Texila International Journal of Public Health
Special Issue-2024
DOI: 10.21522/TIJPH.2013.SE.24.06.Art001

Fabrication and Characterization of PVA Tricalcium Phosphate and Quercetin-doped Strontium Membranes for Guided Bone Regeneration

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Abstract

Guided Bone Regeneration (GBR) is a technique used in reconstructive dentistry to address small defects around dental implants and to facilitate bone regeneration in alveolar defects. It involves the use of bone grafts and barrier membranes to promote the selective growth of bone tissue while preventing soft tissue infiltration. Surface structural morphology analysis using scanning electron microscopy (SEM) revealed notable differences between the fabricated membrane samples. A comparative assessment indicated an increase in both porosity and nanofiber size upon the addition of strontium nanoparticles. This alteration was further corroborated by the observed decrease in hydrophilicity, as evidenced by an increase in the contact angle from the typical 30 degrees to 58.7 degrees. Our study offers a promising avenue for advancing guided bone regeneration through the development of PVA-TCP membranes tailored with quercetin and strontium.

Keywords: *Biodegradable Materials, Guided Bone Regeneration, Scanning Electron Microscopy.*

Introduction

Guided Bone Regeneration (GBR) is a technique used in reconstructive dentistry to address small defects around dental implants and to facilitate bone regeneration in alveolar defects. It involves the use of bone grafts and barrier membranes to promote the selective growth of bone tissue while preventing soft tissue infiltration [1]. The success of GBR depends heavily on the properties of the membranes employed, including biocompatibility, stiffness, and resorption period. Recent advancements in membrane development have improved their mechanical properties, bioactivity, and therapeutic efficacy [2]. Innovations such as Collagen/PVA dual-layer membranes have demonstrated improved strength and bioactivity, making them suitable for periodontal bone regeneration applications [3]. Multifunctional fiber mats incorporating compounds like ascorbyl palmitate and strontium polyphosphate nanoparticles have shown promise in GBR, offering tunable microstructures and therapeutic activity for enhanced bone regeneration [4]. Biodegradable materials such as Zn-0.5Fe alloy membranes have also been developed, exhibiting superior mechanical properties, corrosion resistance, and biocompatibility [5].

GBR techniques find relevance in the treatment of various pathologic lesions affecting the alveolar bone. The method's ability to halt the growth of epithelial and connective tissues plays a crucial role in facilitating bone healing by maintaining the integrity of the defect space and providing mechanical support during bone development [6]. Fundamental to the concept of GBR is the use of a membrane to prevent non-osteogenic

Received: 01.08.2024 Accepted: 11.11.2024 Published on: 30.12.2024
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tissues from interfering with bone repair. Recent research has turned to the incorporation of strontium hydroxyapatite (SrHA) into GBR membranes, building upon growing evidence of strontium's role in bone formation and remodeling [7]. Strontium membranes typically offer osteogenic properties, stimulating osteoblast activity and enhancing bone regeneration through the release of strontium ions [8]. Modified PVA membranes, including super-oleophobicity and high underwater oil contact angles, have potential for applications beyond bone regeneration. PVA's biocompatibility, barrier properties, and controlled release capabilities make it a versatile material suitable for various biomedical applications, including drug delivery systems [9].

Periodontitis, a chronic infectious disease characterized by the pathological loss of periodontal ligament and alveolar bone, highlights the importance of effective GBR techniques in dentistry. Current periodontal diagnostics primarily rely on clinical criteria, emphasizing the need for innovative approaches in treatment modalities [10]. Advancements in GBR membrane development, coupled with an understanding of bone biology and the pathophysiology of periodontal diseases, offer promising avenues for improving patient outcomes in reconstructive dentistry [11]. Through interdisciplinary research efforts spanning materials science, biology, and clinical dentistry, there is potential to revolutionize treatment paradigms and address the unmet needs of patients with bone defects and periodontal conditions [12].

This article presents a comprehensive investigation into the fabrication and characterization of novel PVA-tricalcium phosphate (PVA-TCP) membranes doped with quercetin and strontium for guided bone regeneration [13]. The study aims to elucidate the influence of these components on membrane properties, including biocompatibility, mechanical strength, degradation rate, and osteogenesis.

Materials and Methods

Preparation of Polymer Solutions

A 10% w/v solution of polyvinyl alcohol (PVA) was prepared by stirring poly(ε-caprolactone) (PCL) in a solvent mixture of 1:1 (v/v) at 500 rpm [14]. This preparation was conducted at both room temperature and 37 degrees Celsius. A stock solution of 100% PVA was first prepared. Beta-tricalcium phosphate (β-TCP) at a concentration of 0.5% was then added to the final polymeric solution, and the mixture was stirred slowly with a magnetic stirrer for 24 hours. Following this, strontium nanoparticles were introduced into the final polymeric solution at a concentration of 5 micrograms/milliliter (µg/mL). The stirring process continued for another 24 hours to ensure thorough mixing and uniform dispersion of the components. This resulting mixture served as the control group, comprising a uniform polymeric blend of PVA/strontium/β-TCP.

Fabrication of Nanofibrous Substrates

Using the electrospinning technique, Nanofiber substrates were fabricated from the polymer blend (PVA/strontium/β-TCP). The polymer blend was loaded into a 5 ml plastic syringe fitted with a 22Gx32 mm needle [15]. A syringe pump maintained a constant flow rate of 0.9 ml/h, ensuring a consistent deposition of the polymer solution. A high voltage of 10 kV was applied at the tip of the needle, and the distance between the needle and the collector was maintained at 10 cm. The electrospinning process was carried out at a controlled temperature of 25 ± 1 degree Celsius and a humidity of 45%. Under these conditions, nanofibrous substrates were successfully fabricated from the polymer blend, with varying ratios achieved by adjusting the composition of the polymer solution.

Calcium Deposition Analysis

The differentiation of MG63 cells over a 14-day period in differentiation media supplemented with 10 mM β-glycerophosphate and 0.05 mM ascorbic acid, alongside the material of interest, was evaluated for calcium deposition using Alizarin Red staining. Following a 10-minute incubation with 2% Alizarin Red solution and subsequent washing with 1X PBS, quantitative analysis was performed by adding 200 µl of DMSO to each well and incubating for 1 hour [16]. The quantity of Alizarin was then measured using a spectrophotometer at 405 nm.

Surface Characteristics

SEM and FTIR analysis will assess the surface characteristics of PVA tricalcium phosphate and quercetin-doped strontium membranes.

Results

Surface structural morphology analysis using scanning electron microscopy (SEM) revealed notable differences between the fabricated membrane samples in Figures 1A and 1B. A comparative assessment indicated an increase in both porosity and nanofiber size upon the addition of strontium nanoparticles. This alteration was further corroborated by the observed decrease in hydrophilicity, as evidenced by an increase in the contact angle from the typical 30 degrees to 58.7 degrees, as shown in Figure 2.

The MG63 cell culture showed an increase in calcium uptake upon the addition of strontium. Figure 1 D compares quercetin, Quercetin-strontium, tricalcium phosphate, and quercetin-doped strontium membranes.

Fourier-transform infrared spectroscopy (FTIR) analysis was employed to characterize the chemical composition of the analyzed compound. The FTIR spectrum exhibited distinct peaks at specific wavenumbers, each corresponding to particular functional groups within the compound. Notably, the peak observed at 3337.78 cm indicated OH stretching, suggesting hydroxyl groups commonly found in compounds such as alcohols, phenols, and carboxylic acids. Furthermore, the peak at 2938.02 cm represented CH stretching, signifying the stretching vibration of C-H bonds present in various organic molecules. The peak observed at 2061.23 cm was attributed to C≡C stretching, indicating the presence of triple bonds in alkynes. Moreover, the peak at 1710.98 cm corresponded to C=O stretching, suggesting the presence of carbonyl groups in compounds such as aldehydes, ketones, and carboxylic acids. Additionally, peaks at 1421.08 cm, 1335.28 cm, and 1091.91 cm were identified as CH bending and C-O stretching vibrations, respectively, providing further insight into the molecular structure of the analyzed compound. The FTIR spectrum also exhibited a peak at 848.87 cm, representing C-H bending in aromatic compounds, and a peak at 607.72 cm corresponding to out-of-plane bending vibrations of aromatic C-H bonds. Overall, the FTIR analysis elucidated the presence of specific functional groups within the compound, contributing to a comprehensive understanding of its chemical composition and molecular characteristics.

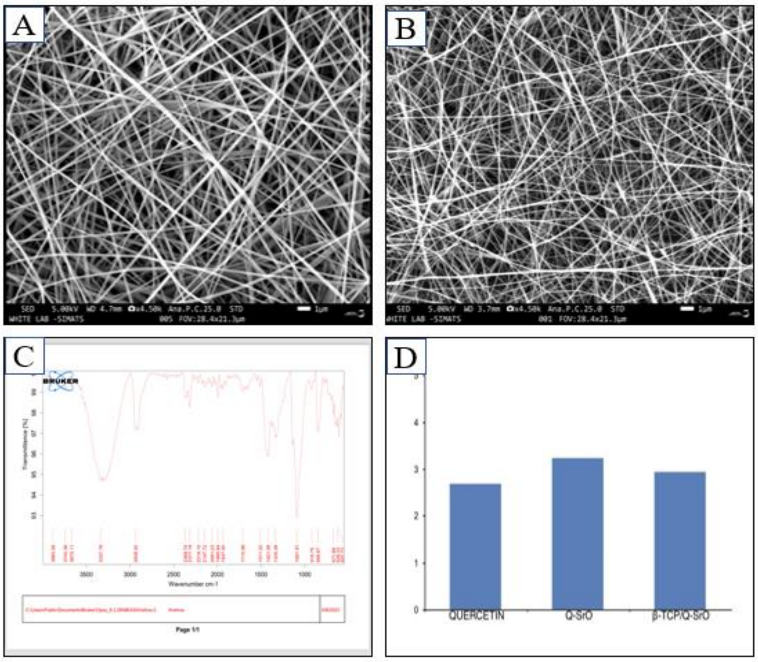

Figure 1 (A-D). SEM revealed notable differences between the fabricated membrane samples in 1A and 1B. FTIR shown in 1C. Comparison between quercetin, Quercetin-strontium, and tricalcium phosphate, and quercetin-doped strontium membranes is shown in 1D

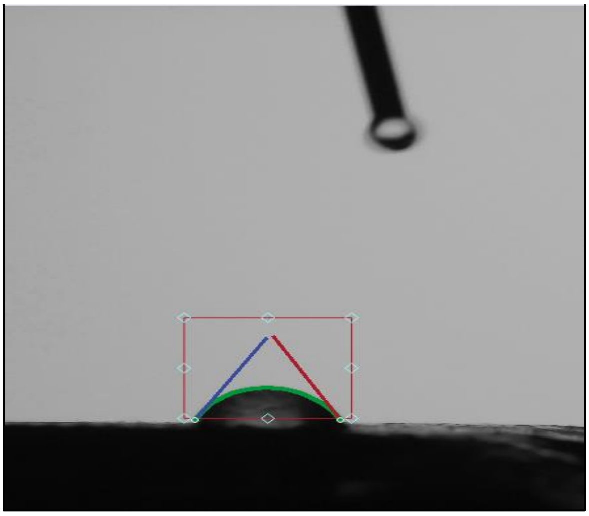

Figure 2. Increase in the contact angle to 58.7 degrees

Discussion

The present study represents a significant advancement in the field of guided bone regeneration (GBR), building upon seminal research conducted by Dahlin et al. (1988) and earlier investigations by Bassett et al. (1956), Ashley et al. (1959), and Murray et al. (1957). GBR, facilitated by barrier membranes, has emerged as a pivotal strategy in regenerative medicine, offering promise for restoring lost or damaged bone tissue [17]. This study extends the paradigm by developing novel PVA-tricalcium phosphate (PVA-TCP) membranes doped with quercetin and strontium and characterizing their properties [18].

The selection of polyvinyl alcohol (PVA) as a base material is grounded in its well-established biocompatibility and versatile utility across various medical applications [19]. Central to our investigation is the exploration of how quercetin and strontium dopants influence critical membrane properties that are essential for effective bone regeneration [20]. Employing a meticulous approach, polymer solutions are prepared and electrospun to create nanofibrous substrates with tailored compositions [21]. Examination of membrane morphology using scanning electron microscopy (SEM) reveals notable structural modifications following the incorporation of strontium nanoparticles [22]. Specifically, there is evidence of increased porosity and nanofiber size, indicating improved scaffold architecture conducive to cellular infiltration and tissue regeneration [23]. Moreover, the observed reduction in hydrophilicity, as indicated by an elevated contact angle, underscores the impact of dopants on surface characteristics [24].

Beyond structural characterization, our study delves into the osteogenic potential of the fabricated membranes through calcium deposition assays using MG63 cell cultures [25]. Remarkably, enhanced mineralization is observed in cells treated with strontium-doped membranes, suggesting a favorable influence on bone regeneration [26, 27]. Fourier-transform infrared spectroscopy (FTIR) provides complementary insights into membrane composition, which identifies characteristic peaks associated with specific functional groups [28]. Mineralisation of artificial substitutes like calcium carbonate, PRF, nano hydroxyapatite have shown to have clinical benefits across various fields [29-31].

However, it is essential to acknowledge the inherent limitations of our study when interpreting the findings. While our experimental approach allows for a comprehensive assessment of membrane properties in vitro, translating these findings to clinical settings necessitates further validation through vivo studies. Additionally, elucidating the underlying mechanisms driving the observed enhancements in biocompatibility and osteogenic activity warrants additional mechanistic studies.

In summary, our study represents a significant contribution to the ongoing efforts aimed at advancing GBR strategies through the development of novel biomaterials. By systematically investigating the influence of quercetin and strontium dopants on membrane properties and osteogenic potential, we provide valuable insights that may inform the design of next-generation regenerative therapies. Moving forward, continued research endeavors, including in vivo studies and mechanistic investigations, are essential to realizing the clinical potential of these innovative membranes in promoting bone regeneration and addressing critical limitations in current treatment modalities.

Conclusion

In conclusion, our study offers a promising avenue for advancing guided bone regeneration through the development of PVA-TCP membranes tailored with quercetin and strontium. By conducting a systematic evaluation of the influence of these components on membrane properties and osteogenic potential, we contribute to the ongoing efforts in bone tissue engineering. Further research aimed at refining membrane design and elucidating underlying mechanisms will facilitate the translation of these findings into clinically relevant therapies for bone regeneration. The results of this study could have significant implications in the field of bone tissue engineering, and our findings warrant further exploration to enhance our understanding of the potential of tailored PVA-TCP membranes in bone regeneration.

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