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The incorporation of poly(lactic-*co*-glycolic) acid nanoparticles into porcine small intestinal submucosa biomaterials

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Abstract

Small intestinal submucosa (SIS) derived from porcine small intestine has been intensively studied for its capacity in repairing and regenerating wounded and dysfunctional tissues. However, SIS suffers from a large spectrum of heterogeneity in microarchitecture leading to inconsistent results. In this study, we introduced nanoparticles (NPs) to SIS with an intention of decreasing the heterogeneity and improving the consistency of this biomaterial. As determined by scanning electron microscopy and urea permeability, the optimum NP size was estimated to be between 200 nm and 500 nm using commercial monodisperse latex spheres. The concentration of NPs that is required to alter pore sizes of SIS as determined by urea permeability was estimated to be 1 mg/ml 260 nm poly(lactic-*co*-glycolic) acid (PLGA) NPs. The 1 mg/ml PLGA NPs loaded in the SIS did not change the tensile properties of the unmodified SIS or even alter pH values in a cell culture environment. More importantly, PLGA NP modified SIS did not affect human mammary endothelial cells (HMEC-1) morphology or adhesion, but actually enhanced HEMC-1 cell growth.

Keywords: Scaffold; Small intestinal submucosa; Porosity; Nanoparticle; Biocompatibility

1. Introduction

Small intestinal submucosa (SIS) is a xenogenic dense connective tissue harvested from porcine small intestine. The tunica mucosa and the serosa muscularis are mechanically removed from the inner and the outer sides of the intestine, respectively, producing a thin, translucent graft (0.1 mm wall thickness) composed mainly of the submucosal layer of the intestinal wall [1]. SIS is rich in type I collagen and has a resorption rate of 4–16 weeks *in vivo* [2]. SIS is non-immunogenic in over 1000 cross-species transplants with no adverse response being reported [3,4]. SIS has been investigated to repair and replace a variety of tissues including tendon [5], arterial and venous tissues [1,6,7], skin [8], wound closure [9], as well as urinary bladder [10–12] in a variety of animal models including rodents [13,14], rabbits [15], and dogs [2,16,17].

SIS is superior to synthetic biomaterials because it supports cell ingrowth and differentiation without prior cell seeding such as in the case of bladder regeneration [18]. The superiority of SIS in tissue regeneration may result from intrinsic

Abbreviations: SIS, small intestinal submucosa; PLGA, poly(lactic-*co*-glycolic) acid; NPs, nanoparticles; EC, endothelial cells; ECM, extracellular matrix; PVA, poly(vinyl alcohol); PEI, poly(ethyleneimine); PBS, phosphate buffer saline; TNE, Tris–HCl/NaCl/EDTA; HMEC, human mammary endothelial cells.

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growth factors [19], glycosaminoglycans, glycoproteins, and proteoglycans [20] residing in SIS that assist in cell migration, proliferation, and differentiation, as well as cell–cell and cell–biomatrix interactions during the regenerative process [21]. However, a number of pre-clinical trials have clearly demonstrated that not all SIS biomaterials are the same. Thus, using SIS is constrained by obtaining reliable, reproducible products in large-scale preparations, and is subjected to the concerns of heterogeneity in its structural features [18].

In order to address the heterogeneity issue related to SIS, we proposed the possibility of altering the microarchitecture of SIS and providing a more uniform SIS for better tissue repair and regeneration using nanotechnology. Biodegradable poly(lactic-*co*-glycolic) acid (PLGA) nanoparticles (NPs) have been manufactured using double emulsification–solvent evaporation method and used as a vehicle for delivering various bioactive molecules including nucleotides [22–24] and drugs [25–27] for therapeutic purposes [28–30].

In this study, we investigated the possibility of modifying SIS using PLGA NPs. NPs modified the SIS porous structure as demonstrated by scanning electron microscopy (SEM) while the membrane's permeability to urea decreased. In addition, NPs did not alter the physical characteristics of the original SIS. More importantly, the NP modified SIS continued to support cell adhesion and proliferation; and cell growth was significantly higher in PLGA NP modified SIS as compared to the unmodified SIS.

2. Materials and methods

2.1. Materials

PLGA with a 50:50 monomer ratio, molecular weight of 106 kDa, and viscosity of 1.05 dl/g was purchased from Absorbable Polymers International (Pelham, AL). Negatively charged polystyrene latex spheres (six sizes between 50 nm and 2000 nm), urea, poly(vinyl alcohol) [PVA], poly(ethyleneimine) [PEI], and MCDB cell culture medium were obtained from Sigma–Aldrich (St. Louis, MO). Single layer SIS (Surgisis®) was obtained from Cook® Biotech (West Lafayette, IN). Chloroform was purchased from EMD Chemicals (San Diego, CA). Urea assay kit was purchased from Diagnostics Chemicals Limited (Oxford, CT). Fetal bovine serum (FBS) and penicillin–streptomycin were obtained from Invitrogen (Carlsbad, CA). Human mammary endothelial cells (HMEC-1) were provided by Dr. Mike Ihnat at the University of Oklahoma Health Sciences Center [31].

2.2. Synthesis of PLGA NPs

PLGA NPs were synthesized using a modified double emulsion solvent evaporation technique [32]. Briefly, 30 mg of PLGA was first dissolved in 1 ml of chloroform. An aliquot of 200 μl of 7% PEI (used to produce positively charged nanoparticles) was added to the PLGA/chloroform solution followed by sonication on ice with a probe sonicator (model VC60; Sonics & Materials, Danbury, CT) set in a continuous mode for 30 s at 100% amplitude. The primary emulsion was transferred into 10 ml of 1% PVA; and the entire solution was sonicated on ice for another 1 min. The organic solvent in the final solution was allowed to evaporate overnight with continuous stirring. PLGA NPs were recovered by centrifugation at 30,000 × *g* for 20 min at 4 °C. The pellet consisting of aggregated NPs was weighed and washed three times with water to remove any residual PVA. PLGA NPs were then resuspended in water using sonication to obtain a final concentration of 2 mg/ml. The NPs were either used immediately or freeze-dried and then stored at −80 °C for later use.

2.3. Characterization of PLGA NPs

PLGA NPs were assessed for the particle size, polydispersity index, and zeta potential using diffraction light scattering Zeta PALS (Brookhaven Instruments, Holtsville, NY) at room temperature. Viscosity and refraction indices were set equal to those specific of water. Particle concentration was measured using a FACSCalibur flow cytometer (Becton–Dickinson, San Jose, CA). For this purpose, synthesized NPs were diluted in water at four different concentrations. Particle concentrations were calculated using a calibration curve developed using commercially available latex particles with four different known concentrations.

2.4. Microarchitecture analysis of NP modified SIS

SIS was cut into 1.2 cm × 1.2 cm pieces and assembled in 1.5 ml Eppendorf tubes between the lid and the tube with mucosal side facing upwards. NPs were loaded onto the mucosal side of the SIS inserts. The inserts were incubated overnight at room temperature with a constant shaking on an orbital shaker. To evaluate the microarchitecture of the NP modified SIS, the modified biomatrix was dehydrated using increasing concentrations of ethanol followed by a brief vacuum drying. Samples were then sputter coated with a 15 nm thick layer of gold at 40 mA and analyzed using an SEM (Joel scanning microscope).

2.5. Characterization of physical properties of PLGA NP modified SIS

SIS was converted into wells constructed using silicone glue on the mucosal side; and PLGA NP suspensions with the concentration of 1.273 mg/cm^2 were added onto the mucosal side of SIS. The assembly was placed on an incubator shaker at 37 °C overnight. The NP modified SIS membranes were rinsed with water to remove unattached NPs. The thickness of the NP modified SIS was measured using our previously described method [33]. Briefly, the NP modified SIS membranes were cut into small (2 mm × 10 mm) strips. Digital micrographs of the cross section were recorded using an inverted microscope equipped with a CCD camera. The cross section distances were measured using Sigma Scan Pro software (Systat Software, Inc., Point Richmond, CA) which was calibrated using an image of a hemocytometer.

Tensile properties were also determined by our previously described method [33,34]. In brief, 6 cm × 1 cm strips of NP modified SIS membranes were cut from each sample and analyzed using an INSTRON 5842 (INSTRON Inc., Canton, MA) with a constant crosshead speed of 10 mm/min. Tests were performed under hydrated conditions at 37 °C using a custom designed chamber.

2.6. Urea permeability studies of NP modified SIS

Permeability was analyzed using the apparatus built in-house as previously described [33]. Briefly, latex spheres and PLGA NPs were suspended in phosphate buffered saline (PBS), and placed on the mucosal side of SIS placed in the permeability chamber. NPs were allowed to settle onto the SIS through gravity on an orbital shaker at 37 °C overnight. The NP modified SIS membranes were washed three times with PBS in the chamber, and filled with 550 mM urea (typical concentration at physiological conditions) in PBS. PBS was then added to the serosal side of SIS in the second chamber. Aliquots of samples (20–50 μl) were collected from the second chamber between 0 min and 2 h. Samples collected immediately after the assembly of the chambers were used as time-zero values (i.e. C_2 at $t = 0$). Concentrations of urea were determined using a urea quantitation kit (Diagnostic Chemicals Limited, Oxford, CT).

Membrane permeability was calculated as described previously [33]. In brief, the following equation is obtained using a quasi-steady state approximation

$$\ln\left(\frac{C_0 - 2C_2}{C_0}\right) = -\left(\frac{A_m}{V}P\right)t$$