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Structure, physical properties, biocompatibility and *in vitro*/*vivo* degradation behavior of anti-infective polycaprolactone-based electrospun membranes for guided tissue/bone regeneration

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ABSTRACT

Nanofiber membranes composed of polycaprolactone (PCL), PCL/metronidazole (MNA), PCL/gelatin/MNA, and PCL/gelatin/MNA/acetic acid (HAC), named P0, P30, PG30, and PGH30, respectively, were fabricated by electrospinning for application in guided tissue/bone regeneration (GTR/GBR) therapies. The architectural features, mechanical properties, hydrophilicity, drug-encapsulation efficiency, drug-release pattern, antimicrobial properties, cell barrier functions, *in vitro*/*vivo* degradability and biocompatibility were investigated. All membranes were found to have high tensile strength, which is required for GTR applications. Strong interactions among PCL, gelatin, and MNA resulted in high drug loading efficiency, which was further improved by the incorporation of gelatin and HAC. MNA incorporation gave the membranes good antimicrobial property, while reducing host versus graft reaction, improving the hydrophilicity and accelerating the degradation. Gelatin incorporation considerably improved cytocompatibility, while accelerating the degradation dramatically. Very low quantities (0.1% v/v with respect to polymer solution) of HAC effectively prevented the phase separation of PCL and gelatin, resulting in homogeneous nanofiber, which facilitates stable physical properties. The drug-release profiles of all drug-loading membranes were consistent with the inflammation cycle characteristics. High drug loading and trace amounts of HAC did not cause any adverse reactions, as evidenced by subcutaneous implantation. Both P0 and P30 maintained their cell barrier function *in vivo* for as long as 24 weeks; PGH30, for 8 weeks; and PG30, for less than 8 weeks. These findings enabled a comprehensive understanding of the influence of different compositions on the structure and performance of the membranes, thereby supporting the design of membranes with superior overall performance for GTR/GBR application.

1. Introduction

Guided tissue/bone regeneration (GTR/GBR) techniques have been successfully applied for treating periodontal lesions and have provided the opportunity to form new bone [1]. This technique utilizes membranes as mechanical barriers to create a space around the defects, permitting bone regeneration to occur in the absence of competition for space by the surrounding connective tissues. Membranes used in GTR/GBR therapy must be biocompatible, have the proper degradation profile and adequate mechanical and physical properties, and sufficient sustained strength [2].

Electrospun nanofibrous scaffolds/membranes more closely mimic the scales and morphologies of extracellular matrix (ECM) proteins (fiber diameters range from 50 to 500 nm). The inherently high surface to volume ratio of electrospun scaffolds can enhance cell attachment, drug loading, and sustained and controlled local drug delivery [3,4]. The average pore size of the electrospun nanofiber membrane is approximately 4–6 μm. Eukaryotic cell diameters range from 10 to 100 μm. It is difficult for gingival fibroblasts and periodontal cells to pass through an electrospinning membrane. These structural characteristics make the electrospun nanofibrous membranes very suitable for use as a GTR/GBR membrane.

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GTR membranes can be non-biodegradable or biodegradable. Non-biodegradable membranes require a second surgical procedure for membrane removal. Biodegradable membranes allowing a single-step procedure reduce patient discomfort. Collagen is the most widely used natural biomaterial for GTR application. However, there are some limitations, such as the loss of space maintenance under physiological conditions, risks of disease transmission to humans from animal-derived collagen, and high cost [5]. To overcome these limitations, polymeric GTR membranes were developed. Poly (lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers have been widely considered for GTR membrane applications. However, the accumulation of acid during degradation may significantly reduce pH, resulting in chronic aseptic inflammation [6].

Compared to PLA and PGA, PCL degradation does not produce a local acidic environment. Thus, because of its comparatively low cost and high mechanical strength, PCL is an attractive biomedical polymer. However, only a few studies have examined PCL-based GTR membranes [7,8]. PCL degrades very slowly. The absorption time of PCL *in vivo* is approximately 2–3 y [5,9], which is too long for application in GTR/GBR treatment. Furthermore, its poor hydrophilicity reduces cell adhesion. Therefore, PCL is blended or copolymerized with other polymers before biomedical application.

Among electrospun biodegradable polymers, electrospinning of hybrids, particularly natural and synthetic polymer blends, has received increasing attention [10,11]. Gelatin, the hydrolysis product of collagen, can be completely absorbed quickly. It is also biocompatible, biodegradable, and a major component of the native ECM. Gelatin is non-immunogenic and retains informational signals such as the arginine–glycine–aspartic acid sequence, which promotes cell adhesion, differentiation, and proliferation [12]. Thus, gelatin was combined with PCL to form a composite GTR membrane.

However, poorly blended polymeric fibers are generated with porous or phase-segregated internal structures as a result of the weak molecular interactions between natural and polymeric polymers. This is particularly true when the natural biopolymer component reaches a threshold percent. Intense phase separation was observed in electrospun collagen/PCL hybrids when the PCL component was increased to 30% [13], and severe phase segregation occurred when the components were blended in equal parts for the cases of electrospun gelatin/PCL [14] and electrospun collagen/PLCL [15]. Therefore, controlling the formation of homogeneous internal structures of electrospun nanofibers by modulating the miscibility of solutions containing both natural and synthetic components is critical. Incorporating a tiny amount of acetic acid may allow the originally turbid solution to become immediately clear and to be single-phase stable for more than 1 week [16]. Some structural and physical properties of the PCL/gelatin/HAC composites have been studied. However, biosecurity, degradation, and foreign body reaction following incorporation of acetic acid have not been reported. In addition, PCL/gelatin/HAC composites used to prepare the drug loading GTR/GBR membrane have not been determined.

The presence of periodontopathogens such as *Porphyromonas gingivalis* and *Prevotella intermedia* may negatively affect the success of periodontal regeneration. Therefore, it is very important to control and/or reduce bacterial contamination of the periodontal defect to enhance periodontal regeneration [17]. Metronidazole (MNA) has been used to treat infections for more than 45 y and is still successfully used for treating anaerobic bacterial infections. Although systemic administration of antibiotics is useful, high oral doses are necessary to achieve effective concentrations in the gingival fluid. However, long-term use may lead to the development of resistant bacterial strains. These limitations have led researchers to examine localized delivery of antibiotics directly at the diseased site [18]. Electrospun nanofiber membranes are used to achieve different controlled drug release profiles in drug delivery applications [19]. MNA has been integrated into PLA and PLGA nanofibers for local periodontitis treatment. This drug delivery system showed sustained drug release and significantly decreased bacterial viability [20]. In addition, significant improvement in periodontal regeneration following GTR was observed in dogs [21]. Although a derivative of MNA has been mixed with PCL to fabricate nanofibrous scaffolds, but PCL-based matrix loading of MNA for GTR/GBR application has not been reported. The effects of the interaction between the drug and polymer matrix on the physical–chemical properties, drug release profile, and long-term *in vivo* biocompatibility and biodegradability of the drug delivery system have not been thoroughly investigated.

In this study, electrospinning technology was used to fabricate 4 types of PCL-based GTR membranes composed of PCL, PCL/MNA, PCL/gelatin/MNA, and PCL/gelatin/MNA/HAC. The effects of the material components on the physical and chemical structure, mechanical and thermal properties, barrier function, antimicrobials performance, histocompatibility, and *in vitro* and *in vivo* degradation of different membranes were investigated in detail. The results of this study would render technology and material references for preparation of GTR/GBR membranes with excellent comprehensive properties.

2. Materials and methods

2.1. Preparation of electrospun PCL-based nanofiber membranes

The materials used in this study are listed in Electronic Supplementary Information (ESI) S1. Composition and preparation of the spinning solution are stated in ESI S2. Membranes with different compositions were fabricated according to the conventional electrospinning procedure are also described in S2.

2.2. Characterization of PCl-based nanofiber membranes

2.2.1. Morphology

Membrane morphology was observed using a scanning electron microscope (SEM) (S4700, Hitachi Co., Tokyo, Japan) at a voltage of 20 kV. The membranes were coated with gold and observed under the microscope. Fiber diameter and pore size were measured using the ImageJ software from SEM micrographs at random locations ($n = 100$). Membrane thickness was measured using a micrometer, and its apparent density and porosity was estimated using equations eq.s1 and eq.s2 (see ESI S3) [22].

2.2.2. Molecular dynamic simulation

Molecular dynamics simulation was used to investigate the effect of drug content in the polymer. The Discover and Amorphous Cell modules of the Materials Studio suite were used (Accelrys, San Diego, CA, USA) [23]. All theoretical calculations were performed using the condensed-phase optimized molecular potentials for atomistic simulation studies (COMPASS) force field (see ESI S4).

2.2.3. Thermal, crystal, and chemical structure and mechanical property

Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) analyses (see ESI 5) were performed to investigate the chemical and thermal properties of the membranes. Tensile strength (TS) and elongation at break (EB) of the membranes both in the dry and wet states were evaluated using a BOSE ElectroForce 3200 test instrument (Framingham, MA, USA) (see ESI S5).