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Biofunctionalization of electrospun PCL-based scaffolds with perlecan domain IV peptide to create a 3-D pharmacokinetic cancer model

Olga Hartman [a,*], Chu Zhang [b,f], Elizabeth L. Adams [b,c], Mary C. Farach-Carson [b,e], Nicholas J. Petrelli [d], Bruce D. Chase [a], John F. Rabolt [a]

[a] Department of Materials Science and Engineering, University of Delaware, Newark 19716, USA
[b] Department of Biological Sciences, University of Delaware, Newark 19716, USA
[c] Delaware Biotechnology Institute, University of Delaware, Newark 19711, USA
[d] Helen F. Graham Cancer Center, Christina Care Health System, Wilmington, DE 19899-1668, USA
[e] Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005-1827, USA
[f] Center for Translational Cancer Research, University of Delaware, Newark 19716, USA

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ABSTRACT

Because prostate cancer cells metastasize to bone and exhibit osteoblastic features (osteomimicry), the interrelationships between bone-specific microenvironment and prostate cancer cells at sites of bone metastasis are critical to disease progression. In this work the bone marrow microenvironment *in vitro* was recreated both by tailoring scaffolds physical properties and by functionalizing electrospun polymer fibers with a bioactive peptide derived from domain IV of perlecan heparan sulfate proteoglycan. Electrospun poly (ε-caprolactone) (PCL) fibers and PCL/gelatin composite scaffolds were modified covalently with perlecan domain IV (PlnDIV) peptide. The expression of tight junction protein (E-cadherin) and focal adhesion kinase (FAK) phosphorylation on tyrosine 397 also were investigated. The described bioactive motif significantly enhanced adherence and infiltration of the metastatic prostate cancer cells on all modified electrospun substrates by day 5 post-seeding. Cells cultured on PlnDIV-modified matrices organized stress fibers and increased proliferation at statistically significant rates. Additional findings suggest that presence of PlnDIV peptide in the matrix reduced expression of tight junction protein and binding to PlnDIV peptide was accompanied by increased focal adhesion kinase (FAK) phosphorylation on tyrosine 397. We conclude that PlnDIV peptide supports key signaling events leading to proliferation, survival, and migration of C4-2B cancer cells; hence its incorporation into electrospun matrix is a key improvement to create a successful three-dimensional (3-D) pharmacokinetic cancer model.

1. Introduction

Cancer cells reside in an organ-specific host microenvironment that long has been underemphasized as a unique niche for cancer progression. A large number of the published literature suggests that tumor–microenvironment interaction controls cancer growth, invasion and distant metastasis. Tumor associated stroma, the connective, non-functional supportive framework surrounding growing cancer cells, actively fuels the progression of prostate cancer. Reactive stromal cells can exert a pro-invasive signal, increasing motility, and decrease apoptosis of cancer cells [1,2].

Studies of prostate, breast and several other cancer types that migrate into bone [2] suggest that bone matrix proteins confer increased cancer cell growth, adhesion, migration and invasion [3–5]. A reciprocal cancer cell–microenvironment interaction facilitates osteomimicry by cancer cells [6], a process by which cancer cells mimic gene expression profiles of cells in their microenvironment. Prostate cancer cells can express bone-like osteomimetic properties; proteins commonly associated with bone cells including osteocalcin, bone sialoprotein, osteopontin, osteonectin, and parathyroid hormone-like related proteins have can be expressed by prostate cancer cells [3,6–8]. Hence, a bone-directed targeting strategy would be highly selective and effective against bone metastasis. To achieve this, a more comprehensive understanding of the cross-talk between cancer cells and organ-specific stromal microenvironments is needed [3,6].

The small fiber diameters and interconnected porous network of electrospun polymer fiber mats create ideal conditions for the

* Corresponding author at: 201 Dupont Hall, Department of Materials Science and Engineering, University of Delaware, Newark, DE 19716, USA. Tel.: +1 (302) 831 4476; fax: +1 (302) 831 4545.
E-mail address: ohartman@udel.edu (O. Hartman).

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production of artificial extracellular matrices (ECM) and cell culture applications. In principle, such scaffolds can mimic the structure and morphology of the ECM components in the body, and provide topographical cues to control and enhance tissue regeneration [9,10]. However, it is critical to reconstruct not only physical and structural characteristics, but also to provide effective biochemical stimuli found in native microenvironment, including various growth factors, hormones, and adhesion molecules [2–7]. Electrospun scaffolds also may be functionalized through the inclusion of drugs and proteins to provide biochemical cues [11–15]. Cells that come into contact with such modified scaffolds respond to the biochemical stimuli through highly specific interactions between cell surface receptors with the fiber immobilized ligands [16].

Poly (ε-caprolactone) (PCL) is a semi-crystalline biodegradable member of the polymer family of poly α-hydroxy aliphatic polyesters. Compared with other polyester family members such as poly(lactide) (PLA), poly-(glycolide) (PGA), and poly(lactide-*co*-glycolide) (PLGA), PCL has been less frequently used as a material for fabricating biomaterial scaffolds, mainly because of concern over its slower degradation kinetics and adequate cell binding properties. Synthetic and composite 3-D matrices comprised of completely synthetic PCL fibers for culture of fetal bovine chondrocytes (FBCs) were fabricated [17]. Electrospun gelatin and PCL/gelatin composite scaffolds were evaluated for the culture of bone marrow stromal cells (BMSC) [18], and engineering of a three-dimensional (3-D) bone marrow microenvironment for stem cell research has been undertaken [19]. PCL and PCL/gelatin electrospun fibrous mats have not been investigated, however, for their compatibility with cancer cells. The similarity between cancer and stem cell developmental biology and osteomimicry with bone marrow stromal cells suggests that prostate cancer cells are compatible with the described electrospun substrates. We previously produced and characterized 3-D electrospun collagen matrices that structurally, functionally and chemically mimic bone microenvironment with collagen type I as a main component. However, study of the function of any particular motif in a highly biomimetic collagen environment can be problematic because collagen has a large number of cell-binding sites including the well-known RGD peptide sequence [20].

In cancer research there is an increasing need for a physiologically mimetic 3-D tissue culture model to study how structural and biochemical cues provided by the tissue microenvironment modify tumorigenic phenotypes [21]. For example, the role of cancer adhesion molecules (CAMs), found in the extracellular domains of various ECMs, has been investigated [22]. These include, for instance, the junction adhesion molecules (JAMs) in breast cancer invasion/metastasis [23] and VEGF and VEGFR in angiogenesis [24]. Changes in the extracellular domain may "transduce" information via the intracellular molecular skeleton to organelles, such as the nucleus, and by doing so contribute to metastasis [25]. This may modify behavior including cancer cell binding, focal adhesion kinase (FAK) activation (via enhanced cell–matrix interactions), and proliferation. Although some CAM molecules contributing to the cancer progression have been identified, the role of many other ECM components remains unknown. A novel adhesive peptide sequence found in domain IV of perlecan heparan sulfate proteoglycan (PlnDIV) was identified and found to improve cell attachment and spreading *in vitro* in a variety of cell lines [26,27]. A highly hydrophilic amino acid sequence TWSKVGGHLRPGIVQSG which protrudes outward like a "finger" from the beta-sandwich structure may allow a better access to the active site by cell surface receptors. It is possible that this domain is responsible for homophilic binding of perlecan molecules or other protein–protein interactions [26]. This work investigated the role of this peptide in cancer progression in an engineered bone-like microenvironment comprised of synthetic and composite electrospun fibers. We investigated the utility of electrospun polymer fibers functionalized with PlnDIV peptide for their ability to partially mimic bone marrow ECM to culture human bone metastasis derived C4-2B prostate cancer cells [3]. These synthetic and composite scaffolds were designed to provide not only sufficient mechanical and structural properties, but also configured for the successful coupling of PlnDIV peptide to the electrospun fibers to introduce biochemical stimuli supporting prostate cancer cells growth in 3-D. Because of the potential of electrospun PCL and PCL/gelatin membranes as an artificial bone marrow ECM [17–19], we hypothesized that incorporation of PlnDIV peptide into the electrospun polymeric matrix would provide the appropriate chemical cues creating a more biomimetic environment for cancer cells.

2. Materials and methods

2.1. Materials

Poly (ε-caprolactone) (PCL) (Scientific Polymer Products, Inc, Ontario, NY) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (Sigma Aldrich, St. Louis, MO) at 10% (w/v) for fabrication of PCL/HFIP membranes, and in chloroform ($CHCl_3$) (Sigma Aldrich) at 12% (w/v) for fabrication of PCL/$CHCl_3$ membranes (hereafter referred to as PCL/HFIP and PCL/$CHCl_3$ respectively). The blends of PCL and gelatin in HFIP and in 2,2,2-trifluoroethanol (TFE) (Fisher Scientific, Pittsburgh, PA) were prepared using gelatin (Kodak, Rochester, NY) that was dissolved in HFIP and TFE respectively at 10% (w/v), and then mixed with 10% (w/v) PCL solutions in HFIP and TFE respectively at 1:1 volume ratio (hereafter referred to as PCL/gelatin/HFIP and PCL/gelatin/TFE). A 70% ethanol solution (Fisher Scientific) was used to sterilize the electrospun mats. Electrospun collagen fibers used for the control experiments [Figs. 11 and 12] were electrospun from 12% (w/v) solution in HFIP using the same processing parameters as for other membranes (Table 2). Collagen type I (rat tales) was obtained from BD Bioscience, San Jose, California.

2.2. Scaffold preparation: electrospinning

Electrospinning parameters including needle gauge, voltage, distance to target, and viscosities of the polymer solutions were optimized to achieve smooth and uniform fibers free of beads. The electrospinning apparatus consisted of a 3 mL syringe (Hamilton, Columbus, OH) connected to a syringe pump (KDS100, KD Scientific Holliston, MA). A high-voltage power supply (Glassman Series EH, Whitehouse Station, NJ) was used to apply a voltage to the tip of a needle. The collector consisted of a 4" × 4" metal sheet covered in non-stick aluminum foil, which was placed 16 cm from the tip of the needle. A needle with 0.51 mm inner diameter (Hamilton, USA), 2 mL/h flow rate, +15 kV applied voltage and 16 cm working distance were employed. Electrospinning processing parameters are listed in Table 2.

2.3. Synthesis of PlnDIV and biotinalation

PlnDIV peptide was synthesized by Lisa Haines-Butterick (Chemistry and Biochemistry, University of Delaware) using solid state peptide synthesis. In order to biotinylate the PlnDIV, 500 μl of 10 mM sulfo-NHS-LC-biotin solution (EZ-Link Sulfo-NHS-LC-Biotinylation Kit, Pierce Biotechnology Inc., Rockford, IL) was added to 500 μl of the PlnDIV water solution (5 mg/ml). The biotin and PlnDIV molecules cross-link via an amide bond. This mixture was placed on ice for 2 h, separated by gel filtration chromatography, and the protein fractions were identified by monitoring at 280 nm. The high molecular weight fractions were pooled and filtered (Amicon Ultra-15, MWCO = 5000 g/mol, Millipore, Burlington, MA).

2.4. Conjugation of PlnDIV-Biotin and BSA

The protocol suggested by the manufacturer was followed for the Imject® Immunogen EDC conjugation Kit (Pierce, Rockford, IL). EDC (10 mg) was added to a 700 μl solution containing 2 mg/ml of PlnDIV-Biotin and 10 mg/ml of BSA in conjugation buffer. BSA (molecular weight of 65 kDa) was obtained from Sigma–Aldrich. The reaction mixture was incubated for 2 h at room temperature in the dark. Following incubation, conjugated peptide was separated from unconjugated fraction using gel filtration with D-Salt™ Dextran Desalting Columns (Pierce). Columns were prepared according to manufacturer's protocol. In brief, columns were washed with 15–20 ml of purification buffer followed by addition of 0.5 ml of the Biotin-PlnDIV-BSA mixture that was applied directly to the center of the column disc. The elution of Biotin-PlnDIV-BSA was completed using 8–10 aliquots of 0.5 ml of Purification Buffer. All fractions were collected in separate tubes. Absorbance at 280 nm was measured to locate fractions containing conjugates. The Biotin-PlnDIV-BSA complexes were detected in the first absorbance peak. All fractions with acceptable levels of conjugates were pooled, syringe filtered for sterilization and