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Enhanced mechanical properties and bone bioactivity of chitosan/silica membrane by functionalized-carbon nanotube incorporation

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ABSTRACT

A space-maintaining ability of degradable guided bone regeneration (GBR) membranes is still a challenge due to weak mechanical strength despite their excellent concept. Here we incorporated functionalized-carbon nanotube (*f*-CNT) for this purpose. In particular, a hybrid composition of chitosan/silica (CS/Si) was used as the matrix of the GBR membrane. With increasing *f*-CNT content, hydrophilic nature increased while hydrolytic degradability was reduced. Among the membranes with various contents of *f*-CNT (up to 3 wt%), 2 wt% *f*-CNT-doped CS/Si showed the highest mechanical properties, including tensile strength and elastic modulus as well as significantly improved bone bioactivity with respect to CS/Si free of *f*-CNT. *In vivo* animal study showed favorable tissue responses of the CNT-incorporated membrane. Taken all, the newly-developed CNT/CS/Si may find potential usefulness as a GBR membrane with excellent mechanical properties while exhibiting good bioactivity and tissue reaction.

1. Introduction

An effective strategy for bone defect recovery in dentistry is a guided bone regeneration (GBR), a surgical procedure utilizing barrier membranes to direct bone growth while avoiding invasion of fibrous cells or tissues. Materials of commercially available for GBR membranes include non-degradable expanded-polytetrafluoroethylene, and degradable polymers, such as collagen, polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), and polyglactin. On the one hand, non-degradable membranes have excellent space-maintaining properties to impel bone growth, but they are still skeptical of their biocompatibility and convenience in terms of the immune response and second removal surgery procedure, respectively. On the other hand, degradable membranes have a benefit to evade second removal surgery that reduces the burden of patients and show better biocompatibility than their counterparts, but there are still challenges, such as other tissue invasion in case of their rapid degradation and their mechanical stability to sustain surgery treatment or during bone healing [1]. In order to challenge these issues, recent interest in the use of organic–inorganic hybrid membranes for GBR purpose has increased because they benefit from the flexibility and reactivity of organic components and the structural robustness of chemically inert inorganic ones. Furthermore, the hybrid material with homogeneous nanostructures as an extracellular matrix (ECM) can better mimic the nanostructured bone tissue, because cells directly respond or interact with nanostructured ECMs. As a result, the biomimetic feature and physicochemical property of nanostructured hybrid materials can stimulate cell growth and guide bone healing [2].

Of these, the GBR membrane of silica xerogel-hybridized polymers, such as collagen [3], chitosan [4], poly(ε-caprolactone)

Abbreviations: CNT/CS/Si, *f*-CNT-incorporated chitosan–silica; ECM, extracellular matrix; *f*-CNT, functionalized-carbon nanotube; FT-IR, Fourier-Transform Infrared; GBR, guided bone regeneration; HA, hydroxyapatite; HCl, hydrochloric acid; PCL, poly(ε-caprolactone); PLA, polylactic acid; PLGA, poly(lactic-co-glycolic acid); PBS, phosphate buffered saline; SBF, simulated body fluid; SEM, Scanning electron microscope; TGA, thermogravimetric analysis; TMOS, tetramethylorthosilane; XRD, X-ray diffraction.

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[5], and PLA [6], produced by a sol–gel process has gained great attention in recent studies on bone regeneration, because silica incorporation into these polymers leads to a strong interfacial interaction [7] and improves mechanical/biological properties of these polymers. These synergetic effects are explained by the silica stiffness and bioactivity structured from the atomic composition similar to the bioactive glass clinically used as a bone defect filler. In addition, a sol–gel process to hybridize organic/inorganic materials allows them to lead to the homogeneous mixture at nanoscale. Compared to silica-free membranes, however, these hybrids were rapidly degraded due to the rapid absorption of hydrophilic silica into aqueous media, i.e., their sparse structure attributed to silica absorption was a key reason to accelerate their degradation. Indeed, chitosan–silica hybrid membrane [4] in our previous study was more rapidly degraded than silica-free membrane, probably resulting in the hindrance of bone healing in case of too faster degradation of materials than bone regeneration rate.

In order to reduce degradation rate of hybrid membranes while enhancing physical stability and bone regeneration, therefore, we incorporated the functionalized-carbon nanotube (*f*-CNT) into the chitosan–silica hybrid membrane via a sol–gel process in this study. Unlike pristine carbon nanotubes (CNTs), *f*-CNT can increase their water miscibility and make their composites further robust and biocompatible with negligible *in vivo* toxicity. For instance, *f*-CNT-incorporated biodegradable biomaterials such as collagen [8], chitosan [9], or hydroxyapatite (HA) [10] enhanced their physical strength and cellular functions without *in vivo* toxicity. Thus, this study reports the investigations of *f*-CNT-incorporated chitosan–silica (CNT/CS/Si) xerogel nanohybrid membrane concerning physicochemical properties and bone bioactivity *in vitro* and *in vivo*.

2. Experimental section

2.1. Materials and membrane fabrication

Chitosan powder with medium molecular weight and 75~85% of deacetylated degree, tetramethylorthosilane (TMOS), and multi-walled *f*-CNTs (CNT–COOH═CNTs, >95%, 20–30 nm outer diameter, 10–30 μm length) treated by acid condition were purchased from a commercial vendor (Sigma–Aldrich). All supplementary chemicals were of analytical grades and were used without further purification.

CNT/CS/Si hybrid membrane was prepared in a form of membranes by a sol–gel process at room temperature. Briefly, a silica solution was prepared by adding 1 mL (6.7 mmol) of TMOS to 1 mL of hydrochloric acid (HCl) at pH 2.0, while stirring for 3 h at room temperature. A CNT/CS solution was separately prepared by homogenizing the given concentrations of *f*-CNT via ultrasonication in a 5% chitosan solution (0.5 N HCl). Subsequently, both solutions were combined and then the mixture solution was homogeneously stirred for 3 h to induce the sol–gel transition. Ethanol was slowly poured into the homogeneous CS/Si/CNT hybrid solution under stirring to obtain fine hybrid fiber precipitates. After the precipitates were filtered and freeze–dried, they were hot pressed at 40 °C and 2 psi for 30 min to obtain a thick membranous form with a dimension of 10 cm × 10 cm × 0.1 cm (length × width × thickness).

2.2. Characterization

The dried CNT/CS/Si nanohybrid composite was analyzed by Fourier-Transform Infrared Spectrometry (FT-IR, Perkin Elmer, Spectrum BXII spectrophotometer) and thermogravimetric analysis (TGA, TG/DTA6100, SEICO INST) at a heating rate of 10 °C/min from 25 to 900 °C in air. Scanning electron microscopic (SEM) surface morphology of the membrane was obtained on the microscope of a JEOL JSM7000F and a HITACHI S-3000H. The samples were fixed on carbon tape and then coated with a thin layer of gold by a sputtering technique. The mechanical properties of the samples were recorded using an Instron universal testing machine (Instron 3344) at room temperature. Four specimens per group with a dimension of 10 mm × 3 mm × 1 mm (length × width × thickness) were drawn at a speed of 5 mm/min and the stress–strain curves were recorded. An initial slope at the linear region was considered as an elastic modulus, and a maximum stress value prior to a break was taken as a tensile strength. A strain value at break point was considered as an elongation rate.

2.3. In vitro assays

Water uptake capacity of CNT/CS/Si membranes was assessed by soaking the specimens (10 mm × 10 mm) in water for given periods of up to 24 h. As with water uptake assessment, their degradation and mineral induction tests were evaluated by incubating the specimens (10 mm × 10 mm) in phosphate buffered saline (PBS) and simulated body fluid (SBF) for the given periods at 37 °C, respectively. SBF solution with similar ion concentrations to human blood plasma was prepared according to a Kokubo's method [11]. Measurements of the water uptake and degradation rates were taken by normalizing the final weight to the initial weight of specimens at a final time point of the measurement. For the assessment of mineral induction, morphological changes of the samples were analyzed by SEM micrographs. The phase and composition of the samples were detected by X-ray diffraction (XRD, Rigaku).

2.4. In vivo study

The *in vivo* biocompatibility of the nanohybrid samples was evaluated in a rat calvarial defect model using six 11-week-old male Sprague–Dawley rats. Effects of the inserted nanohybrid samples on tissue reaction and bone formation were investigated. A protocol of this study was approved by the Dankook University Institutional Animal Care and Use Committee, South Korea. Prior to the surgical operation, the animals were anesthetized with intramuscular injections of 80 mg/kg ketamine and 10 mg/kg xylazine. All surgical procedures were carried out under general anesthesia using sterile techniques. The cranial bone was exposed following a midline incision through the skin and the excision of the periosteum using #10 blades with a bardparker scalpel. Two critical-size calvarial defects of 5 mm in diameter were created at the mid-portion of each parietal bone using a trephine burr, and the defects were randomly allocated to two groups with CNT-free CS/Si and CNT/CS/Si membranes. The subcutaneous area was sutured with a 4–0 absorbable suture material, and the skin was subsequently sutured with a 4–0 non-absorbable monofilament suture material. After surgery, each rat per cage was housed, and kept on a 12 h light/12 h dark cycle, while providing standard pellet food and water ad libitum. The tissue specimens harvested from the animals after 4 weeks were fixed in a 10% neutral buffered formaldehyde solution, and undergone by a process of decalcification and dehydration in a graded series of increasing ethanol concentrations. Paraffinized tissue sections perpendicular to the cranial bone were histologically analyzed with Hematoxylin–Eosin (HE) staining.

2.5. Statistical analysis

All experiments were repeated three or more times, and the experimental data are expressed as the mean ± one standard