Available online at www.sciencedirect.com
ScienceDirect
Biomaterials 29 (2008) 314–326
Biomaterials
www.elsevier.com/locate/biomaterials

The effect of enzymatically degradable poly(ethylene glycol) hydrogels on smooth muscle cell phenotype

Catharina Adelöw[a], Tatiana Segura[a,b], Jeffrey A. Hubbell[a,c], Peter Frey[a,d,*]

[a]*Institute of Bioengineering, Ecole Polytechnique Fédérale de Lausanne, Switzerland*
[b]*Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, CA, USA*
[c]*Institute of Chemical Sciences and Engineering, Ecole Polytechnique Fédérale de Lausanne, Switzerland*
[d]*Department of Pediatric Surgery and Urology, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland*

Received 8 June 2007; accepted 23 September 2007
Available online 22 October 2007

Abstract

The formation of scar tissue due to dedifferentiation of smooth muscle cells (SMCs) is one of the major issues faced when engineering bladder tissue. Furthermore, cell sources for regenerating the SMC layer are also limiting. Here we explore if human mesenchymal stem cells (MCSs), cultured in enzymatically degradable poly(ethylene glycol) (PEG) hydrogel scaffolds can be differentiated into SMC-like cells. We explored the degree to which a less synthetic SMC phenotype can be achieved when primary human SMCs are cultured within these scaffolds, It was observed that when both MSCs and SMCs are cultured in the PEG hydrogel scaffolds, but not on traditional tissue culture plastic, they up-regulate markers associated with the less synthetic SMC phenotype, decreased expression of α_5 integrin and THY-1, and increased expression of α-smooth muscle actin (αSMA) and myosin. Furthermore, we show that MSCs and SMCs cultured in the PEG hydrogels are able to proliferate and express matrix metalloproteinases for up to 21 d in culture, the duration of the study. This study addresses the importance of the cellular microenvironment on cell fate, and proposes synthetic instructive biomaterials as a means to direct cell differentiation and circumvent scar tissue formation during bladder reconstruction.

Keywords: Hydrogel; PEG; ECM; Bladder; Mesenchymal stem cell; Smooth muscle cell

1. Introduction

Bladder malformations and diseases including bladder exstrophy, congenital and traumatic neurogenic bladder, interstitial cystitis and cancer are associated with scar tissue formation within the bladder wall. Procedures to repair damaged or diseased bladders include tissue substitution with autologous tissue such as small or large bowel [1], gastric segments [2], or tissue engineered constructs [3]. However, these procedures also often result in scarring. The formation of scar tissue has been correlated to a phenotypic switch of quiescent contractile smooth muscle cells (SMCs) into a synthetic proliferative phenotype, also referred to as myofibroblasts [4,5], producing excessive amounts of collagen. Normally, myofibroblasts residing in healing wounds enter apoptosis after contracting the wound. However, within scar tissue, myofibroblasts remain and continue to produce collagen [6,7]. The disproportionate quantity and fibrous character of the collagen gives rise to a poorly compliant tissue unable to sustain the function of the normal bladder, e.g. to expand up to fifteen times its volume at constant pressure.

Due to a persistent lack of donor tissue and limitations met using autologous tissue, such as metabolic complications [8], tissue engineering has emerged as an attractive alternative for bladder regeneration and repair. One challenge of bladder tissue engineering is to determine the specific signals involved in maintenance of the normal contractile SMC cell phenotype. It is widely recognized that bladder SMCs undergo phenotypical changes in response to environmental stress and injury [9], including

*Corresponding author. EPFL SV-LMRP, Station 15, CH-1015 Lausanne, Switzerland. Tel.: +41 21 693 9662, +41 21 314 3070; fax +41 21 693 9685, +41 21 314 3101.
E-mail address: peter.frey@epfl.ch (P. Frey).

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.09.036

mechanical stress such as over-distension of the bladder during bladder outlet obstruction or chemical stress such as urine leakage due to perforations of the urothelium as seen in interstitial cystitis.

Many functions of SMCs such as adhesion, migration, proliferation and contraction are determined by ligation of cell surface receptors involved in cell–cell and cell–ECM interactions [10,11]. Although considerable effort has been made, the complex signaling pathways that underlie bladder SMC response to their microenvironment [10,12] have still not been completely elucidated. The state of SMC differentiation is characterized by expression of smooth muscle-specific variants of cytoskeletal and contractile proteins such as α-smooth muscle actin (αSMA), myosin, caldesmon, and calponin, among others [13–15]; moreover, the expression of specific integrins [16] as well as levels of secreted proteases [16] and ECM proteins [17] depends on SMC differentiation.

As a tool with which to explore the dependence of cell phenotype upon the extracellular milieu, our laboratory has developed an approach to synthetic hydrogels as mimics of the extracellular matrix. These materials are composed of a reactive poly(ethylene glycol) (PEG) derivative, crosslinked with matrix metalloproteinase (MMP)-degradable peptides [18,19]. These materials allow control of many features of the extracellular milieu, including the density and identity of extracellular matrix adhesion molecules and elastic modulus. Both features are known to influence cell differentiation in a number of contexts [20,21]. Such enzymatically degradable hydrogels have been investigated extensively as scaffolds for tissue engineering and have proved successful for a variety of applications including bone [22,23], vascular tissue [24,25] and cartilage [26]. We sought to use these PEG–peptide hydrogels to explore the signals that lead to retention of SMC phenotype. Further, based on the role of the mesenchyme during bladder development [27], we sought to explore the signals that would lead to mesenchymal stem cell (MSC) differentiation into SMCs *in vitro* and potentially *in vivo*. Finally, we sought to compare our differentiation phenomena in our 3D hydrogel cultures to traditional 2D cultures on polystyrene plasticware.

The primary SMC phenotype showed decreased expression of α_5 integrin and THY-1 and increased expression of the αSMA and myosin when transferred into 3D PEG hydrogels. Further, MSCs significantly up-regulated expression of smooth muscle specific proteins such as αSMA and myosin. Both cell types acquired a spindle-shaped morphology within 3D gels. The MSCs remained fairly quiescent, spreading and forming less extensive networks than did SMCs, which infiltrated and created extensive networks throughout the gels. SMC proliferation within 3D gels was almost as high as in 2D. Furthermore, both MSCs and SMCs down-regulated expression of α_3 and α_5, and up-regulated expression of α_v integrin. The MSCs also up-regulated expression of α_1, β_1 and β_3 integrin subunits. The expression of MMPs involved in gel degradation was determined, and the expression of newly synthesized ECM proteins was demonstrated. This study further elucidates the importance of 3D geometry and extracellular microenvironment on cell fate and proposes synthetic instructive biomaterials as a means by which to direct cell functions and circumvent scar tissue formation during bladder reconstruction.

2. Materials and methods

2.1. Cell isolation and culture

Primary human SMCs were isolated from bladder wall tissue harvested during open surgery in children for the treatment of congenital vesico–ureteral reflux, performed by one of the authors (P.F.). Negative isolation of SMCs was performed by positive selection of urothelial cells and fibroblasts with magnetic beads (Dynabeads®, Invitrogen/Dynal Biotech, Basel, CH) conjugated to mouse monoclonal antibodies against epithelial antigen (anti-human epithelial antigen clone Ber-EP4, DakoCytomation, Baar, CH) and THY-1 (clone AS02, Dianova, GmbH, Germany) respectively. Human MSCs isolated from bone marrow were received as a generous gift from Pierre Charbord, Université François-Rabelais, Tours, France. Cells were maintained in alpha-MEM without deoxyribonucleosides or ribonuclosides (Cambrex Bio Science Verviers, Verviers, BE) supplemented with 10% bovine growth serum (BGS, Hyclone, Perbio Science, Lausanne, CH), 2 mM glutamine (Invitrogen, Basel, CH), and 1% penicillin/streptomycin (Invitrogen, Basel, CH). The MSC medium during expansion of cells before 3D hydrogel and 2D experiments was also supplemented with 1 ng/ml basic fibroblast growth factor (recombinant human FGF basic, R&D Systems, Basel, CH) for maintaining the undifferentiated MSC phenotype [28]. Cells between passages 4 and 6 were used for all experiments. Medium was changed every third day. For cells in gel and 2D experiments, only half the medium was removed and replaced by fresh complete medium.

2.2. PEG-VS synthesis

Four-armed PEG-OH (20 kDa, Nektar, Aerogen Ltd., Galway, IE) was modified with divinyl sulfone as previously described with minor modifications [18]. Briefly, PEG-OH was dissolved in tetrahydrofuran (THF, Aldrich/Fluka, Basel, CH) under inert atmosphere and heated to reflux in a Soxhlet apparatus filled with molecular sieves for 3–4 h. The solution was allowed to cool to the touch and sodium hydride (NaH, Fluka, Basel, CH), at 5-fold molar excess over OH groups, was added followed by the addition of divinyl sulfone, at 30-fold molar excess over OH groups. The reaction was carried out at room temperature (RT) under argon atmosphere with constant stirring for 3 d. Afterwards, the reaction solution was neutralized with acetic acid, filtered, concentrated and precipitated in ice cold diethyl ether (Acros Organics/Chemie Brunschwig AG, Basel, CH). Precipitation was repeated three times to remove unreacted divinyl sulfone. The final product was dried under vacuum and stored under argon at −20 °C. The degree of functionalization was confirmed with 1 H NMR (in $CDCl_3$): 3.6 ppm (PEG backbone), 6.1 ppm (d, 1 H, dCH_2), 6.4 ppm (d, 1 H, dCH_2), and 6.8 ppm (dd, 1 H, -SO_2CHd). The degree of end group conversion, as shown by NMR, was found to be 99%.

2.3. Bioactive peptides

Bioactive peptides were introduced to the PEG macromer via Michael-type addition. The PEG macromer was modified with the cell adhesion peptide Ac-GCGWGRGDSPG-NH_2 (RGD, 300 μM; NEOMPS, Strasbourg, France) prior to hydrogel formation. 11.3 mg of PEG-VS was dissolved in 108.4 μl TEOA buffer (TEOA, Fluka, Basel, CH) and mixed with 2.65 μl of RGD peptide solution (0.1 mg/μl in TEOA buffer). The