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Preparation and in vitro evaluation of polycaprolactone/PEG/bioactive glass nanopowders nanocomposite membranes for GTR/GBR applications

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ABSTRACT

In the present study, nanocomposite membranes are investigated using poly-ε-caprolactone (PCL), polyethylene glycol (PEG) and bioactive glass nanopowders (BGs) synthesized via solvent casting method with different reinforcement rates of BGs consisting of 3, 5 and 7 wt% for regenerating the periodontal tissue in vitro. These prepared membranes were evaluated by a vast range of essential tests; including Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), Transmition-electron microscopy (TEM), tensile testing before and after soaking in PBS solution, degradation and contact angle assessments as well as cell culture assays. In spite of the fact that the percentage of Cu incorporated into BGs was trivial, this negligible amount exerted major cytotoxic impact upon cells during in vitro cell tests. According to the results, the blended-membrane contained 7 wt% copper-free BGs indicated optimum characteristics including satisfactory mechanical and biodegradation features, more wettable surface, higher proliferation rates of adipose-derived stem cells (ADSCs), superior ALP activity and brilliant bone mineralization capacity which was confirmed by Alizarin red assay. As a consequence, it can be used as a desirable candidate for guided tissue/bone regeneration (GTR/GBR) to accelerate bone tissue healing.

1. Introduction

There is a growing tendency among scientists towards dental biomaterials due to the increased rate of aging population. Nowadays, periodontal diseases have dramatic impact on approximately 90% of the worldwide population. The moderate form of these diseases, which is named gingivitis, is created by bacterial biofilm aggregating on teeth adjacent to gingiva; this form of illness has no influence on destructing the teeth supporting structures and would be reversible by effective oral hygiene and drug consumption. If it is not treated in early stages, it would develop profound inflammation into connective tissues resulting in recession along with loss of supporting connective tissue and alveolar bone. The inflammatory disease is known as periodontitis. Intensive periodontitis can eventually cause tooth loss [1,2]. The progressing of periodontitis will have serious consequences such as the separation of epithelial tissue from tooth, the interruption of periodontal ligament (PDL) and the absorption of alveolar bone tissue [3]. On this occasion, conventional treatments like root surface debridement and cleaning are available. While ideal strategies must be capable of regenerating all damaged structures including the cementum, the periodontal ligament and the alveolar bone [3,4], these treatments are not effective in restoring the compromised periodontal attachment apparatus. The connection form between the cementum and bone depends on the size of the defect and the number of cells that engross the treated region upon surgery [4]. The first and the most considerable cells that migrate into the defect site are epithelial cells. These cells can grow beside the tooth root, lead to a problem like bone formation inhibition [3,4]. To overcome this problem, GTR and GBR strategies are prevalence. In these techniques an occlusive membrane is implemented to prevent the early epithelial migration into the defect site which allows time for periodontal apparatus to regenerate [3–9]. According to their degradation properties, GTR/GBR membranes are divided into two broad categories: non-resorbable (e.g. Polytetrafluoroethylene (PTFE) membranes) and resorbable (e.g. collagen and polylactide (PLA) polyglycolic acid (PGA)-based membranes) [1,5]. One of the drawbacks of non-resorbable membranes is performing additional surgery required for eliminating them. This imposes further economic burden and causes additional pain for the patient. For eliminating the second surgery process, resorbable membranes have been expanded [1]. Some requirements are needed for good performance of GTR/GBR membranes.

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Briefly, these membranes should show: 1. space maintenance and creation, 2. cell occlusive, 3. biocompatibility, 4. tissue integration, and 5. easy manipulation [1,4,5].

One of the most commonplace materials used to generate GTR/GBR membranes in the market is collagen. However, collagen is derived from human or animal sources and there are some limitations on applying it as a GTR/GBR membrane such as the risk of disease transmission, religious beliefs, its fast resorption rate, and insignificant mechanical properties. Furthermore, these important limitations resulted in the application of new materials with far more satisfactory features [1,4].

PCL is a hydrophobic, semi-crystalline polymer that has a very low melting point (59–64 °C) and glass transition temperature (−60 °C). Its low melting point, exceptional compatibility with most compounds, and good solubility in most solvents (dichloromethane, chloroform, benzene, carbon tetrachloride, toluene, cyclohexanone and 2-nitropropane), have inspired wide research into its potential application in biomedical fields. In spite of the fact that PCL can be utilized in various forms of biomaterials such as films, membranes, foams, gels, particles and scaffolds [10–16], PCL contains polymers which imposes a great deal of restrictions on bone regeneration due to low stiffness, hydrophobic nature, and lack of bioactivity. For these reasons, an approach named composite approach should be adopted in which polymers are combined with bioactive materials like hydroxyapatite (HA), tricalcium phosphate (TCP), and bioactive glass (BG). The combination of polymers with bioactive inorganics would improve biological activity, mechanical properties and osteoconductivity [11,17].

BGs have been found in wide applications due to facilitated reactions leading to the implant's connection with the surrounding living tissues. It seems that bonding to the living bone would be expanded by a carbonate hydroxyapatite (CHA) layer formed on the BG's surface. BGs are more bioactive than calcium phosphate compounds. Moreover, bioactive reactions are faster than hydroxyapatite in some silica-based BGs. Genetic control of osteoblasts is known as one of the crucial characteristics of BGs. Thus, human osteoblasts' proliferation is boosted by ionic products of BG dissolution which induces the expression of insulin-like growth factor II mRNA and the synthesis of protein.

In addition, polyethylene glycol (PEG) will be added to PCL as plasticizer with the aim of enhancing the properties of PCL. Generally speaking, this plasticizer is no doubt that will diminish material hardness, glass transition temperature as well as the drying procedure in solvent-involving processes by decreasing the quantity of solvent required [18,19].

Regarding these properties, the study aimed at preparing and characterizing a novel, bioactive and biodegradable nanocomposite membrane made from PCL, PEG with BG nanopowders for GTR/GBR applications.

2. Materials and methods

2.1. 45S and Cu45S BG nanopowders synthesis

45S sol-gel glass with a glass composition of SiO_2–P_2O_5–CaO (44.5 mol% SiO_2, 48.1 mol% CaO and 3.5 mol% P_2O_5) was synthesized by the following steps. The materials for preparing BG nanopowders included deionized water, tetraethoxysilane (TEOS; Merck), triethyl phosphate (TEP; Merck), calcium nitrate tetrahydrate (Merck), ethanol, copper nitrate (Merck), and 2N hydrochloric acid (Merck). The process commenced with mixing and stirring the proper amount of TEOS (15.6 ml) and ethanol (50 ml) as an alcoholic media with 2N hydrochloric acid (2.5 ml) and deionized water (15 ml) for 30 min at room temperature down to gain a transparent mixture. The volume ratio of ethanol: TEOS was 3:1. Afterwards, TEP (0.5 ml) was added to the former solution and stirred for another 20 min. Finally, calcium nitrate (19.8 g) was added to the solution and stirred for an additional hour. Then, the obtained gel was transferred into a sealed Teflon container for aging process and then heated for 52 h at 60 °C and dried for 48 h at 130 °C. Eventually, the dried samples were calcined for 1 h at 600 °C. The prepared specimens were ground with a mortar and pestle to restrain the agglomeration of nanopowders and sieve in order to produce a particle size < 180 nm.

Likewise, Cu45SBG nanopowders, SiO_2-P_2O_5-CaO-CuO (44.7 mol% SiO_2, 45.51 mol% CaO, 3.95 mol% P_2O_5, and 1.311 mol% CuO), were synthesized according to the aforementioned procedure only with a partial difference which was the addition of copper nitrate (0.38 g) after adding calcium nitrate.

2.2. Preparation of the nanocomposite membranes

The solvent casting method was applied to prepare the membranes. Briefly, BG nanopowders were dispersed ultrasonically in 10 ml of dichloromethane (DCM-CH_2CL_2, M = 84.93 g/mol, Merck-Germany) and added to PCL (Sigma-Aldrich, *Mn* = 70,000–90,000) as well as proper amount of polyethylene glycol 400 (PEG-HO $(C_2\ H_4\ O)_n$ H, M = 380–420 g/mol, Merck-Germany) as a plasticizer while stirring. The solutions with three diverse proportions of the constituents (PCL: nanopowders 97:3, 95:5, and 93:7 by weight percent) were prepared. Immediately, the mixed solution was casted in the glass molds (10 cm in diameter) and the solvent was then allowed to evaporate at the room temperature (21 °C) for 24 h. Prepared samples with different names and compositions were demonstrated in the Table 1.

2.3. Structural analysis of membranes

2.3.1. Morphological characterization of the membranes

The morphology and microstructure of the membranes were determined by Scanning Electron Microscopy (SEM, ZEISS- Germany). The samples were coated with a conductive gold layer by a thickness of 100 $\acute{Å}$ using physical vapor deposition (PVD) method.

In order to assess the distribution of BG nanopowders in the membranes, Transmission Electron Microscopy (TEM, ZEISS, Germany EM10C80KV) was utilized. To achieve that, the membranes were initially cut with a diamond blade knife employing Ultra Microtome (OmU3, REICHERT, Austria) technique. After that, prepared thin films were collected on the small metal networks and transferred into the microscope.

2.3.2. Chemical characterization

Fourier transform infrared (FTIR) spectroscopy was performed for evaluating the functional groups and the structure of the pure PCL, and nBGs along with nanocomposite membranes using (FT infrared spectroscope, JASCO, FT/IR-6300, Japan) in the 400 to 4000 cm^{-1} spectral region.

2.4. Mechanical characterization

Mechanical characteristics of the prepared samples were measured by the tensile test before and after soaking in PBS solution (after soaking the membranes in solution for 7 days) using universal testing machine (CB6-K006, Hounsfield, Korea) at 25 °C regarding ASTM F451-86 standard. The specimens were sectioned into rectangular strips

Table 1
Different samples names and compositions.

Specimens names	Compositions
S1	Pure PCL
S2	PCL/PEG/3% wt BG
S3	PCL/PEG/3% wt BGCu
S4	PCL/PEG/5% wt BG
S5	PCL/PEG/7% wt BG