Materials Science and Engineering C 74 (2017) 146–151

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Short communication

Impact of matrix stiffness on fibroblast function

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ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form 11 November 2016
Accepted 2 February 2017
Available online 7 February 2017

Keywords:
Wound dressing
Matrix stiffness
Fibroblasts
Angiogenic activity

ABSTRACT

Chronic non-healing wounds, caused by impaired production of growth factors and reduced vascularization, represent a significant burden to patients, health care professionals, and health care system. While several wound dressing biomaterials have been developed, the impact of the mechanical properties of the dressings on the residing cells and consequently on the healing of the wounds is largely overlooked. The primary focus of this study is to explore whether manipulation of the substrate mechanics can regulate the function of fibroblasts, particularly in the context of their angiogenic activity. A photocrosslinkable hydrogel platform with orthogonal control over gel modulus and cell adhesive sites was developed to explore the quantitative relationship between ECM compliance and fibroblast function. Increase in matrix stiffness resulted in enhanced fibroblast proliferation and stress fiber formation. However, the angiogenic activity of fibroblasts was found to be optimum when the cells were seeded on compliant matrices. Thus, the observations suggest that the stiffness of the wound dressing material may play an important role in the progression of wound healing.

1. Introduction

Chronic wounds, resulting from various underlying disorders including diabetes, reduced production of cytokines/growth factors, and vascular compromise [1,2], fail to proceed in an orderly fashion through the precise stages of wound healing. These wounds represent a significant burden to patients, health care professionals, and health care system with an estimated incidence of 5–7 million cases per year in United States [3–5]. More than $50 billion are spent annually for clinical management of these wounds [4]. The goal of wound management therapies is regeneration of tissues at the wound bed with restored structural and functional properties. Wound dressing biomaterials are being designed to promote the healing process. Following injury, the mechanical environment of the wound bed and consequently the biology of the resident cells are altered [6,7]. However, the role of mechanical cues in designing wound dressings has been largely overlooked [8]. The primary focus of this study is to explore whether the function of dermal fibroblasts can be regulated by altering the compliance of biomaterials.

The commonly used materials for mechanotransduction studies involve either biologically derived materials including collagen I, Matrigel, alginate, hyaluronic acid, and fibrin [9–12] or synthetic polymers such as poly (acrylamide) (PA) or poly (ethylene glycol) (PEG) [13–15]. Although the natural materials can effectively recapitulate the properties of ECM, high batch-to-batch variation often results in inconsistencies [16]. Another limitation in utilization of natural hydrogels is that the manipulation of gel modulus via alteration of protein concentration alters other ECM properties such as cell-binding sites, pore size, porosity, and degradability [17,18]. Since, decoupling various intertwined ECM properties is not easy, parsing the specific contributions of the microenvironmental cue is difficult. On the other hand, the synthetic polymers have inherent advantages such as well-defined mechanical properties and chemical inertness [19]. Even though these materials lack the cell-binding sites for promoting attachment and proliferation of cells, they provide a "blank" slate on which cell-responsive and protease-cleavage motifs can be easily incorporated [20,21].

In this study, we have developed mechanically tunable gelatin methacrylate (GelMA)-poly (ethylene glycol) diacrylate (PEGDA) composite hydrogels which leverage the biocompatibility of natural materials with the stability and reproducibility of synthetic polymers. PEG is a bio-inert polymer that resists non-specific adsorption of proteins [22]. GelMA, obtained via conjugation of methylacrylate groups to gelatin (denatured collagen), provides the cell-adhesive as well as matrix metalloproteinase (MMP) sensitive sites [23]. This composite platform provides a cell culture platform to evaluate the cellular responses to the changes in the mechanical microenvironment. The rigidity of the gels was manipulated by varying the concentration of PEGDA while maintaining a uniform concentration of GelMA and consequently cell-binding sites. The stiffness of the matrices were varied from 1.3 ± 0.5 kPa to 23.0 ± 1.0 kPa to span the mechanical properties of the physiologically relevant tissues. We evaluated the morphology/spread area, proliferation, and angiogenic capability of human dermal fibroblasts. Our results indicate that cell area and proliferation of fibroblasts increased with increasing stiffness of the hydrogel matrices. On the

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http://dx.doi.org/10.1016/j.msec.2017.02.001
0928-4931/

other hand, angiogenic activity of fibroblasts was upregulated by substrate of appropriate stiffness.

2. Materials and methods

2.1. Materials

Poly (ethylene) glycol diacrylate 10,000 (PEG10kDA), phosphate buffer saline (PBS), gelatin from porcine skin, photo-initiator (2-Hydroxy-4¢-(2-hydroxyethoxy)-2-methylpropionphenone), ethylene glycol, acetone and methacrylic anhydride were procured from Sigma Aldrich (St. Louis, MO). Penicillin Streptomycin L-Glutamine (Pen Strep), 0.25% Trypsin-EDTA, and Fetal bovine serum (FBS) from Gibcos (Grand Island, NY). LIVE/DEAD Cell Viability assay-kit, Alexa Flour 488 conjugated phalloidin antibody, and Geltrex TM LDEV-free reduced growth factor basement membrane matrix were purchased from Life Technologies (Grand Island, NY). XTT proliferation assay kit, fibroblast basal medium, and fibroblast growth kits were obtained from American Type Culture Collection (ATCC, VA).

2.2. Synthesis of methacrylated gelatin

Gelatin methacrylate was synthesized as described elsewhere [24]. Briefly, gelatin type A (gelatin from porcine skin) was added into Dulbecco's phosphate buffered saline (DPBS) and stirred continuously at 50 °C until fully dissolved to prepare 10% (w/v) solution. Methacrylation was achieved by adding 10% (w/v) of methacrylic anhydride at a rate 0.5 ml/min and reacting it at 60 °C for 3 h. Following a 5× dilution with warm DPBS (50 °C), the mixture was dialyzed using dialysis cassette (Slide-A-lyzer™, Thermo Fisher Scientific) against distilled water at 50 °C for 15 days to remove salts and acid. The sample was then freeze dried and GelMA was generated as a porous foam and stored at −80 °C until further use.

2.3. Fabrication of mechanically tunable scaffolds

For fabrication of the scaffolds, 50 μl of the pre-polymer solution consisting of PEG10kDA, 5% GelMA (w/v) and 1% photoinitiator was added to each well of the 96 well plate and then exposed to UV (CL-1000 UV Crosslinker (UVP), 365 nm) for 5 min. The concentration of PEGDA was varied from 2.5% to 10% (w/v).

2.4. Mechanical properties

To characterize the mechanical properties of the hydrogels, cylindrical samples of diameter 10 mm were fabricated and compressed along the thickness to measure stiffness. The polymer precursor solutions were placed in 10 mm diameters cylindrical mold (~3 mm in thickness,) and UV polymerized. The cylinders were then incubated in PBS for 72 h to facilitate removal of the unreacted monomers. Prior to analysis, excess fluids on the surface of the hydrated samples were removed by dabbing with wipers (Kimwipe™, Kintech Science). The compressive tests were carried out by using uniaxial testing machine (TestResources, USA) at a loading rate of 1.2 mm/min with a precision load up to 9 N. At least 3 samples per group were used for each test. Maximum strain and stress at the moment of fracture was recorded and compression modulus was calculated from the initial 10% compression.

2.5. Mesh size

To determine the mesh size, the hydrogels were incubated in PBS for 48 h in a 37 °C shaker (50 rpm, excess fluids removed from the swollen hydrogels, and weights of the samples were measured. The samples were then dried at 50 °C for 24 h and the dry weights were measured. The average mesh size ξ, was calculated from modified Flory-Rehner theory.

$$\xi = \nu_2^{-1/3}\left(\bar{r}_0^2\right)^{1/2}$$

where, ν_2 = polymer volume fraction in the equilibrium swollen hydrogel and $(r_0^2)^{1/2}$ is the average end-to end distance.

2.6. Cell culture and angiogenesis assay

Human dermal fibroblast cells were procured from American Type Culture Collection (ATCC, Manassas, VA) and expanded in Fibroblast basal medium supplemented with 1% (v/v) penicillin streptomycin and fibroblast growth kit (containing ascorbic, fetal bovine serum (FBS), rh EGF, heparin sulfate, L-glutamine, hydrocortisone and bovine brain extract). Cells up to passages 5 were used in this study.

2.7. Morphology

Fibroblasts were dissociated from flask using 0.25% Trypsin–EDTA and suspended in cell growth media. Cells at a density of 1×10^3 were seeded on each of scaffolds of varying stiffness and the cell morphology was measured 72 h after seeding. For this purpose, images of the cells were captured using Zeiss Axio Observer A1 with integrated CCD camera. At least five random images were captured for each scaffold and the area of the cells was measured in each image. Experiments were performed in triplicates.

2.8. Proliferation

To investigate the effect of matrix compliances on proliferation of fibroblast cells, the scaffolds were fabricated in 96 well plates following exposure to UV for 5 min. After 48 h, the proliferation was measured using XTT assay kit as per manufacturer's instructions. Briefly, 150 ml of cell culture media containing 50 ml of activated XTT-solution was added to each well. The cells were incubated for 5 h. Then 100 ml of solution from each of wells was transferred to a new 96 well plates and the absorbance of plates at a wavelength of 475 nm was measured using Biotek Eon Microplate spectro-photometer. The percentage of cells growth was calculated as follows:

$$\textit{Cell growth percentage} = \frac{A475\text{nm}_{sample} - A475\text{nm}_{blank}}{A475\text{nm}_{control} - A475\text{nm}_{blank.}}$$

2.9. Immunostaining

For immunostaining the samples, the fibroblasts cultured on scaffolds of varying stiffness were incubated in 5% (v/v) Hoechst 33342 for 5 min. Following which, the cells were fixed and permeabilized with 50% acetone at −20 °C for 20 min. Then the cells were blocked with 5% bovine serum albumin (BSA) for1h and washed with 1× PBS for three times. After that the cells were incubated with 5% (v/v) Alexa Fluor 488-phalloidin antibody at 37 °C. The cells were then again washed with 1× PBS three times and images were captured to monitor the organization of the actin stress fibers.

2.10. Angiogenesis assay

Fibroblasts were cultured on scaffolds of varying stiffness and the conditioned media was collected after 48 h. For the angiogenesis assay, 50 μl of thawed Geltrex TM LDEV-free reduced growth factor basement (Life Technologies, CA) was added to 96 well plates and incubated at 37 °C for 5 min to facilitate gel formation. Once the gels were prepared, human umbilical vein endothelial cells (HUVECs) at a