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Guided bone regeneration by poly(lactic-*co*-glycolic acid) grafted hyaluronic acid bi-layer films for periodontal barrier applications

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Abstract

A novel protocol for the synthesis of biocompatible and degradation controlled poly(lactic-*co*-glycolic acid) grafted hyaluronic acid (HA-PLGA) was successfully developed for periodontal barrier applications. HA was chemically modified with adipic acid dihydrazide (ADH) in the mixed solvent of water and ethanol, which resulted in a high degree of HA modification up to 85 mol.%. The stability of HA-ADH to enzymatic degradation by hyaluronidase increased with ADH content in HA-ADH. When the ADH content in HA-ADH was higher than 80 mol.%, HA-ADH became soluble in dimethyl sulfoxide and could be grafted to the activated PLGA with N,N′-dicyclohexyl carbodiimide and N-hydroxysuccinimide. The resulting HA-PLGA was used for the preparation of biphasic periodontal barrier membranes in chloroform. According to in vitro hydrolytic degradation tests in phosphate buffered saline, HA-PLGA/PLGA blend film with a weight ratio of 1/2 degraded relatively slowly compared to PLGA film and HA coated PLGA film. Four different samples of a control, OSSIX^{TM} membrane, PLGA film, and HA-PLGA/PLGA film were assessed as periodontal barrier membranes for the calvarial critical size bone defects in SD rats. Histological and histomorphometric analyses revealed that HA-PLGA/PLGA film resulted in the most effective bone regeneration compared to other samples with a regenerated bone area of 63.1% covering the bone defect area.

Keywords: Hyaluronic acid; Poly(lactic-*co*-glycolic acid); Periodontal barrier membrane; Controlled degradation; Bone regeneration

1. Introduction

A variety of membrane materials has been developed for guided bone regeneration (GBR) and guided tissue regeneration (GTR) [1–6]. The materials that are used as a barrier membrane for GBR/GTR procedures should meet several prerequisites. As the membrane is supposed to be implanted in the body, it must be biocompatible, non-immunogenic, and non-toxic. To avoid the removal of the membrane after healing, it would be better to be composed of biodegradable materials. The degradation time should be long enough to achieve bone regeneration before membrane disintegration. Other properties such as tissue integration, cell occlusivity, nutrient transfer, space making ability and ease of use in the clinic are also of interest [7]. There are various commer-

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cially available products, ranging from non-resorbable materials such as expanded polytetrafluorethylene (e-PTFE) to bioabsorbable membranes composed of poly(lactic acid), poly(glycolic acid), polyurethane, and so on [7–11]. More recently, many investigations focused on the use of products derived from type I and type III porcine or bovine collagen [12]. Some advantageous properties of collagen over other materials include homeostatic function to allow early wound stabilization, chemotactic properties to attract fibroblasts, and semi-permeability to facilitate nutrient transfer [13]. However, the porcine and bovine collagens are known to have a major drawback of immunogenicity in the body.

Poly(lactic-*co*-glycolic acid) (PLGA) has been extensively investigated and used for various medical applications for a few decades due to its biodegradability and biocompatibility [14]. The biodegradation of PLGA can be controlled by changing its molecular weight, composition (the ratio of LA to GA in PLGA), crystallinity and other parameters [14]. More significantly, PLGA has the outstanding biocompatibility with bio-absorbable and non-toxic degradation products. PLGA exhibits a wide range of physicochemical diversities depending on the structural characteristics. For example, high-molecular-weight crystalline PLGA can be fabricated into surgical sutures, bone fixation nails and screws with a feasible mechanical strength. On the other hand, low molecular weight amorphous PLGA is found to be useful for controlled drug delivery applications [15]. Recently, hyaluronic acid (HA) and modified HA have been used for various medical applications such as drug delivery and tissue engineering [16–21]. As a natural linear polysaccharide, HA is biodegradable, biocompatible and non-immunogenic [22]. HA is also known to be osteoconductive, promote angiogenesis, and moderate immune responses [22]. A number of strategies for the chemical modification of HA through the functional groups of carboxyl and hydroxyl groups have been reported as described elsewhere [23–27]. Most of HA chemical modifications have been carried out in aqueous solution. In order for the chemical modification of HA in an organic solvent, such as dimethyl sulfoxide (DMSO), tetrabutyl ammonium (TBA) salt of HA was prepared in aqueous solution using ion-exchange resins [23]. For example, benzyl ester of HA, Hyaff®, has been synthesized by the esterification of TBA salt of HA with benzyl bromide in DMSO [23].

In this work, we have developed a novel biocompatible and degradation-controlled HA-PLGA for the applications to periodontal barrier membranes. HA was chemically modified with adipic acid dihydrazide (ADH) in the mixed solvent of water and ethanol. The addition of ethanol resulted in highly modified HA-ADH, which exhibited the enhanced stability to enzymatic degradation by hyaluronidase. Interestingly, when the ADH content in HA-ADH was higher than 80 mol.%, HA-ADH became soluble in DMSO and could be grafted to the activated PLGA with N, N′-dicyclohexyl carbodiimide (DCC) and N-hydroxysuccinimide (NHS). The resulting HA-PLGA was used for the preparation of amphiphilic bi-phasic films. After in vitro degradation tests in phosphate buffered saline (PBS), four different samples of a control (no treatment), OSSIX™ membrane, PLGA film, and HA-PLGA/PLGA blend film were assessed as periodontal barrier membranes for bone regeneration in the calvarial critical size bone defect of SD rats. Histological and histomorphometric analyses were carried out after hematoxylin–eosin (H&E) staining of regenerated bones in 8 and 12 weeks.

2. Experimental

2.1. Materials

PLGA with a molecular weight (MW) of 66,000 was obtained from Wako Pure Chemicals Co. (Osaka, Japan). HA with MW of 20,000 and 132,000 was purchased from Lifecore Co. (Chaska, MN). Adipic acid dihydrazide (ADH), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC hydrochloride), N-hydroxysuccinimide (NHS), N,N′-dicyclohexyl carbodiimide (DCC), and PBS tablet were purchased from Sigma–Aldrich (Milwaukee, WI). Ethanol, hydrochloride (HCl), sodium hydroxide, acetonitrile, dimethyl sulfoxide (DMSO) and chloroform ($CHCl_3$) were obtained from Junsei Chemicals (Tokyo, Japan), and hyaluronidase SD (*Streptococcus dysgalactiae*) from Seikagaku Biobusiness Co. (Tokyo, Japan). All reagents were used without further purification.

2.2. HA-ADH synthesis

To increase the degree of ADH modification in HA-ADH, the protocol for HA-ADH preparation by Luo et al. was slightly modified as follows [27]. HA (100 mg, 250 μmol) was dissolved in 20 ml of water to prepare HA solution of 5 mg ml^{-1}. Forty times molar excess of solid ADH (10 mmol) was added to the solution and dissolved completely by mixing for 10 min. The pH of the mixed solution was adjusted to 4.8 by the addition of 1.0 N HCl. Then, ethanol (20 ml, 50 vol.%) was added and mixed for 30 min. After that, four times molar excess of EDC (1 mmol) was added in a solid form. The pH of the mixed solution was maintained at 4.8 by the addition of 1.0 N HCl. The reaction was stopped in 2 h by raising the pH of reaction mixture to 7.0 with 1.0 N NaOH. The reaction solution was poured into the pre-washed dialysis membrane tube (MWCO of 7000) and dialyzed against a large excess amount of 100 mM NaCl solution, followed by the dialysis against 25 vol.% ethanol and pure water. The resulting solution was finally lyophilized for 3 days. The purity of HA-ADH was determined by gel permeation chromatography (GPC, Waters, Milford, MA) and the degree of ADH modification was measured by ^1H nuclear magnetic resonance (NMR, DPX300, Bruker, Germany) analysis [27].

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