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Skeletal muscle derived stem cells microintegrated into a biodegradable elastomer for reconstruction of the abdominal wall

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ABSTRACT

A variety of techniques have been applied to generate tissue engineered constructs, where cells are combined with degradable scaffolds followed by a period of in vitro culture or direct implantation. In the current study, a cellularized scaffold was generated by concurrent deposition of electrospun biodegradable elastomer (poly(ester urethane)urea, PEUU) and electrosprayed culture medium + skeletal muscle-derived stem cells (MDSCs) or electrosprayed culture medium alone as a control. MDSCs were obtained from green fluorescent protein (GFP) transgenic rats. The created scaffolds were implanted into allogenic strain-matched rats to replace a full thickness abdominal wall defect. Both control and MDSC-integrated scaffolds showed extensive cellular infiltration at 4 and 8 wk. The number of blood vessels was higher, the area of residual scaffold was lower, number of multinucleated giant cells was lower and area of connective tissue was lower in MDSC-integrated scaffolds ($p < 0.05$). GFP + cells co-stained positive for VEGF. Bi-axial mechanical properties of the MDSC-microintegrated constructs better approximated the anisotropic behavior of the native abdominal wall. GFP + cells were observed throughout the scaffold at ~5% of the cell population at 4 and 8 wk. RNA expression at 4 wk showed higher expression of early myogenic marker Pax7, and b-FGF in the MDSC group. Also, higher expression of myogenin and VEGF were seen in the MDSC group at both 4 and 8 wk time points. The paracrine effect of donor cells on host cells likely contributed to the differences found in vivo between the groups. This approach for the rapid creation of highly-cellularized constructs with soft tissue like mechanics offers an attractive methodology to impart cell-derived bioactivity into scaffolds providing mechanical support during the healing process and might find application in a variety of settings.

1. Introduction

A variety of techniques have been applied to generate tissue engineered constructs, where cells are combined with degradable scaffolds followed by a period of in vitro culture or direct implantation [1–3]. For example, three dimensional (3D) printing where a cell-suspending bioink is printed onto a collector plate is attractive in that it does not require cell seeding and culture [4]. Furthermore, the fabrication can be altered by selecting cell type and bioink carrier. However, mechanical weakness is often an issue because the mechanical properties of the created construct is determined by the bioink utilized. This material has to both carry the cells and then be amenable to a process of stiffening to obtain the desired mechanical properties (often using crosslinking chemistries). On

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the other hand, polymer electrospinning has the advantage of being able to deposit polymers of relatively high mechanical strength using solvent processing, but with the disadvantage that subsequently achieving high cell seeding densities in the interfiber space can be problematic due to the high fiber densities normally generated. To overcome this limit, researchers have used methods to reduce the density of fibers during the deposition process, providing subsequently seeded cells larger pathways for migration inwards [5]. Scaffolds have also been processed after the fiber deposition process by laser ablation [6] or dissolution of secondary fiber populations [7] to provide for better cellular ingress. An alternative approach to this challenge is to utilize a process of concurrent polymer electrospinning and cell electrospraying, where fibers and cells are deposited together to result in a "microintegrated" cellularized construct [8,9]. This technique has the advantage of occurring in one step such that subsequent cell seeding and cellular ingress into the scaffold are not required. With judicious selection of polymer and cell type, this concurrent electrodeposition technique offers the potential to rapidly generate conduit and sheet constructs for a variety of applications.

Our previous work has demonstrated the general feasibility of creating "microintegrated" tissue constructs by the concurrent electrodeposition technique [5,8,9]. This technique is unique in that cells are seeded three dimensionally while building the synthetic scaffold. The electrodeposited cells were well preserved in the processing (over 95% viability) and maintained proliferative ability while the scaffold achieved attractive tensile mechanical properties from the concurrently deposited fibers. However, the stability of the seeded cells and their potential contribution to the construct remodeling process has not been evaluated in vivo. To investigate the performance of one of these types of scaffolds, we have concurrently electrospun a biodegradable elastomeric polymer while electrospraying skeletal muscle-derived stem cells (MDSCs) in culture medium. The resulting micro-integrated tissue construct was evaluated in a rat abdominal wall muscle replacement model. The cell type selected, MDSCs, are considered a promising somatic stem cell for use in tissue engineering in that they may not be restricted to the myogenic lineage or mesenchymal tissues, and can differentiate into multiple lineages [10,11]. These cells also are known to secrete many growth factors and cytokines to impact tissue regeneration [11–16]. The polymer utilized, poly(ester urethane)urea (PEUU), has been previously characterized in a variety of soft tissue settings, including the abdominal wall [17–20], and can be processed to possess mechanical properties that approximate certain soft tissue behavior. Since tracking of the MDSCs associated with the implanted construct was desired, green fluorescent protein positive (GFP+) transgenic rats were utilized as tissue donors to isolate GFP + MDSCs. The GFP + tissue constructs were implanted in GFP- strain-matched rats. Over an 8 wk implantation period, with construct explantation occurring at 4 and 8 wk, the presence of the loaded cells in the construct was determined. Also determined was the mechanical behavior and histological characteristics of the MDSC-loaded construct versus a control construct where culture medium without cells was electrosprayed. Biaxial, as opposed to uniaxial, tensile testing was performed since in situ the construct or tissue fibers would be constrained and forced to undergo more stretching than rotation. This would not be the case with uniaxial tension.

2. Materials and methods

2.1. MDSC isolation

MDSCs were isolated from the skeletal muscle of 3-wk-old green fluorescent protein (GFP) transgenic SD rats hind limb, as previously described [21], by a modified preplating technique. The GFP transgenic rats were kind gifts from Dr. Kimimasa Tobita, University of Pittsburgh, originally provided by Prof. Masaru Okabe of Osaka University. The obtained MDSCs were expanded in proliferation media (Dulbecco's Modified Eagle's Medium, Invitrogen, Carlsbad, California), 10% fetal bovine serum (Invitrogen), 10% horse serum (HS) (Invitrogen), 1% penicillin/streptomycin, and 0.5% chick embryo extract (Sera Laboratories International, West Sussex, United Kingdom) and used for scaffold fabrication. Fifth to eighth passage number cells were used in this study.

2.2. Scaffold fabrication

Cellular microintegration into the forming scaffolds was performed based on a previously described fabrication method [8]. Specifically, MDSCs (1×10^7/mL) were suspended in proliferation medium and fed by syringe pump (Harvard Apparatus) at 0.2 mL/min through a sterile Type 316 stainless-steel nozzle (I.D. = 1.2 mm) charged at 8.5 kV and located 4.5 cm above the target. PEUU was synthesized according to previously described method [22]. PEUU in hexafluoroisopropanol (HFIP) (12% w/v) was fed at 1.5 mL/min through tubing into a sterile nozzle (I.D. = 1.2 mm) charged at 12 kV and located 23 cm from the target in a perpendicular configuration to the electrospray nozzle. The target consisted of a sterile stainless-steel mandrel (6.0 mm diameter) charged at - 3 kV and rotating at 250 rpm while translating in a reciprocating fashion 8 cm along its axis at a rate of 1.5 mm/s. High voltage was supplied for each component using a combination of three high voltage generators (Gamma High Voltage Research). A fabrication time of 30 min was used to produce a microintegrated sheet (Fig. 1A). Culture medium (CM) control scaffolds were created by the same fabrication method except that the electrosprayed fluid did not contain MDSCs. After fabrication the sample was placed in a sterile bottle with enough proliferation medium to soak the scaffold sheet attached to the mandrel. The bottle was placed in an incubator for 24 h to allow for cell attachment to the electrospun fibers and then a sheet was removed (approximately 650 μm thick) from the mandrel under aseptic conditions. The sheet was then further divided into 1 × 2.5 cm pieces for implantation.

2.3. Scanning electron microscopy

Sections of each construct (8 mm^2, separate from the implanted pieces) were reserved for scanning electron microscopy (SEM) imaging before implantation. Acellular constructs were rinsed in deionized water and dried in ambient conditions before further processing. Sections of cellularized constructs were fixed for 1 h in 2.5% glutaraldehyde followed by 1 h in 1% OsO_4. The sections were then dehydrated in a graded series of ethanol washes and were finally dried using hexamethyldisilazane. All constructs were Au/Pd sputter coated prior to SEM imaging.

2.4. Animal study

The rat implantation studies were performed following US National Institutes of Health guidelines for animal care, and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. The research was performed in compliance with the Animal Welfare Act Regulations and other Federal statutes relating to animals and experiments involving animals and adhered to the principles set forth in the Guide for Care and Use of Laboratory Animals, National Research Council, 1996. Adult female Sprague Dawley rats were obtained from a local vendor (Harlan Sprague Dawley, Inc.). For the abdominal wall reconstruction procedure, 10–12 wk old (200–250 g) rats were