Biomaterials 63 (2015) 47–57

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Poly-L-arginine based materials as instructive substrates for fibroblast synthesis of collagen

Hannah C. Bygd [a], Dana Akilbekova [a], Adam Muñoz [a, b], Kiva D. Forsmark [c], Kaitlin M. Bratlie [a, c, d, *]

[a] Department of Materials Science & Engineering, Iowa State University, Ames, IA 50011, USA
[b] Department of Nanoengineering, University of California, San Diego, La Jolla, CA 92093, USA
[c] Department of Chemical & Biological Engineering, Iowa State University, Ames, IA 50011, USA
[d] Ames National Laboratory, Ames, IA 50011, USA

ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form
27 May 2015
Accepted 28 May 2015
Available online 3 June 2015

Keywords:
Fibroblast
Collagen
Collagen structure
Cell morphology

ABSTRACT

The interactions of cells and surrounding tissues with biomaterials used in tissue engineering, wound healing, and artificial organs ultimately determine their fate *in vivo*. We have demonstrated the ability to tune fibroblast responses with the use of varied material chemistries. In particular, we examined cell morphology, cytokine production, and collagen fiber deposition angles in response to a library of arginine-based polymeric materials. The data presented here shows a large range of vascular endothelial growth factor (VEGF) secretion (0.637 ng/10^6 cells/day to 3.25 ng/10^6 cells/day), cell migration (~15 min < persistence time < 120 min, 0.11 μm/min < speed < 0.23 μm/min), and cell morphology (0.039 < form factor (FF) < 0.107). Collagen orientation, quantified by shape descriptor (D) values that ranges from 0 to 1, representing completely random (D = 0) to aligned (D = 1) fibers, exhibited large variation both *in vitro* and *in vivo* (0.167 < D < 0.36 and 0.17 < D < 0.52, respectively). These findings demonstrate the ability to exert a certain level of control over cellular responses with biomaterials and the potential to attain a desired cellular response such as, increased VEGF production or isotropic collagen deposition upon exposure to these materials in wound healing and tissue engineering applications.

1. Introduction

The extracellular microenvironment affects cells and is instrumental in controlling cellular behavior and responses [1]. Implanted biomaterials, tissue engineering constructs, and artificial organs all interact to some extent with surrounding tissues and cells. Ultimately, their *in vivo* fate depends on the outcome of this interaction. The impact of biomaterials on cells has been widely studied [2–5]; however, the ability to tune the response of fibroblasts for applications in wound healing and tissue engineering, in particular the substrate's ability to alter the distribution of collagen fiber orientation through changing chemical functional group alone, has not been explored.

Wound healing is a complex process involving the interaction of various cell lineages and tissue types [6,7]. While healing is often rapid and efficient, the resultant tissue is not always aesthetically appealing or functionally suitable [6]. Scar tissue is visually unpleasant and is mechanically weakened, sometimes dysfunctional. At maximum, remodeled tissue only reaches 80% of the strength of unwounded skin [7]. Reorganization of collagen by fibroblasts is directly responsible for this decrease in strength [8]. In healthy tissue, collagen is found to have isotropic fiber orientation, while scar tissue contains smaller bundles of collagen aligned parallel to one another [8]. This variation is especially problematic for tissue-engineered scaffolds and artificial organs because fibrotic tissue impedes nutrient transport [4].

Achieving random collagen deposition that more closely resembles young, healthy skin would be a vast improvement upon the imperfections of the natural wound healing process and the integration of tissue engineered scaffolds. One approach would be to develop a wound-healing polymer that can influence the way in which fibroblasts synthesize and deposit collagen. Numerous

* Corresponding author. Department of Materials Science & Engineering, Iowa State University, Ames, Iowa 50011, USA.
E-mail address: kbratlie@iastate.edu (K.M. Bratlie).

http://dx.doi.org/10.1016/j.biomaterials.2015.05.045
0142-9612/

wound dressings, other than standard bandages and gauzes, have aimed to provide an optimum environment for cells involved in wound healing [9]. These conditions include a warm, moist environment with oxygen circulation and low bacterial access [9]. Many newly developed dressings decrease pain levels and healing time; they can also serve an antibacterial, absorbent, adherent, or occlusive function. Preeminent dressings also elicit the appropriate cell–material interactions crucial for the immunomodulation and improved would healing processes [9]. Unfortunately, none of the commercially available wound dressings target the remodeling phase of wound healing, in particular how fibroblasts respond to the biomaterials, to reduce scarring [9].

Here, we present a potential platform for engineering fibroblast responses to improve upon the wound healing process. This work directly targets and affects fibroblasts in the remodeling phase. The library of materials used here is based on the amino acid arginine. Arginine-rich peptides have been extensively explored as intracellular delivery vehicles as their guanidine functionality allows them to interact with the phosphate groups in cell membranes [10,11]. We have developed a library based on arginine on the premise that interactions between the arginine moieties and the cell membrane may result in altered cell morphology, cytokine production, and collagen fiber deposition distribution by fibroblasts.

2. Materials and methods

2.1. PLR modification

Fifteen different molecules (Fig. 1a) (Sigma, St. Louis, MO), herein called amidine derivatives, were used to modify poly-L-arginine (PLR) with an 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Oakwood Chemical, West Columbia, SC) coupling reaction. A generalized reaction scheme is given in Fig. 1b. Briefly, 4 mL of a 2.5 mg/mL solution of PLR in phosphate buffered saline (PBS) were placed in glass scintillation vials with 100 molar equivalents of amidine derivative. 200 mg of EDC was added to the vial and the reaction was allowed to stir for 4 h at room temperature. The polymers were then dialyzed in DI H_2O for 24 h to remove EDC and unreacted amidine derivatives, and subsequently lyophilized (4.5 L, Labconco, Kansas City, MO). The modified PLR was resuspended in PBS at 0.1% and stored at −20 °C.

2.2. Material characterization

In order to ensure that the reaction proceeded, NMR was used to characterize the reaction products (see supporting information Supplementary Fig. 1). The modified PLR samples were dissolved in D_2O and freeze-dried three times to replace all OH groups with OD groups before 2 mg of polymer were dissolved a final time in 600 μL of D_2O. The ^1H spectra were recorded on a Bruker Avance III Spectrometer at 70 °C, a sweep width of 6602.1 Hz, a 90° pulse, and an acquisition time of 2.48 s. 128 repetitive scans with 64 k points were acquired and the data were processed in MNova with 128 k points, zero filling, and exponential line broadening of 1.0 Hz [12]. 16 repetitive scans were acquired at room temperature for the ^1H spectra of the amidine derivatives with all other parameters the same as above. In comparing spectra, additional peaks were found near 1.8, 3.5, and 3.8 ppm for each modified PLR. Primary amines typically appear in the region of 1–3 ppm, depending on H-bonding. The 1.8 ppm shift is assigned to the primary amine formed during the amidine reacting with the carboxylic acid. Because peaks were not present at these chemical shifts in the spectra of the amidine derivative reacted with PLR, it was determined that they were a result of the formation of amide bonds in EDC coupling reactions. Protons associated with imidazole show shifts in the range of 7–8.4 ppm, which were not observed the ^1H NMR spectra.

2.3. Water contact angle

The hydrophobicity of each modified PLR sample was measured by determining the water contact angle of each sample. The captive air bubble method was used as all the samples were very hydrophilic. The coated coverslips were attached to microscope slides and placed upside down on top of a container of water. An air bubble (100 μL) was then distributed under the coverslip and imaged with a digital camera (Canon EOS Rebel T3i, Canon, Melville, NY). This process was repeated to obtain five replicates before the angle between the glass and the bubble were measured using ImageJ (NIH, Bethesda, MD) software and subtracted from 180° to determine the final water contact angle. Water contact angles and corresponding standard deviations are shown for each modified PLR sample in Supplementary Table 1. The angle of 24° for a control glass coverslip closely matched other literature values for silicon dioxide [13]. With the highest angle measuring at 69°, this data indicates that all samples are reasonably hydrophilic. Although none of the samples are as hydrophilic as the control, modifications 4, 5, 7, 9 and PLL were the most hydrophilic with water contact angles between 34° and 38° which is in the conditionally non-adhesive region, as defined by Vogler and Saltzman [14]. Modifications 3, 11, and 12 were the most hydrophobic with angles greater than 55°, falling in the adhesive region. The remaining modifications were moderately hydrophilic with angles ranging from 41° –53°.

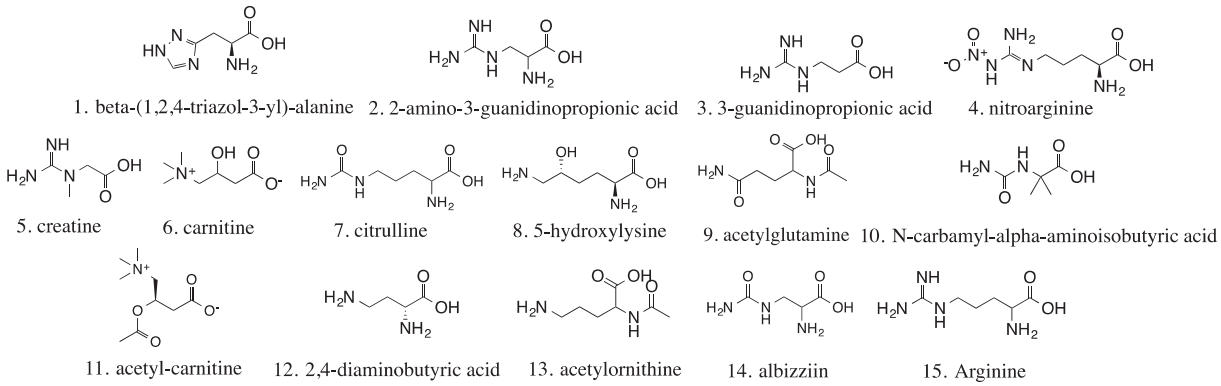

Fig. 1. Synthesis of a new library of poly-L-arginine (PLR) derivatives. Chemical structures of molecules used to modify PLR. The amidine derivatives shown here are numbered for easier identification in experiments and discussion throughout the paper.