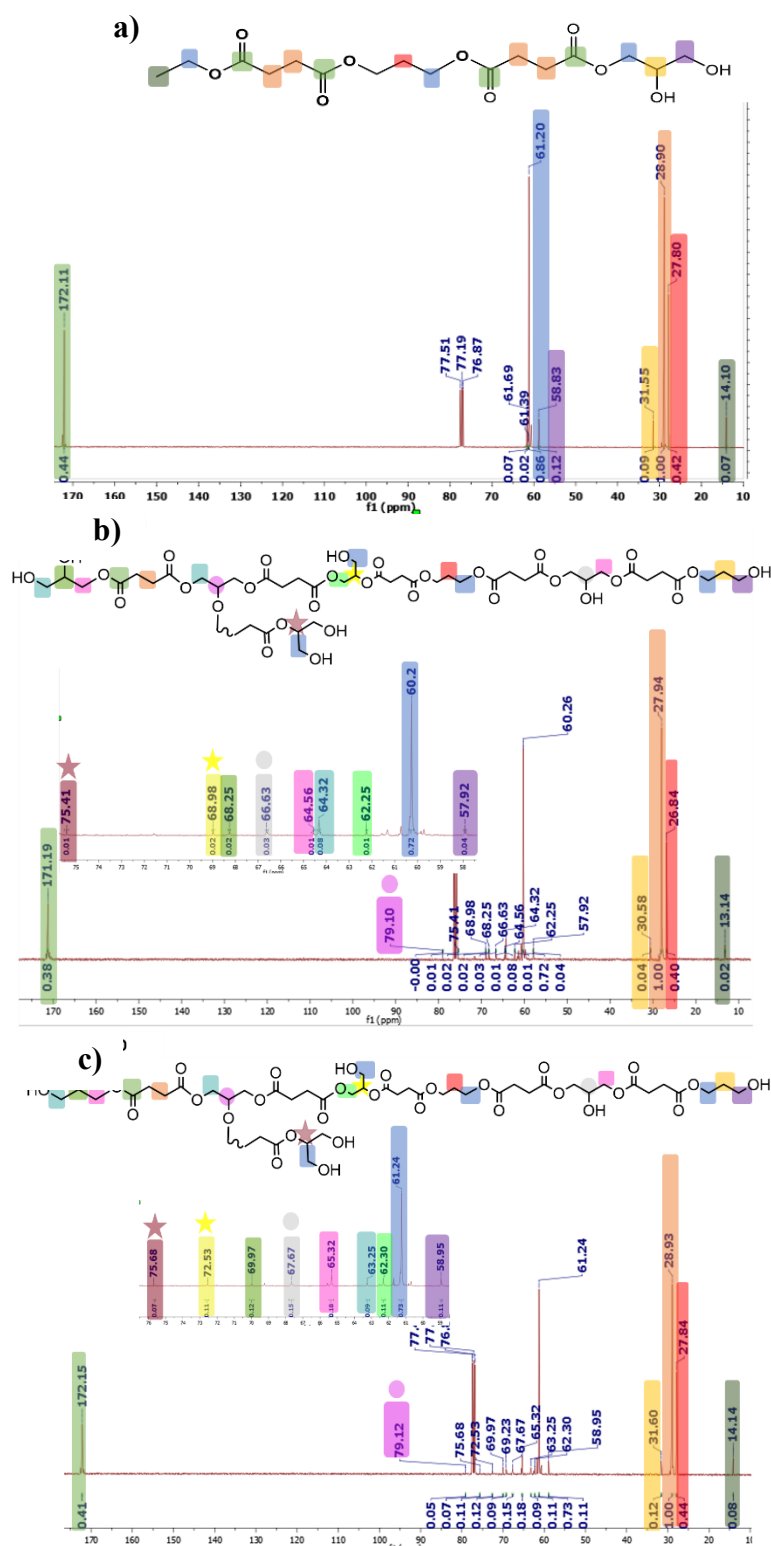


Figure S 5. ^{13}C -NMR spectra of PPS(a), PPSG10 (b) and PPSG20 (c) synthesized with 0, 10 and 20% of the glycerol respectively and 10 wt% of N435 in relation to the monomers at 90 °C for 24 h

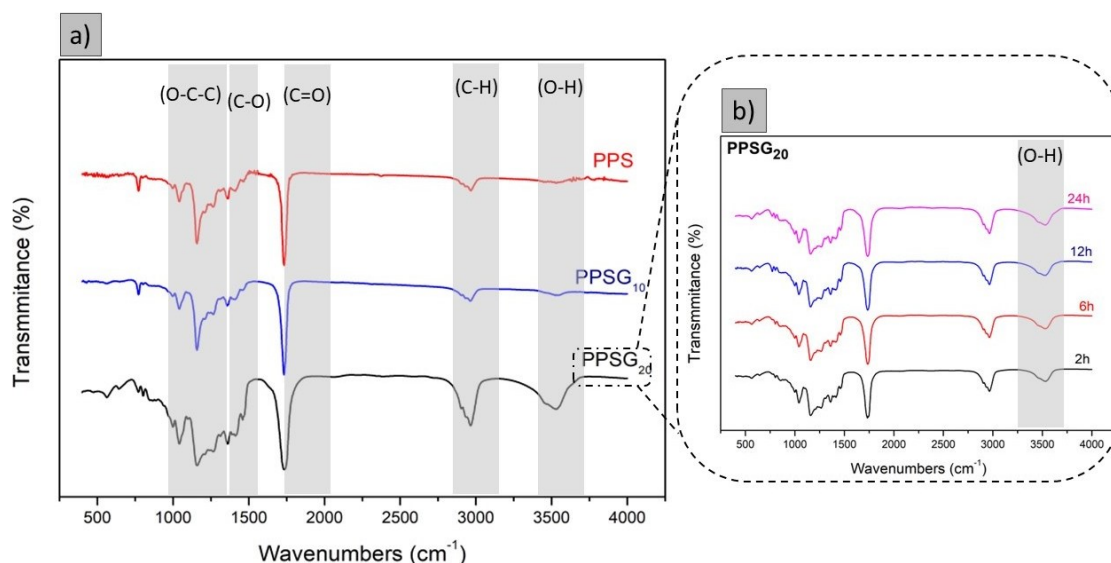


Figure S 6. FTIR analysis of the synthesized samples: (A) Verification of synthesized PPS polyester, and PPS with 10% of glycerol (PPSG10) and 20% of glycerol (PPSG20); (B) Verification of synthesized PSG20 polyester over time.

Table S 2. Thermal properties of electrospun PCL and PCL/PPSG fibers determined by DSC analysis. The table presents the melting temperature (T_m), crystallization temperature (T_c), and enthalpy of fusion (ΔH) for electrospun fiber samples of pure PCL and PCL/PPSG blends (5%, 10%, and 20% PSG).

Sample	T_m (°C)	ΔH (J/g)	T_c (°C)
PCL	53.1	3.8	33.8
PCL/5PPSG	51.9	3.8	33.3
PCL/10PPSG	52.3	3.8	32.9
PCL/20PPSG	52.3	3.1	32.9

Melting temperature (T_m), crystallization temperature (T_c), enthalpy of fusion (ΔH).

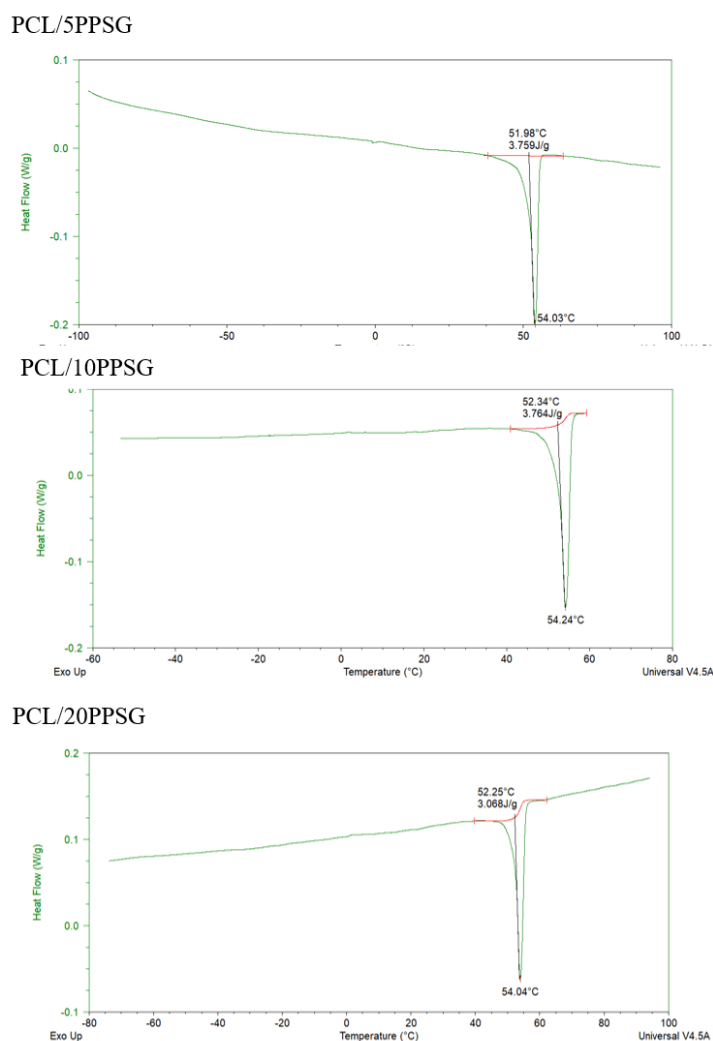


Figure S 7. DSC thermograms of electrospun fibers of PCL and PCL/PPSG blends.

Table S 3. Characteristic FTIR absorption bands of PCL electrospun mats with the “green” PPSG and BG bioactive glass and their designation according to^{195,215}

Absorption band (cm ⁻¹)	Description
2935; 2864; 1365	Stretching of alkyl group (-CH ₂)
1726	Stretching of carbonyl group (C=O)
1296	Backbone C-C and C-O stretching
1166	Stretching of ester group (C-O)
900; 1100	Stretching of silicate group (Si-O-Si and Si-O)
470	Bending of silicate (Si-O-Si)

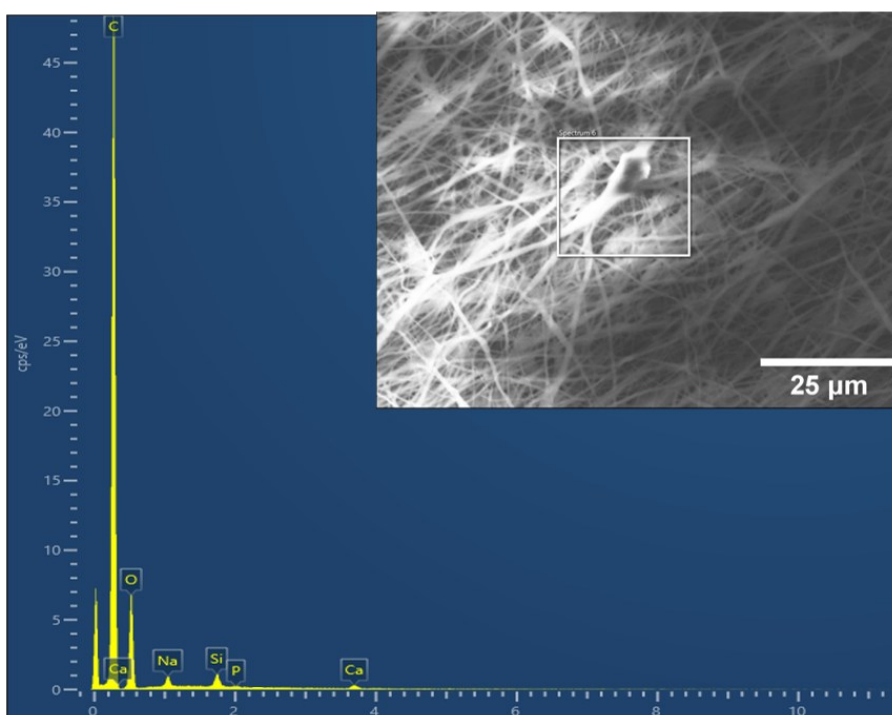


Figure S 8. EDX analysis of electrospun fiber with PCL/PPSG/5BG bioglass

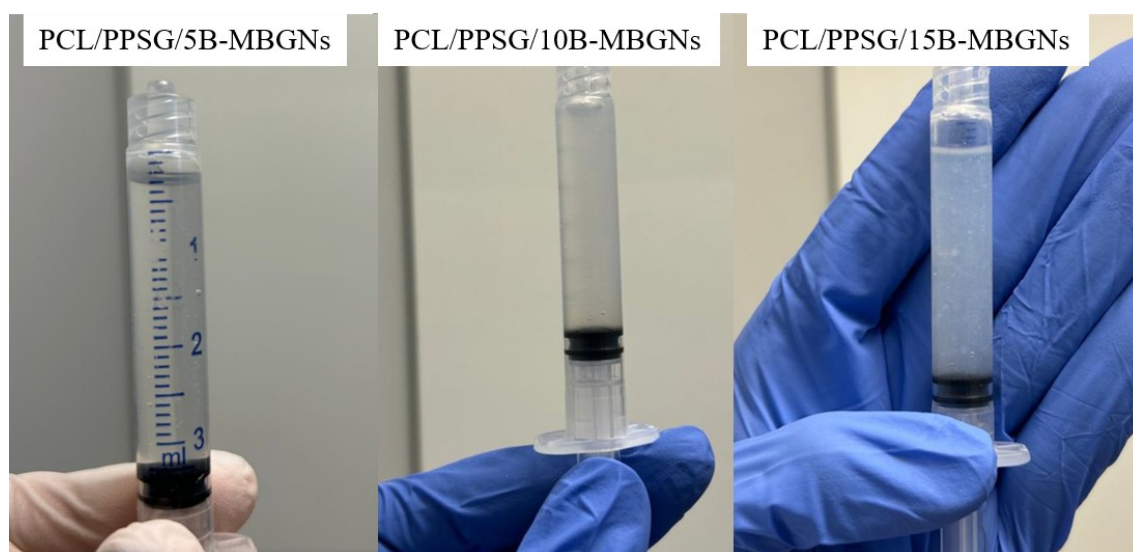


Figure S 9. Polymer solutions of PCL/PPSG/B-MBGNs with 5, 10, and 15 wt% B-MBGNs, showing increased nanoparticle agglomeration at 15 wt%.

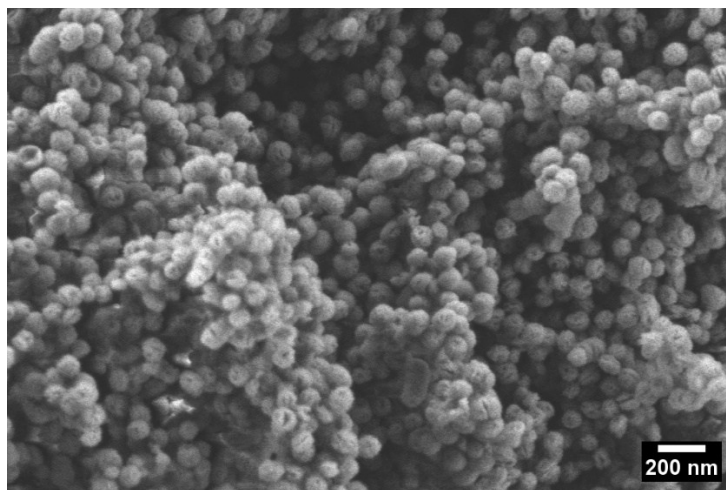


Figure S 10. SEM image of the dispersed B-MBGs nanoparticles.