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Fabrication and characterization of two-layered nanofibrous membrane for guided bone and tissue regeneration application

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ABSTRACT

Membranes used in dentistry act as a barrier to prevent invasion of intruder cells to defected area and obtains spaces that are to be subsequently filled with new bone and provide required bone volume for implant therapy when there is insufficient volume of healthy bone at implant site. In this study a two-layered bioactive membrane were fabricated by electrospinning whereas one layer provides guided bone regeneration (GBR) and fabricated using poly glycerol sebacate (PGS)/polycaprolactone (PCL) and Beta tri-calcium phosphate (β-TCP) (5, 10 and 15%) and another one containing PCL/PGS and chitosan acts as guided tissue regeneration (GTR). The morphology, chemical, physical and mechanical characterizations of the membranes were studied using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), tensile testing, then biodegradability and bioactivity properties were evaluated. *In vitro* cell culture study was also carried out to investigate proliferation and mineralization of cells on different membranes.

Transmission electron microscope (TEM) and SEM results indicated agglomeration of β-TCP nanoparticles in the structure of nanofibers containing 15% β-TCP. Moreover by addition of β-TCP from 5% to 15%, contact angle decreased due to hydrophilicity of nanoparticles and bioactivity was found to increase. Mechanical properties of the membrane increased by incorporation of 5% and 10% of β-TCP in the structure of nanofibers, while addition of 15% of β-TCP was found to deteriorate mechanical properties of nanofibers. Although the presence of 5% and 10% of nanoparticles in the nanofibers increased proliferation of cells on GBR layer, cell proliferation was observed to decrease by addition of 15% β-TCP in the structure of nanofibers which is likely due to agglomeration of nanoparticles in the nanofiber structure. Our overall results revealed PCL/PGS containing 10% β-TCP could be selected as the optimum GBR membrane in view point of physical and mechanical properties along with cell behavior. PCL/PGS nanofibers containing 10% β-TCP were electrospun on the GTR layer for fabrication of final membrane. Addition of chitosan in the structure of PCL/PGS nanofibers was found to decrease fiber diameter, contact angle and porosity which are favorable for GTR layer. Two-layered dental membrane fabricated in this study can serve as a suitable substrate for application in dentistry as it provides appropriate osteoconductivity and flexibility along with barrier properties.

1. Introduction

The aging population is one of the main reasons that forces both scientists and clinicians to develop the appropriate methods for replacement of lost teeth [1]. Dental implants have attracted more interest as a standard procedure in recent years. Adequate bone volume and soft tissue augmentation in the site of implant is an important prerequisite for implant placement and success in dental implantation with regard to the aesthetic and functional reconstruction of patients [1–5]. However in some cases, insufficient volume of healthy bone at implant site due to trauma, tumors and periodontal diseases cause only partial fulfillment of such prerequisites [3,5–6]. A variety of techniques have been attempted to regenerate alveolar bone defects in the implant site including bone grafting, distraction osteogenesis and guided bone/tissue regeneration (GBR/GTR) to allow successful dental implant placement [2–3]. Guided bone regeneration (GBR) and guided tissue regeneration

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(GTR) have been increasingly used as efficient methods for the reconstruction of both the structure and function of alveolar bone defects and damaged tissues [1,7].

GBR technique is a surgical procedure in which an occlusive membrane is used to stimulate new bone formation, guide the bone regeneration process and protect blood clots which are beneficial for treatment of failing implants [1,4]. It is also known that the osteoinductive, osteogenic and angiogenesis properties of membrane leads to satisfactory results in bone regeneration [4,8–9]. Similar to GBR, the concept of GTR also involves the application of a membrane to avoid migration of epithelial cells to the defected area as epithelial cells inhibit bone formation and encourage the down-growth of the connective tissue into the area of injured tissue [10–13]. The membranes used in GBR/GTR method needs to have appropriate flexibility and stiffness to shield the defect and provide diffusion of nutrition, oxygen and bioactive substances which is vital for bone and soft tissue regeneration [4,14–16]. Although appropriate mechanical, physical and bioactive properties are required for the membranes [17–18], biodegradability rate is also an important parameter such that second surgery can be avoided [19–20]. Moreover successful regeneration of the defected site requires at most 6 months which determines biodegradation rate of the membrane [1,4,13,21–22]. Membranes can be fabricated from various synthetic polymers including the poly glycolic acid (PGA), poly lactic acid (PLA), polycaprolactone (PCL) and their co-polymers and natural polymers such as collagen, gelatin and chitosan [7,11,13,23–24].

Polycaprolactone (PCL) is a biocompatible polyester with extraordinary mechanical properties which make it a good candidate for bone reconstruction [7,25,14]. Polyglycerol sebacate (PGS) is an elastomeric polymer which provides flexibility and has been used for fabrication of scaffolds in tissue engineering [9,26]. As dental membrane needs to be flexible, PGS can be a good candidate for fabrication of dental membrane [9,27]. Although PGS in the form of sheets and sponge has been used as a polymer for fabrication of scaffolds for bone tissue engineering [26,27], to the best of our knowledge, it is the first time that nanofibers containing PGS is used for bone regeneration.

Bioceramics have gained huge interest in dental membranes as they enhance bioactivity, physico-chemical and biological properties of resultant membrane. Several studies have incorporated bioceramics such as calcium phosphate (CP), bioactive glasses (BG) and hydroxyapatite (HA) in the structure of dental membrane due to prominent biological properties such as osteoconductivity and osteoinductivity and ability to mimic the natural inorganic bone component [24,28–30]. Beta tri-calcium phosphate (β-TCP), has been utilized as an excellent bioceramic in many clinics for bone repair applications and dental membranes due to its biocompatibility, osteoconductivity and high *in vivo* resorbability which are useful for successful bone regeneration [12,27,31].

Different shapes of membranes such as gels, mats, foams and fibers fabricated by a variety of techniques including freeze-drying, solvent casting, particulate leaching, molding, self-assembly, phase separation and electrospinning have been used [2–3,32]. The ability of fibrous structure to mimic the native extracellular matrix (ECM) with high surface area to volume ratio and high porous structure with interconnected pores make nanofibers interesting for dental applications [32–33].

Electrospinning has been widely used as a resourceful and cost effective technique for fabrication of random and aligned nanofibers with controllable morphology and has gained more interest in tissue engineering, drug delivery, oral and dental applications [7,32,34–38].

Considering the importance of dental membranes for regeneration of impaired alveolar bone, we aimed the fabrication of two layered electrospun membrane (Fig. 1). PCL and PGS were used for fabrication of electrospun membrane and the effect of incorporation of different amount of β-TCP towards the properties of composite was evaluated. *In vitro* cell culture study was also done for understanding the effect of incorporation of bioceramics on bioactivity of GBR side. The optimum combination of PCL/PGS with β-TCP was selected and used for the fabrication of GBR. Furthermore chitosan was blended with PCL and PGS and PCL/PGS/chitosan nanofibers were coated on GBR side to provide a two layered GBR/GTR membrane.

2. Materials and methods

2.1. Materials

Glycerol and sebacic acid with purity of 99.9%, polycaprolactone (PCL, Mn 80,000), chitosan (Mw 190,000–310,000, 75–85% deacetylated, viscosity 200–800 cps), trifluoroacetic acid, chloroform and methanol were purchased from Sigma-Aldrich (St Louis, MO, USA). Phosphate buffered saline (PBS) were purchased from HiMedia (Mumbai 400,086, India). Dioctyl sulfosuccinate sodium salt as surfactant, calcium nitrate tetrahydrate ($Ca(NO_3)_2\_4H_2O$), potassium dihydrogenphosphate (KH_2PO_4), ammonia (NH_3) for synthesis of β-TCP nanoparticles were purchased from Merck (USA). All the inorganic salts used for SBF preparation were purchased from Beijing Chemical Engineering Factory (Beijing, China).

CellTiter 96_ AQueous One solution reagent (MTS), used in the cell culture study, was obtained from Promega.

2.2. Synthesis and characterization of PGS

2.2.1. Polymer synthesis

The synthesis of PGS was accomplished according to the same method of Rai et al. [39]. In summary the polymer synthesis was carried out in two steps: pre-polycondensation and then crosslinking of pre-polymer. For the polycondensation process, equal molar mixtures (1 M) of glycerol and sebacic acid were reacted at 120 °C under inert nitrogen atmosphere in oil bath for 24 h to form the pre-polycondensed polymer. After that mixture was put on vacuum oven at 50° C and pressure was

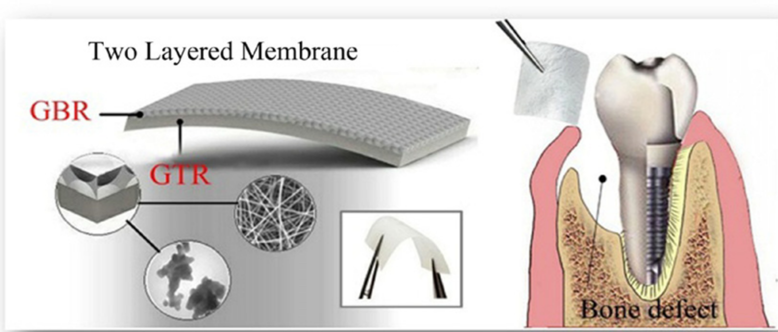

Fig. 1. Schematic illustration of application of two layered membrane in defected site.