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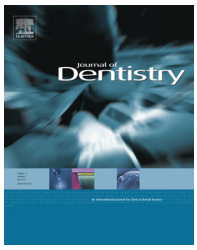

Guided bone regeneration with tripolyphosphate cross-linked asymmetric chitosan membrane

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ABSTRACT

Objectives: The objective of this study was to prepare a novel asymmetric chitosan guided bone regeneration (GBR) membrane, which is composed of a dense layer isolating the bone defect from the invasion of surrounding connective fibrous tissue and a loose layer which can improve cell adhesion and stabilize blood clots, thus guided bone regeneration.
Methods: The chitosan membrane was fabricated through liquid nitrogen quencher combined with lyophilization and cross-linked by sodium tripolyphosphate (TPP). The physical properties of asymmetric chitosan membrane were measured by scanning electron microscope (SEM), Fourier-transform infrared (FTIR), x-ray diffraction (XRD) and tensile test machine. MTT assay and Live/Dead cell staining for MC3T3-E1 osteoblasts cultured on the membrane were used to characterize the biocompatibility of the membrane. In animal experiments, full-thickness and critical sized skull defects were made to evaluate the effect of the membrane on bone regeneration.
Results: The results of this study indicate that the asymmetric chitosan membrane can be built and cross-linked by TPP to enhance the tensile strength of the membrane. *In vitro* experiment showed that no significant numbers of dead cells were detected on the chitosan membrane, indicating that the membrane had good biocompatibility. In animal experiments, the chitosan membrane had faster new bone formation, showing the capability to enhance bone regeneration.
Conclusions: The chitosan membrane prepared in this study has an asymmetric structure; its tensile strength, biodegradation and biocompatibility fulfil the requirements of guided bone regeneration. Therefore, the asymmetric chitosan membrane is a promising GBR membrane for bone regeneration.
Clinical significance: Guided bone regeneration (GBR) is an effective method for healing bone defects caused by periodontitis and implantitis, in which GBR membrane is a key biomaterial.

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1. Introduction

Both periodontitis and peri-implantitis can cause inflammation around the soft tissue as well as progressive bone loss.[1] The resulting bone defect surrounding tooth or implant can lead to tooth loss and implant failure.[2–4] Recently, guided bone regeneration (GBR) using barrier membranes has been routinely employed in dealing with bone defects and accomplished considerable success in clinical practice.[5] GBR membranes, as physical barriers, prevent the surrounding fibrous connective tissue from invading into bone defects and create a space for bone regeneration.[6] Therefore, GBR technique is effective in halting bone destruction and promoting new bone formation.[7]

GBR membranes are made of various nondegradable and degradable materials.[8] Expanded polytetrafluoroethylene (ePTFE), a typical nondegradable GBR membrane, has achieved good clinical results;[9] however, a second surgical procedure is required to remove the membrane, which creates additional surgical trauma to patients and raises their treatment costs. So far, the GBR membranes made of degradable materials, such as poly-lactic acid (PLA), poly (DL-lactic-co-glycolic acid) (PLGA), collagen, and chitosan have been developed, which avoid the second surgical procedure due to their degradability.[10–14] Among them, Bio-Gide®, a commercially available collagen membrane, has already been used in clinical practice and achieved excellent clinical effects.

More recent studies have paid much attention to developing GBR membranes with an asymmetric structure including a dense layer and a loose layer.[8,15,16] The dense layer of asymmetrical membranes can effectively isolate the bone defect from the invasion of surrounding connective fibrous tissue, while the loose layer can improve adhesion to bone and stabilize blood clots.[15] The techniques for fabricating asymmetric membranes mainly include the phase inversion (usually combined with different drying techniques)[8,16,17] and electrospinning.[18] In general, the phase inversion can be accomplished through solvent vapourization and subsequent immersion precipitation (coagulation),[8,16] and liquid nitrogen quencher.[19] The difference in the rate of solvent vapourization between the surface and the bulk of polymer solution generates a rich polymer phase and a poor polymer phase, forming a heterogeneous structure which can be stabilized by coagulating solution.[16,17] By contrast, the liquid nitrogen quencher can quickly achieve the effects mentioned above, and there is no coagulating solution remaining either. Thus, in this study, we chose the method of liquid nitrogen quencher to prepare asymmetric GBR membranes.

Chitosan(poly (1,4-D-glucosamine)), one deacetylated derivative of chitin, is a cationic natural biopolymer, which is easily processed into nanoparticles, nanofibres, gels, scaffolds and membranes.[20] Recently, some studies have focused on the membranes made of chitosan for bone tissue regeneration and skin tissue regeneration[17,21] due to its biocompatibility, biodegradability, antibacterial ability, and non-toxicity.[19,22–24] Up to now, some researchers have already developed asymmetric chitosan membranes for wound healing and guided periodontal tissue regeneration.[16,17,25] However, to our knowledge, the effect of asymmetric chitosan membrane on bone regeneration has not been investigated, lacking the relevant evidence from animal study. In order to fill this gap, we would investigate the effect of asymmetric chitosan membranes on bone regeneration through animal experiments.

GBR membranes should be equipped with satisfactory mechanical properties to accomplish the functions of barrier action, space maintenance and clinical manageability.[24] However, these requirements may not be fulfilled by using pure chitosan membrane due to its poor mechanical properties.[26,27] In general, cross-linking agents such as glutaraldehyde, genipin and sodium tripolyphosphate (TPP) are used to improve the mechanical properties of chitosan membranes.[19,28–30] Chitosan can be cross-linked with glutaraldehyde and genipin through the formation of covalent bonds. Glutaraldehyde is limited for the application on biomaterials because of its toxicity.[19,31] Genipin is a biocrosslinker but somewhat expensive. In comparison, the anionic groups of TPP react with amino groups of chitosan, producing ionic cross-linking, which is the simplest and mildest one among chitosan cross-linking methods.[32–34]

The objective of this study was to develop asymmetric chitosan GBR membranes through liquid nitrogen quencher combined with lyophilization. The mechanical properties of the chitosan membranes were enhanced by TPP cross-linking. The hypothesis was that the asymmetric bioabsorbable chitosan membrane can guide bone regeneration. In this study, the morphology, tensile strength, porosity, biodegradation, nutrient permeability, and biocompatibility of the membranes were investigated. The rat skull defect model was established to examine the bone regeneration behaviour of the membrane.

2. Materials and methods

2.1. Materials

Chitosan (*Mw*, ~70,000 and 87% deacetylated), ice acetic acid (*Mw*, ~60.05) and ethylenediaminetetraacetic acid (EDTA *Mw*, ~292.24) were purchased from Life Science Products& Services (Shanghai, China). Sodium tripolyphosphate (TPP *Mw*, ~367.86), MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) and Live/Dead cell double staining kit were purchased from Sigma (St. Louis, MO, USA). Dulbecoco's modified Eagle medium (DMEM), horse serum, penicillin, and streptomycin were all purchased from HyClone (USA). Bio-Gide® (collagen typesI/III bilayer membrane), commercialized GBR membrane, was purchased from Geistlich Pharma AG (Switzerland). Distilled water (ultrapure grade, <18 mΩ) was produced by Mili-Q purification system (EMD Millipore Corporation, Billerica, MA, USA).

2.2. Fabrication of asymmetric chitosan membranes

Chitosan solution (2 wt%) was prepared by dissolving 2 g chitosan powder in 100 mL aqueous acetic acid (2 wt% in water). Afterward, the chitosan solution was slowly poured on a polytetrafluoroethylene mould (60 mm in diameter, 15 mm in depth) at the room temperature to obtain an even liquid

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