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An in vivo study of the host tissue response to subcutaneous implantation of PLGA- and/or porcine small intestinal submucosa-based scaffolds ☆

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Abstract

An innate immune response is often found at the site of biomaterial implantation. Since the effective use of biomaterials in vivo requires good biocompatibility and biofunctionality, it is vital that we assess and compare the inflammatory reactions provoked by various implanted biomaterials in vivo. In the present study, we assessed the host tissue response to poly(lactic-*co*-glycolic acid) (PLGA)- and small intestinal submucosa (SIS)-based scaffolds subcutaneously implanted in Fischer rats. Our results revealed that the PLGA-based scaffolds resulted in severe post-implantation inflammation, whereas the SIS-based scaffolds induced only a slight post-implantation inflammation and a PLGA/SIS-based copolymer yielded intermediate results.

Keywords: Biomaterials; Scaffold; PLGA; Small intestinal submucosa; Inflammation

1. Introduction

Tissue engineering, which holds great promise for the repair and regeneration of defective or damaged tissues and organs [1], requires the presence of a suitable scaffold on which the repair/regeneration takes place [2,3]. Because proliferation of most mammalian cell types is anchorage-dependent [4], the utilized scaffold must provide a suitable surface for cell attachment, proliferation, differentiation, and migration [5–7]. In addition, the scaffold material should be easy to handle and apply, while also being cost-effective. More than anything else, the scaffolds must be biocompatible and non-toxic.

The biomaterials presently used as scaffolds in tissue engineering can be classified into three types: naturally derived materials (e.g. collagen and alginate), acellular tissue matrices (e.g. bladder submucosa and small intestinal submucosa), and synthetic polymers [e.g., polyglycolic acid (PGA), polylactic acid (PLA), and poly(lactic-*co*-glycolic acid) (PLGA)] [8–11]. Naturally derived materials and acellular tissue matrices have various advantages in the biological environment. Collagen, which is the most abundant and omnipresent structural protein in the body [12,13], and small intestinal submucosa (SIS) derived from the submucosal layer of porcine intestine [14–16], both generate a minimal inflammatory response following implantation and have been approved by the FDA for many types of biomedical applications. SIS consists of more than 90% types I and III collagen, plus a wide variety of cytokines, including basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor-1 (IGF-1), as well as glycosaminoglycans, fibronectins, chondroitin sulfates, heparins, heparin sulfates, and hyaluronic acids [17–20]. These constituents are expected to facilitate the function of SIS as a tissue engineering scaffold by supporting cell attachment, proliferation, differentiation, and migration.

☆ For Festschrift of Professor David Williams.

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However, the synthetic polymers have advantage of being able to be manufactured reproducibly on a large scale with controlled properties of strength, degradation rate, and microstructure. PGA, PLA, and PLGA, three copolymers that are widely used in tissue engineering, have gained FDA approval for human use in biomedical fields. However, these copolymers have the disadvantage that their degradation results in an acid environment due to the formation of lactic and glycolic acid [21–23].

Williams and coworkers noted that the innate immune system plays an important role in determining the biocompatibility of biomaterials, and found that in vivo implantation of biomaterials led to tissue-specific responses by macrophages or foreign body giant cells [24–27]. Since the practical use of clinical biomaterials in tissue engineering may be affected by the induction of a post-implantation host tissue response, the development of suitable scaffolding biomaterials must include the minimization of post-implantation host tissue responses.

In light of this, we herein compared the host tissue response to representative synthetic and natural biomaterials by assessing inflammation at the implanted area. We prepared and characterized PLGA-, SIS- and PGLA/SIS-based scaffolds, performed subcutaneous implantation of these scaffolds into rats, and then compared the host tissue response by immunohistochemical staining with ED1 (an anti-CD68 antibody macrophage marker).

2. Materials and methods

2.1. Materials

PLGA (molecular weight 90,000 g/mole, 75:25 by mole ratio of lactide to glycolide; Resomer RG756) was purchased from Boehringer Ingelheim (Germany).

2.2. Preparation of native SIS

Sections of porcine jejunum were harvested from market pigs within 4 hrs of sacrifice (Finnish pig, F_1; Land race + Yorkshire, around 100 kg at 6 months; Woomi Food Company, Korea). The fat was removed from porcine jejunum, which was carefully washed with water, cut into lengths of approximately 10 cm, and then washed with saline solution. The tunica serosa and tunica muscularis were mechanically removed, leaving the SIS. The decellularized SIS was washed with saline solution followed by 0.1% peracetic acid, and then sliced at about 7 cm in the longitudinal direction. The slices were then frozen in 0.5% gentamycin until use. For experiments, the slices were freeze-dried using a freeze dryer (Model FDU-540, EYELA, Japan) at −80 °C for 48 h, and the dried SIS was pulverized using a freezer mill (6700, SPEX Inc., USA) at −198 °C to yield a SIS powder of 10–20 μm in size.

2.3. Preparation of PLGA- or PLGA/SIS-based scaffolds

Ice particles were prepared by spraying deionized water into liquid nitrogen, and particles of 180–250 μm were selected by sieving. PLGA alone or PLGA and SIS powder (20 wt% for PLGA) (PLGA/SIS20) was prepared by dissolution in methylene chloride (MC). The solution was cooled to −20 °C, the above-prepared ice particles were added (90 wt% for PLGA) and the dispersion was gently vortexed and poured into a pre-cooled silicon mold. The mold containing the dispersion was frozen at −78 °C for 2 days and then freeze-dried at −50 °C to remove the MC and ice particles, leaving the desired porous scaffolds behind.

2.4. Preparation of the SIS-based scaffold

SIS powder (1 wt% concentration) was mixed with 10 ml of a solution of 3% acetic acid and 0.1% pepsin. The mixture was stirred for 48 h to specifically cleave the non-triple-helical domains of the collagen. Thereafter, collagen molecules having a native triple-helical structure were solubilized from the tissue. The SIS solution was carefully poured into a homemade silicone mold ($30 \times 5\,mm^2$), and the mold was stored at −20 °C to allow formation of ice particles inside the SIS-based scaffold. After 24 h, the mold was freeze-dried to remove the formed ice particles. The resulting SIS-based scaffold was crosslinked with 100 mM 1-ethyl-(3,3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in a solution of deionized water and ethanol (5/95, v/v) for 24 h, dipped in water at 40 °C for 1 h, and then freeze-dried.

2.5. SEM measurements

Scanning electron microscopy (SEM, S-2250N; Hitachi, Japan) was used to examine the morphology of the generated scaffolds. Each scaffold was mounted on a metal stub and coated with a thin layer of platinum using a plasma-sputtering apparatus (K575; Emitech, Japan) under an argon atmosphere.

2.6. Measurement of porosity and pore size

A porosimeter (Auto Pore IV 9520 V 1.03; Instruments Co., USA) was used to examine the porosity and mean pore diameter of the PLGA-, PLGA/SIS20-, and SIS-based scaffolds. The porosimeter was capable of acquiring continuous data at up to 60,000 psi pressure for intrusion of mercury into the PLGA-, PLGA/SIS20-, and SIS-based scaffolds. Higher pressures were used to intrude mercury into smaller pore sizes of 6–0.003 μm, while low pressures were used for pore diameters ranging from 360 to 3.6 μm. Penetration was performed with a penetrometer equipped with five consecutive capillary stems (1.5 cm diameter and 1.0 cm long) representing intrusion volumes of 0.38, 1.1, 1.7, 3.1, and 3.9 cm^2. The resolution of intrusion was better than 0.1 μl and incremental changes in the applied pressure allowed us to measure the incremental volume of mercury intruded (pore volume) into the PLGA-, PLGA/SIS20-, and SIS-based scaffolds. The pore size distributions were determined by relating pore size to pore volume. The porosimeter was equipped with a computer-based acquisition system that generated data files on cumulative and incremental volumes of intruded mercury as a function of pore size.

2.7. Wetting properties

The water contact angles of the PLGA-, PLGA/SIS20-, and SIS-based scaffolds were measured using a contact angle goniometer (SEO-300A; SEO Co., Korea). Briefly, the scaffolds were placed on the sample holder and covered with 40 μl deionized water using a syringe. The measurement was completed within 60 s for the PLGA- and PLGA/SIS-based scaffolds and within 10 s for the SIS-based scaffold. The angle between the baseline of the droplet and droplet was directly visualized on-screen using the goniometer.

2.8. In vivo scaffold implantation

Nine 5-week-old Fischer rats (150–160 g) were housed in sterilized cages with sterile food and water and filtered air, and were handled in a laminar flow hood following aseptic techniques. All animals were treated in accordance with the Catholic University of Korea Council on Animal Care Guidelines. For implantation tests, the rats were divided into three groups ($n = 3$ each), designated PLGA, PLGA/SIS20, and SIS. All