

VOL III

THE GREAT WORLD OF NANOTECHNOLOGY

Emilio Castro Otero
(Organizador)

 EDITORA
ARTEMIS
2025

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FOREWORD

We are thrilled to present the third installment of ***The Great World of Nanotechnology***, a volume dedicated to exploring the cutting edge of nanotechnology applications, from the fundamental science of materials to their tangible impact on health, industry, and the environment. This book is meticulously structured into four thematic blocks, designed to guide the reader through the latest innovations that seek to solve crucial challenges in modern society.

The first pillar of this volume, ***Fundamentals of Nanomaterial Manufacturing and Characterization***, delves into the critical methodologies for creating high-quality nanostructures. Chapter 1 offers systematic research on the Effects of the Transfer Process on the Structure of Graphene Synthesized by Chemical Vapor Deposition. Complementing this, Chapter 2 examines Polymer Nanofibers via Airbrushing.

In ***Nanomaterials for Surface Engineering and Smart Sensors***, the chapters explore how these nanostructures can be applied in functional devices and material protection. Chapter 3 presents advances in the development of sensors based on graphene functionalized with nanoparticles. Similarly, Chapter 4 addresses the electrodeposition of nanostructured metal coatings.

Nanotechnology in Biomedical and Pharmaceutical Applications brings three chapters focusing on the use of nanotechnology to improve human health. Chapter 5 evaluates the Cytotoxic Effect of Nanoemulsion with Pumpkin Seed Oil on Breast Cancer Cell Lines. In the field of tissue engineering, Chapter 6 describes the development of Alginate/Collagen Structures Enhanced with Conductive Nanoparticles (PEDOT) for the Regeneration of Small-Diameter Blood Vessels. Finally, Chapter 7 discusses the development of an antiseptic gel based on bioactive compounds encapsulated in nanoparticles.

Concluding our exploration, ***Nanotechnological Solutions for Environmental Remediation*** addresses the water pollution crisis. The final chapter describes the Removal of Arsenic from Groundwater Using Recycled Iron Nanoparticles through the development of a low-cost filter that uses iron nanoparticles.

We invite you to immerse yourself in reading *The Great World of Nanotechnology*. Vol. III, where science on the smallest scale translates into large-scale solutions. We sincerely hope that this compilation of advanced research will not only be of utmost interest to you, but also inspire new directions of study and application in this infinitely promising field.

Emilio Castro Otero

PRÓLOGO

É com grande entusiasmo que apresentamos a terceira edição de ***The Great World of Nanotechnology***, um volume dedicado a explorar a vanguarda das aplicações nanotecnológicas, desde a ciência fundamental dos materiais até o seu impacto tangível na saúde, na indústria e no ambiente. Este livro está meticulosamente estruturado em quatro eixos temáticos, concebidos para guiar o leitor através das mais recentes inovações que procuram resolver desafios cruciais na sociedade moderna.

O primeiro eixo deste volume, ***Fundamentos da Fabricação e Caracterização de Nanomateriais***, aprofunda as metodologias críticas para a criação de nanoestruturas de alta qualidade. O Capítulo 1 oferece uma investigação sistemática sobre os Efeitos do Processo de Transferência na Estrutura do Grafeno Sintetizado por Deposição Química de Vapor e o segundo capítulo examina as Nanofibras Poliméricas via Aerografia.

Em seguida, o eixo ***Nanomateriais para Engenharia de Superfícies e Sensores Inteligentes***, explora como essas nanoestruturas podem ser aplicadas em dispositivos funcionais e proteção de materiais. O Capítulo 3 apresenta os avanços no desenvolvimento de Sensores Baseados em Grafeno Funcionalizado com Nanopartículas. Paralelamente, e o Capítulo 4 aborda a Eletrodeposição de Revestimentos Metálicos Nanoestruturados.

O eixo ***Nanotecnologia em Aplicações Biomédicas e Farmacêuticas***, centra-se na utilização da nanotecnologia para melhorar a saúde humana. O Capítulo 5 avalia o Efeito Citotóxico da Nanoemulsão com Óleo de Semente de Abóbora em Linhagens de Células de Cancro da Mama. No campo da engenharia de tecidos, o Capítulo 6 descreve o desenvolvimento de Estruturas de Alginato/Colagénio Melhoradas com Nanopartículas Condutoras (PEDOT) para a regeneração de Vasos Sanguíneos de Pequeno Diâmetro. Finalmente, o Capítulo 7 expõe a Elaboração de um Gel Antisséptico Baseado em Compostos Bioativos Encapsulados em Nanopartículas.

Concluindo a nossa exploração, o eixo ***Soluções Nanotecnológicas para Remédios Ambientais***, aborda a crise da contaminação da água. Este último capítulo descreve a Eliminação de Arsénico das Águas Subterrâneas Usando Nanopartículas de Ferro Reciclado, através do desenvolvimento de um filtro de baixo custo que utiliza nanopartículas de ferro.

Convidamo-lo a mergulhar na leitura de *The Great World of Nanotechnology Vol. III*, onde a ciência na escala mais pequena se traduz em soluções em grande escala. Esperamos sinceramente que esta compilação de pesquisas avançadas não só seja do seu interesse, mas também inspire novos rumos de estudo e aplicação neste campo infinitamente promissor.

Emilio Castro Otero

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CHAPTER 6

ENHANCED ALGINATE/COLLAGEN SCAFFOLDS WITH CONDUCTIVE POLY (3,4-ETHYLENEDIOXYTHIOPHENE) NANOPARTICLES FOR NEXT-GENERATION SMALL-DIAMETER TISSUE-ENGINEERED BLOOD VESSELS

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ABSTRACT: Cardiovascular disease remains the leading cause of death worldwide, highlighting the urgent need for improved

vascular grafts. While large-diameter tissue-engineered blood vessels (TEBVs) have shown therapeutic success, developing small-diameter TEBVs (inner diameter ≤ 6 mm) remains challenging. This study reports a novel method for fabricating small-diameter TEBVs using a triple coaxial nozzle to co-extrude alginate, collagen, and a sacrificial polymer, forming scaffolds closely mimicking native vessel microarchitecture. The resulting conduits had an outer diameter of approximately 1.6 mm and wall thickness of about 265 μm . A key innovation involved coating the scaffold's surface with conductive poly (3,4-ethylenedioxythiophene) (PEDOT) nanoparticles using in situ interfacial polymerization. Optimal PEDOT-to-APS ratios (4:2 and 8:2) were identified, which increased scaffold swelling capability, slowed degradation, and significantly improved mechanical strength (burst pressure increased from 1.13 to 3.38 bar) and electrical conductivity (up to 122 $\mu\text{S/cm}$). Enhanced surface roughness and hydrophilicity resulting from the PEDOT layer promoted the adhesion and proliferation of human aortic smooth muscle cells (hASMCs). Notably, lyophilization was found unnecessary, as direct extrusion yielded reproducible, favorable conduit properties. The conductive, biocompatible nature of these hybrid hydrogel scaffolds suggests great potential for use in vascular regeneration and potentially for vascularizing artificial organs, providing a promising route toward functional small-diameter vascular replacements.

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KEYWORDS: blood vessel; PEDOT nanoparticles; conductive hydrogel; vascular regeneration; smooth muscle cells.

ESTRUTURAS DE ALGINATO/COLAGÊNIO MELHORADAS COM NANOPARTÍCULAS CONDUTORAS DE POLI (3,4-ETILENODIOXITIOFENO) PARA VASOS SANGUÍNEOS DE PEQUENO DIÂMETRO DE PRÓXIMA GERAÇÃO, CONCEBIDOS ATRAVÉS DE ENGENHARIA DE TECIDOS

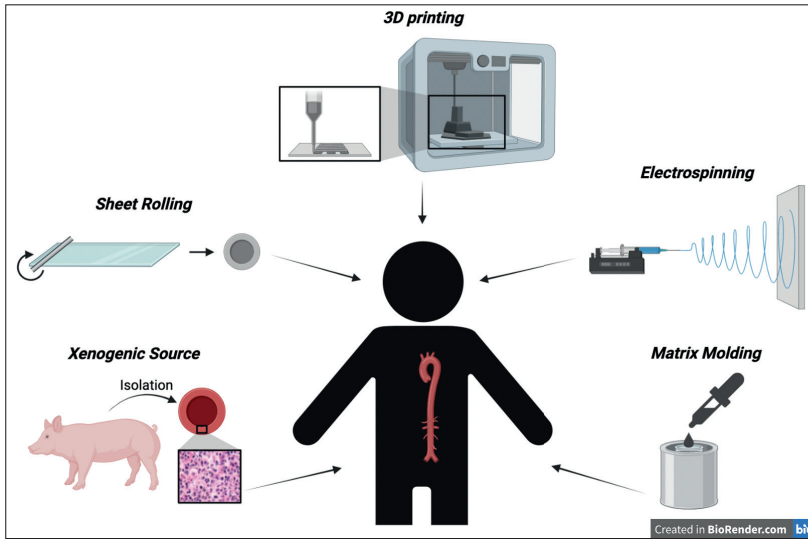
RESUMO: As doenças cardiovasculares continuam a ser a principal causa de morte em todo o mundo, destacando a necessidade urgente de melhorar os enxertos vasculares. Embora os vasos sanguíneos de grande diâmetro criados por engenharia de tecidos (TEBVs) tenham demonstrado sucesso terapêutico, o desenvolvimento de TEBVs de pequeno diâmetro (diâmetro interno ≤ 6 mm) continua a ser um desafio. Este estudo relata um método inovador para fabricar TEBVs de pequeno diâmetro usando um bico coaxial triplo para coextrudar alginato, colagênio e um polímero sacrificial, formando arcabouços que imitam de perto a microarquitetura dos vasos nativos. Os condutos resultantes tinham um diâmetro externo de aproximadamente 1,6 mm e espessura de parede de cerca de 265 μm . Uma inovação importante envolveu o revestimento da superfície da estrutura com nanopartículas condutoras de poli(3,4-etilenodioxietileno) (PEDOT) usando polimerização interfacial in situ. Foram identificadas as proporções ideais de PEDOT para APS (4:2 e 8:2), que aumentaram a capacidade de expansão da estrutura, retardaram a degradação e melhoraram significativamente a resistência mecânica (a pressão de ruptura aumentou de 1,13 para 3,38 bar) e a condutividade elétrica (até 122 $\mu\text{S/cm}$). A rugosidade superficial e a hidrofiliabilidade aprimoradas resultantes da camada de PEDOT promoveram a adesão e a proliferação de células musculares lisas da aorta humana (hASMCs). Notavelmente, a liofilização foi considerada desnecessária, pois a extrusão direta produziu propriedades de conduto reproduzíveis e favoráveis. A natureza condutiva e biocompatível dessas estruturas híbridas de hidrogel sugere um grande potencial para uso na regeneração vascular.

PALAVRAS-CHAVE: vaso sanguíneo; nanopartículas PEDOT; hidrogel condutivo; regeneração vascular; células musculares lisas.

1. THE CRITICAL NEED FOR FUNCTIONAL SMALL-DIAMETER VASCULAR GRAFTS

Cardiovascular disease (CVD) remains the preeminent global health challenge, accounting for the largest share of morbidity and mortality worldwide (Kim et al., 2025). A cornerstone of surgical intervention for advanced CVD, particularly in cases of severe atherosclerosis or trauma, involves the bypass or replacement of occluded or damaged blood vessels. While autologous vascular grafts (vessels harvested from the patient) are the gold standard due to their superior biocompatibility and patency rates, their use is often limited by donor site morbidity, insufficient vessel quality, or the sheer lack of available suitable vessels. This pressing clinical need has driven extensive research into the development of artificial vascular grafts (Lang et al., 2024).

Figure 1. Different techniques for developing small diameter TEBVs.



The field of tissue engineering has delivered notable successes, particularly in the creation of large-diameter tissue-engineered blood vessels (TEBVs diameter > 6 mm), which have shown promising clinical outcomes. However, the engineering of functional small-diameter TEBVs (diameter $\approx$ 6 mm) continues to represent a significant, unmet challenge (È. Bosch-Rué et al., 2023; E. Bosch-Rué et al., 2025). These small-diameter grafts are highly prone to failure due to two primary mechanisms: thrombosis (clotting) and intimal hyperplasia (excessive smooth muscle cell proliferation and extracellular matrix deposition leading to luminal narrowing). A fundamental difficulty lies in recreating the intricate biophysical and biochemical microenvironment of native small arteries, which includes appropriate mechanical strength, compliance, and, crucially, bioelectrical signaling capabilities (Zhang et al., 2021). This current research addresses this critical gap by developing a sophisticated hybrid scaffold that integrates natural polymers with a conductive element to enhance cellular response and mechanical durability.

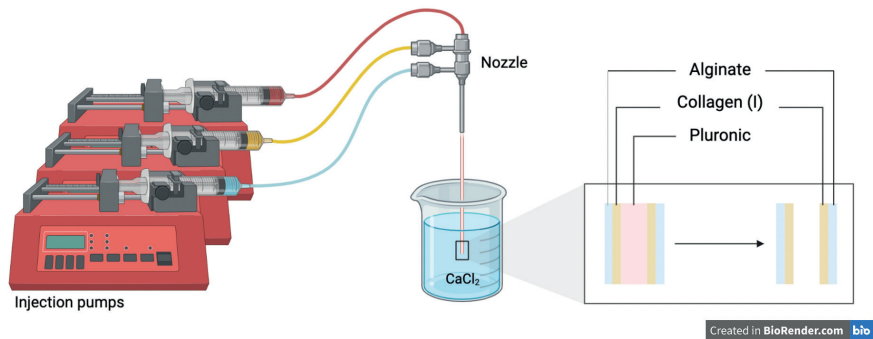
2. FABRICATION AND CONDUCTIVE POLYMER INCORPORATION VIA INTERFACIAL POLYMERIZATION

2.1. SCAFFOLD FABRICATION AND PREPARATION

The foundation of the TEBV mimicry involved the use of a porous, biocompatible hydrogel composed of alginate (Alg) and collagen (Col) (T. Hu & Lo, 2021; X. Hu et al., 2024). These TEBV-like acellular tubular structures were first fabricated using established extrusion techniques, as previously detailed in the literature (E. Bosch-Rué et al., 2020).

Alginate provides structural integrity and is cost-effective, while collagen offers crucial cell-binding sites, mimicking the natural extracellular matrix (ECM). To obtain the nanoparticles exclusively in the outer layer (alginate), the scaffolds (1.5 cm long) were placed on a metal mandrel in which each edge was sutured with non-absorbable silk sutures (Silkam®), preventing the entry of the reagents to the inner layer.

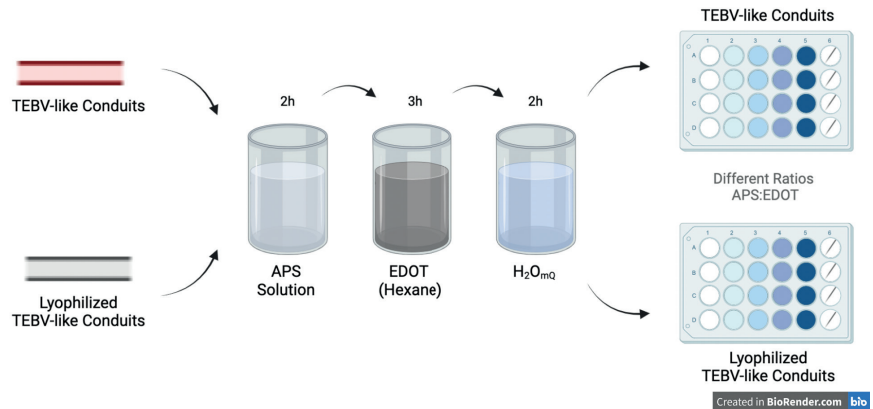
Figure 2. Schematic representation of TEBV-like structures through extrusion method.



2.2. IN SITU INTERFACIAL POLYMERIZATION OF PEDOT NANOPARTICLES

To impart necessary conductivity, the scaffolds were functionalized with poly(3,4-ethylenedioxythiophene) (PEDOT). The interfacial polymerization method via *in situ* synthesis was specifically selected for the incorporation of the 3,4-ethylenedioxythiophene (EDOT) monomer (purity 99.9%) (Wang, Guan, et al., 2017; Wang, Sun, et al., 2017; Xu et al., 2018). This technique leverages the phase boundary between two immiscible liquids – an aqueous phase containing the oxidant and an organic phase (hexane) containing the monomer. The static polymerization of the EDOT monomer at this interface promotes the directed formation of PEDOT nanoparticles onto the scaffold structure.

Figure 3. Preparation of the conductive PEDOT/Alg/Col hydrogels.



Crucially, to mimic the native vessel architecture and ensure unidirectional cell stimulation, the functionalization was strategically confined to the outer layer (alginate-rich side) of the scaffold. Scaffolds (approximately 1.5 cm in length) were carefully mounted onto a non-reactive metal mandrel. Each edge was securely closed using non-absorbable silk sutures (e.g., Silkam®), effectively sealing the inner lumen and preventing the ingress of polymerization reagents to the inner layer.

2.3. OPTIMIZATION OF CONDUCTIVE NANOPARTICLE ASSEMBLY

Two distinct physical treatments of the Alg/Col scaffolds were assessed prior to conductive modification:

1. Directly extruded hydrogels (Hydrated condition).
2. Samples subjected to a freeze-drying process (Freezing at -20°C for 12 hours, followed by lyophilization for 24 hours).

For the *in-situ* polymerization, the Alg/Col structures were first immersed for 2 hours in a 2 mL aqueous solution containing ammonium persulfate (APS) as the oxidant. The swollen samples were subsequently transferred and incubated for 3 hours in a 2 mL hexane solution containing the EDOT monomer (0.4 mol/L). The progress of the PEDOT formation was macroscopically observed by the scaffolds' gradual transition from a colorless or white state to a distinctive dark navy blue/black coloration, indicative of the successful production of PEDOT nanoparticles *in situ*. Systematic optimization was performed by preparing samples across five different molar concentration ratios of APS to EDOT (0, 0.5, 1, 2, and 4) to determine the optimal synthesis conditions. Finally, the ducts were thoroughly purified by immersion in deionized water for 2 hours and careful washing to eliminate any unreacted reagents or byproducts.

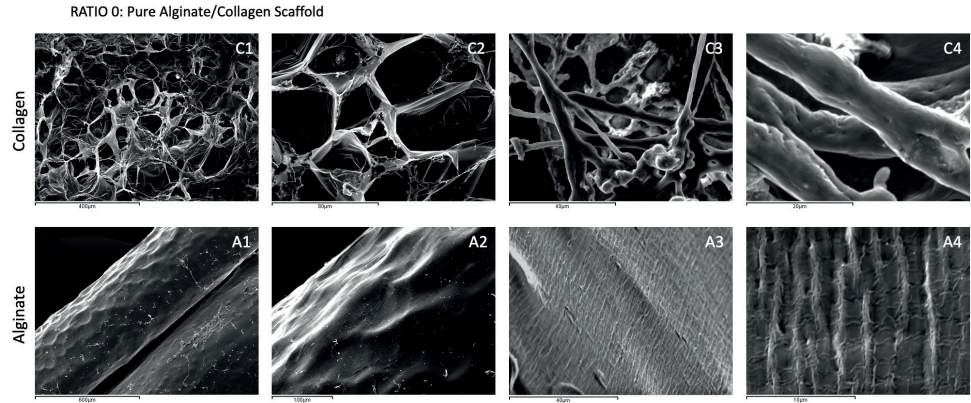
3. PHYSICOCHEMICAL AND BIOLOGICAL CHARACTERIZATION

3.1. MORPHOLOGICAL AND DISTRIBUTION ANALYSIS

The successful polymerization of PEDOT was immediately confirmed by the distinct color shift of the pure white Alg/Col structures to black following the nanoparticle assembly. Scanning Electron Microscopy (SEM) images revealed that the PEDOT nanoparticles, with an average diameter of approximately 50 nm, were successfully grown and localized on the surface of the outermost layer of the samples. Furthermore, the number and surface coverage of these nanoparticles were observed to increase proportionally with higher monomer and oxidant concentrations. Notably, samples where the ends were left

unsutured exhibited PEDOT presence within the inner layer, which critically coincided with a detrimental loss of the characteristic porous structure in that region.

Figure 4. Morphology of the TEBV-like structures was characterized by scanning electron microscope (SEM) (JSM-5410, JEOL).

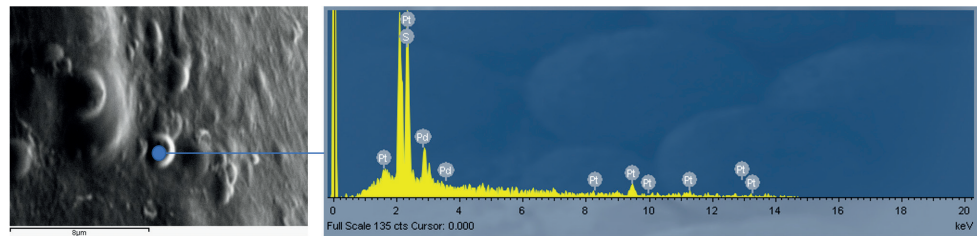


Comparative analysis of the two scaffold pre-treatments (hydrated vs. freeze-dried) showed significant differences in nanoparticle assembly. The data indicated that the freeze-drying process (Group L) prior to PEDOT incorporation resulted in a non-uniform nanoparticle assembly compared to the directly extruded hydrogels. This finding suggests that the structural changes induced by lyophilization (e.g., pore collapse or altered surface chemistry) are detrimental to the final homogeneous distribution of the conductive coating, potentially leading to suboptimal electrical stimulation of cells *in vivo*.

3.2. PHYSICOCHEMICAL AND MECHANICAL PROPERTY ENHANCEMENT

Analysis of the mechanical and swelling properties identified that the molar concentration ratios of APS to EDOT of 4:2 and 8:2 provided the most favorable outcome. These ratios not only demonstrated an increase in the swelling rates of the pure Alg/Col scaffolds but also significantly contributed to decreasing their degradation rate and substantially increasing their overall mechanical properties.

Figure 5. EDS spectra analysis of TEBV surface PEDOT.



The burst pressure test, a key metric for assessing vascular graft strength, showed that the maximum pressure at rupture increased directly with the amount of incorporated PEDOT. This data strongly supports the conclusion that the nanoparticle integration significantly reinforces the duct wall integrity. The presence of PEDOT was further corroborated via Energy Dispersive X-ray Spectroscopy (EDS) spectra analyses, which successfully identified the characteristic sulfur (S) atoms within the PEDOT chemical structure coupled with SEM imaging.

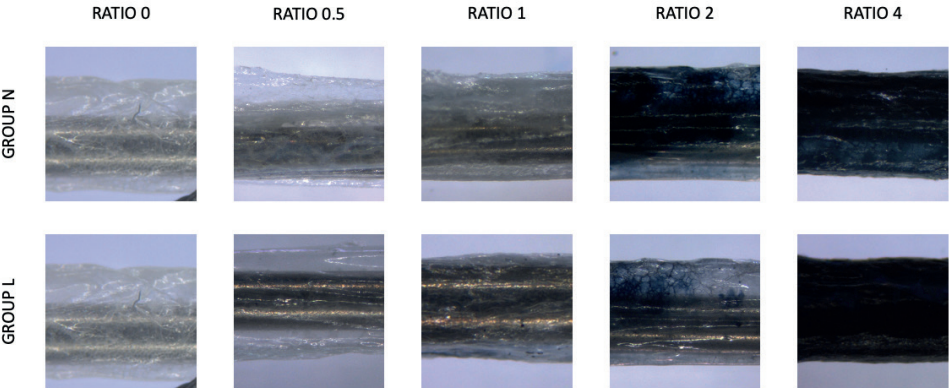
Figure 6. Burst pressure of TEBVs with different APS:EDOT ratios.



3.3. HYDROPHILICITY AND ELECTRICAL CONDUCTIVITY

The incorporation of the conductive nanoparticles dramatically improved the hydrophilicity of the scaffolds compared to the pure hydrogels. This was quantitatively demonstrated by a decrease in the contact angle value corresponding to a higher molar concentration ratio of APS to EDOT. Enhanced hydrophilicity is crucial for promoting protein adsorption and subsequent cell adhesion.

Figure 7. Images of PEDOT nanoparticles assembly on the surface of TEBVs with different APS:EDOT ratios.



The electrical conductivity of the hydrated scaffolds was a primary focus, measured at various time points (30 minutes, 1 hour, and 24 hours after immersion in high-purity water). The results clearly indicated that higher nanoparticle density translated to greater electrical conductivity. Critically, all four scaffolds with the highest PEDOT loading achieved an electrical conductivity value greater than $1.0 \times 10^{-3} \text{ mS/cm}$. This threshold is generally considered sufficient to allow the scaffold to function as an effective electrode for electrical stimulation of vascular cells, a vital function for tissue maturation.

Figure 8. SEM images showing PEDOT nanoparticles on the surface of TEBVs with different APS:EDOT ratios.

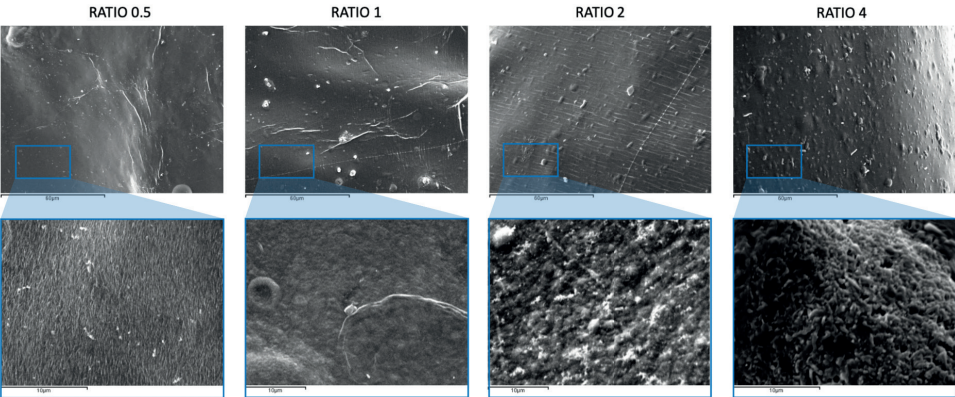


Figure 9. Hydrophilicity of TEBVs with different APS:EDOT ratio.

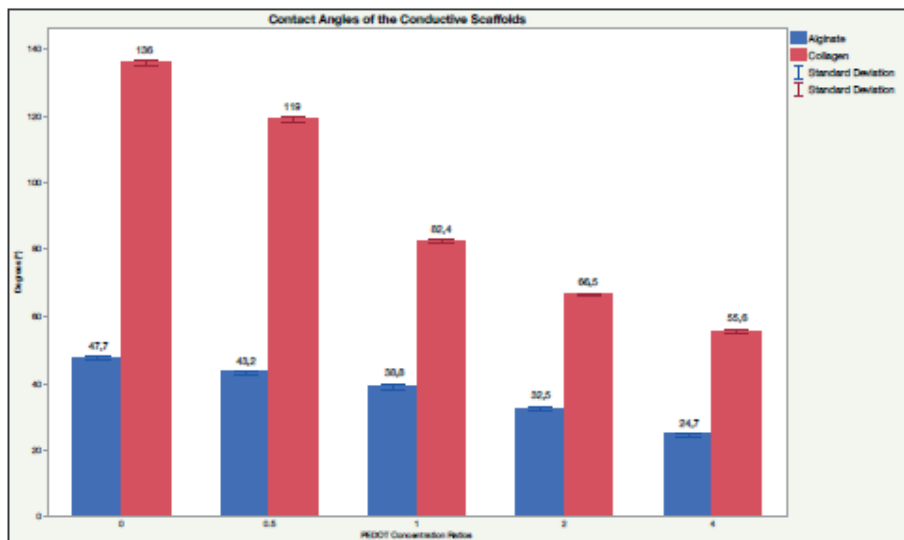


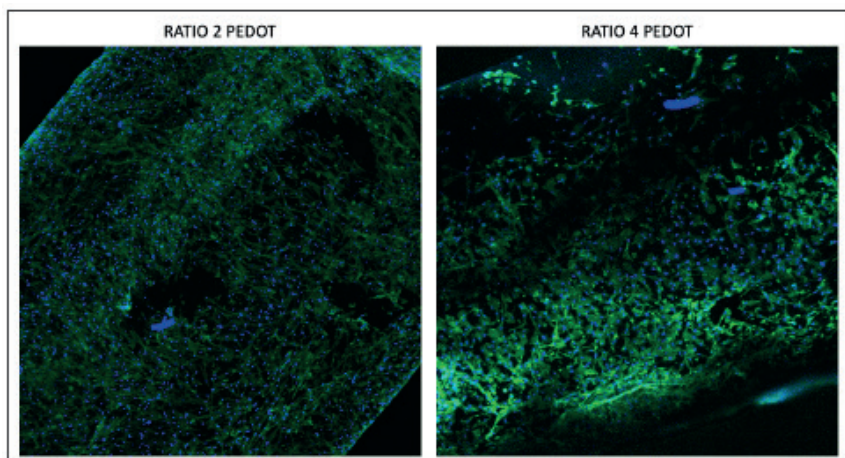
Figure 10. Conductivity of hydrated acellular scaffolds was analyzed using a multiparametric meter (Mettler Toledo TM, S213).

APS:EDOT molar Concentration	Conductivity 30mins (μS/cm)	Conductivity 1h (μS/cm)	Conductivity 24h (μS/cm)
Ratio 0:0	1.19 ± 0.06	1.18 ± 0.08	1.21 ± 0.13
Ratio 1:2	17.77 ± 0.84	20.47 ± 1.15	26.91 ± 1.25
Ratio 2:2	32.39 ± 1.06	33.15 ± 1.36	39.04 ± 1.51
Ratio 4:2	40.82 ± 1.12	53.70 ± 1.37	63.95 ± 1.77
Ratio 8:2	72.65 ± 1.06	109.85 ± 1.73	122.18 ± 1.32

3.4. CELLULAR VIABILITY AND ADHESION

To assess the biological performance, human aortic smooth muscle cells (hASMCs) were seeded and cultured on the outer, sterilized layer of the PEDOT-assembled Alg/Col scaffolds. Smooth muscle cells (SMCs) are essential for regulating vascular tone and compliance (de Laorden et al., 2025). The introduction of the PEDOT nanoparticles is hypothesized to provide a platform for external electrical stimuli capable of inducing SMC contractility and promoting production of their own native ECM, ultimately improving the functional compliance and strength of the engineered vessel (Molina et al., 2024).

Figure 11. Adhesion of hASMCs were evaluated by staining actin filaments (F-actin) using phalloidin on day 3 after seeding. Confocal laser microscopy (Leica SP8, LAS X software version 3.5.5.19976) was utilized at emission wavelengths of 480 nm-520 nm for phalloidin and 405 nm-460 nm for DAPI.



Microscopic evaluation showed excellent cell survival: hASMCs successfully adhered and proliferated, covering almost the entire surface of the vessels containing Alg/Col and various concentrations of PEDOT. This result confirms that the conductive polymer forms a highly biofunctionalized layer that is non-cytotoxic and conducive to cell survival. Furthermore, the scaffold containing the intermediate ratio 2 of PEDOT appeared to enhance superior cell proliferation and attachment compared to the highest molar concentration ratio immediately after seeding, suggesting that there is an optimal concentration for cell compatibility and growth.

4. CONCLUSIONS AND FUTURE OUTLOOK

In conclusion, this study successfully demonstrates the development of hybrid, conductive, porous conduits via the uniform assembly of PEDOT nanoparticles on the surface of Alg/Col scaffolds using a targeted *in situ* interfacial polymerization method. The optimal performance was achieved with the APS to EDOT molar concentration ratios of 4:2 and 8:2. The resulting hydrophilic hydrogel exhibited significantly improved mechanical properties and electrical conductivity suitable for cellular stimulation. Subsequently, *in vitro* testing confirmed that the materials are highly biocompatible, supporting excellent hASMC adhesion and proliferation.

The synergistic combination of the natural, cell-friendly polymers (alginate and collagen) with the electroconductive and mechanically reinforcing properties of PEDOT nanoparticles yields a potentially exceptional scaffold for vascular regeneration (Babeli et

al., 2020). These compelling results strongly suggest that this advanced bioengineering approach is highly suitable for delivering effective electrical stimulation to smooth muscle cells within small-diameter TEBVs. The ability to successfully bioengineer this type of functional artificial artery would constitute a breakthrough, not only serving as a critically needed small-diameter vascular substitute for bypass grafts but also offering a robust, vascularizing platform for the emerging field of artificial organ engineering.

5. ACKNOWLEDGMENTS

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ABOUT THE ORGANIZER

Emilio Castro Otero é professor da área de Bioengenharia na *Universitat Internacional de Catalunya*, Barcelona, Espanha. Pesquisador com ampla trajetória científica nas áreas de biomateriais, nanotecnologia, química física de polímeros e sistemas nanoestruturados aplicados à medicina regenerativa e à liberação controlada de fármacos. Sua produção abrange estudos fundamentais sobre auto-organização molecular, interações entre copolímeros e surfactantes, estabilidade proteica e desenvolvimento de nanopartículas e hidrogéis inteligentes, contribuindo de maneira significativa para a compreensão dos mecanismos físico-químicos que governam materiais avançados e sua aplicabilidade biomédica. Seu trabalho mais recente, publicado em *Acta Biomaterialia* (2023), aborda o papel angiogênico e imunomodulador de íons nas fases iniciais da regeneração óssea, consolidando sua atuação no campo dos biomateriais bioativos. Ao longo de sua carreira, publicou artigos de impacto em periódicos como *Langmuir*, *Biomacromolecules*, *Journal of Biomedical Nanotechnology*, *Macromolecular Bioscience*, *Journal of Physical Chemistry B*, *Chemical Physics* e *Journal of Colloid and Interface Science*, além de contribuir com capítulo de livro na área de nanomedicina, ampliando sua presença internacional na literatura científica.

Entre suas contribuições relevantes, destacam-se estudos sobre auto-organização de elastina recombinante e copolímeros responsivos à temperatura, síntese e caracterização de nanopartículas funcionalizadas, incluindo magnetita pegulada e nanocápsulas multifuncionais, e sistemas poliméricos capazes de encapsular e liberar fármacos de forma controlada, com foco em aplicações oncológicas e terapêuticas avançadas. Sua pesquisa também inclui extensas investigações termodinâmicas envolvendo surfactantes, copolímeros e sistemas micelares complexos, bem como análises detalhadas de interações entre proteínas e fármacos anfífilos, contribuindo para o entendimento de processos de dobramento, estabilidade e agregação proteica. Com forte perfil interdisciplinar, o pesquisador tem colaborado com grupos internacionais de renome em bioengenharia, físico-química de materiais, nanotecnologia e biotecnologia aplicada, contribuindo para o desenvolvimento de estratégias inovadoras em engenharia de tecidos, medicina regenerativa e materiais biomiméticos.

Ao longo de quase duas décadas de produção científica contínua, Emilio Castro Otero consolidou uma carreira marcada pela investigação rigorosa, inovação metodológica e abordagem integrada da ciência dos materiais, oferecendo contribuições relevantes tanto para o avanço do conhecimento fundamental quanto para o desenvolvimento de soluções tecnológicas com potencial translacional na área biomédica.

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