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Surface modifications of photocrosslinked biodegradable elastomers and their influence on smooth muscle cell adhesion and proliferation

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Abstract

Photocrosslinked, biodegradable elastomers based on aliphatic polyesters have many desirable features as scaffolds for smooth muscle tissue engineering. However, they lack cell adhesion motifs. To address this shortcoming, two different modification procedures were studied utilizing a high and a low crosslink density elastomer: base etching and the incorporation of acryloyl-poly(ethylene glycol) (PEG)-Gly-Arg-Gly-Asp-Ser (GRGDS) into the elastomer network during photocrosslinking. Base etching improved surface hydrophilicity without altering surface topography, but did not improve bovine aortic smooth muscle cell adhesion. Incorporation of PEG-GRGDS into the elastomer network significantly improved cell adhesion for both high and low crosslink density elastomers, with a greater effect with the higher crosslink density elastomer. Incorporation of GRGDS into the high crosslink density elastomer also enhanced smooth muscle cell proliferation, while proliferation on the low crosslink density unmodified, base etched, and PEG-GRGDS incorporated elastomers was significantly greater than on the high crosslink density unmodified and base etched elastomer.

Keywords: Biodegradable elastomer; Photocrosslinking; Surface modification; RGD; Base etching

1. Introduction

Smooth muscle is a component of many soft tissues, such as blood vessels, heart valves, cardiac muscle, intestines, lungs and the bladder [1]. A promising strategy to replace smooth muscle-containing tissue with lost or impaired function consists of the implantation of a three-dimensional porous biomaterial scaffold, pre-seeded with autologous tissue-specific cells or stem cells, into the damaged or missing tissue area [2–5]. The three-dimensional polymer scaffold supports cell growth and directs this growth to generate new tissue. To be effective, the polymer chosen must possess a number of specific criteria: it must be biodegradable and degrade at a rate that is matched by the growth of active tissue, it must possess adequate biocompatibility both after implantation and during degradation (i.e. it must not induce a significant or prolonged inflammatory response), and it must promote or allow cellular attachment, growth, and deposition of extracellular matrix. Moreover, as smooth muscle endows the tissue with the ability to be repeatedly contracted and relaxed, the polymer must also be capable of cyclic mechanical stimulation. The necessity of cyclic mechanical stimulation in the growth of elastic soft tissues has recently been well established [6–11].

We have recently developed a biodegradable elastomer that possesses many of the desirable properties outlined above. The elastomer is prepared through the photocrosslinking of ω,ω,ω,-triacrylate [*star*-poly(ε-caprolactone-*co*-D,L-lactide)] (ASCP), yielding elastomers with mechanical properties that are readily controlled through manipulation of the prepolymer molecular weight, composition, and crosslinking conditions [12]. For example, increasing prepolymer molecular weight results in elastomers that have a lower crosslink density and therefore possess a lower

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modulus, but are more extensible. The elastomer is non-cytotoxic, degrades in vivo at a rate controlled by prepolymer molecular weight and monomer composition, and is well tolerated by the host tissue [13]. Furthermore, as the elastomer is crosslinked through covalent linkages, it is creep-resistant and capable of undergoing repetitive mechanical loading.

Nevertheless, it was anticipated that the elastomer would possess poor cell adhesion, much like other caprolactone-based polymers [14–19]. The objective of this study was therefore to examine smooth muscle cell (SMC) interaction with the unmodified elastomer and to investigate relatively straightforward techniques to improve smooth muscle cell adhesion and proliferation. Thus, two different surface modification methods were employed: base-catalyzed surface etching, and the incorporation of the arginine–glycine–aspartic acid (RGD) peptide sequence into the network tethered to a poly(ethylene glycol) (PEG) spacer.

Base etching of non-crosslinked aliphatic polyesters has been used by many others to improve cellular adhesion [14,17,20–22]. However, the mechanism by which this process improves cell adhesion has yet to be resolved. Base etching of polyesters generates surface resident carboxylic acid and hydroxyl groups, which increase the hydrophilicity of the surface [20,21] and produces a surface roughness [17,22]. The mechanism of improved cell attachment has been linked to both effects, and both of these effects have been shown to promote enhanced protein adsorption to the material surface [20,23]. Moreover, the degree of improvement of cell adhesion appears to be dependent on cell type [22]. With respect to the influence of base etching aliphatic polyesters on SMC adhesion specifically, Miller et al. [22] reported that aggressive base etching of poly(lactide-*co*-glycolide) resulted in surfaces with a nanoscale topography, and used silicone casting techniques to show that SMC were responding to the nanotopography and not the changes in surface chemistry. Serrano et al. [14], on the other hand, found that base etching improves SMC adhesion to, and proliferation on, poly(ε-caprolactone) films, but showed through atomic force microscopy (AFM) measurements that there were no significant differences in surface topography and so concluded that the improved adhesion was a result of the improved surface hydrophilicity. One objective of this study, therefore, was to determine which effect, surface roughness or improved hydrophilicity, influences SMC adhesion to the base etched elastomer surface.

Functionalization of polymers with peptide integrin ligands such as RGD has also been shown to improve the surface attachment and proliferation of various cell types (reviewed in Ref. [24]). The RGD peptide sequence can address more than one cell adhesion receptor and is widely distributed throughout the body as cell adhesive sites in vitronectin, collagen, laminin and other extracellular matrix proteins. The RGD sequence has better affinity and specificity when it has flanking amino acids. In particular, the Gly-Arg-Gly-Asp-Ser (GRGDS) sequence has comparable affinity to αvβ3 and α5β1 integrins, which have been found to mediate smooth muscle cell adhesion to fibronectin [25–27] and vitronectin [25,27,28], respectively. Massia and Stark [29] demonstrated that immobilization of the peptide sequence GRGDSP to dextran improved rat aortic smooth muscle cell attachment onto slides coated with the functionalized dextran, and Mann et al. [30] and Peyton et al. [31] have shown that RGDS immobilized within PEG hydrogels improves both aortic smooth muscle cell adhesion and proliferation.

RGD sequences have been incorporated into PEG hydrogel networks through the acrylation of the peptide sequence at the N-terminus, followed by copolymerization of the acrylated peptides with α,ω-diacrylate PEG via photocrosslinking in aqueous solution [30–32]. We reasoned that such an approach would also be effective for preparing biodegradable, photocrosslinked elastomers with improved cellular attachment, through the copolymerization of the acrylated peptides with the ω,ω,ω,-triacrylate [*star*-poly(ε-caprolactone-*co*-D,L-lactide)] in an organic solution (Fig. 1). Upon immersion of the hydrophobic polyester network into an aqueous medium, the more hydrophilic PEG-peptide conjugate would orient itself outward from the surface, and be exposed to seeded cells [33].

To test these hypotheses, two different prepolymer molecular weights were utilized to produce elastomers of low and high crosslink density; a prepolymer of 1800 Da for the high crosslink density elastomer (ELAS 1800) and a prepolymer of 4500 Da for the low crosslink density elastomer (ELAS 4500). The resulting elastomers were either base etched, or were copolymerized with acryloyl-PEG-GRGDS via ultraviolet (UV) light initiated crosslinking. Two different prepolymer molecular weights were used because elastomer crosslink density influences its degradation rate by hydrolysis and so different degrees of surface hydrophilicity due to base etching were expected. Moreover, recent evidence demonstrates that smooth muscle cell proliferation and phenotype, much like other cell types, are affected by substrate stiffness [31,34,35]. By utilizing two different crosslink densities, the influence of the elastomer stiffness could thus also be ascertained. Bovine arterial smooth muscle cell adhesion, morphology, phenotype and proliferation were then examined on these modified, biodegradable elastomers.

2. Methods

Sodiumhydroxide, bicarbonatebuffer, GRGDS, fluorescamine isomer 1, *N*-hydroxysuccinimide, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride, calcium dihydride, glycerol, stannous 2-ethylhexanoate, acryloylchloride, triethylamine, 4-(dimethylamino)pyridine, 2,2-dimethoxy-2-phenyl-acetophenone and glycidyl methacrylate were purchased from Sigma–Aldrich (Canada). Acryloyl-PEG-succinimidyl carboxymethyl (M_n 3400) was purchased from Laysan Bio Inc.(Arab, AL). Methanol, 2-propanol, hexane, tetrahydrofu-

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