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The role of MMP-I up-regulation in the increased compliance in muscle-derived stem cell-seeded small intestinal submucosa

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Abstract

We have previously observed that muscle-derived stem cells (MDSC) seeded onto porcine small intestinal submucosa (SIS) increase the mechanical compliance of the engineered tissue construct [Lu SH, Sacks MS, Chung SY, Gloeckner DC, Pruchnic R, Huard J, et al. Biaxial mechanical properties of muscle-derived cell seeded small intestinal submucosa for bladder wall reconstitution. Biomaterials 2005;26(4):443–9]. To date, however, the initial remodeling events which occur when MDSC are seeded onto SIS have yet to be elucidated. One potential mechanism responsible for the observed increase in mechanical compliance is the release of matrix metalloproteinase-I (MMP-I). To investigate this finding, MDSC ($\sim 1 \times 10^6$) were cultured on single-layer SIS cell culture inserts (4.7 cm^2) for 1–10 days. MDSC MMP-I activity on SIS in the supernatant at 1, 3, 5, 7, and 10 days was determined using a collagenase assay kit. MMP-I activity of the MDSC/SIS was significantly higher ($p < 0.0025$) after one day in culture compared to specimens collected from subsequent time points and the unseeded control. To further study the initial remodeling events, the impact of MMP-I on mechanical compliance was examined. SIS was incubated with 0.16 U/mL collagenase-I for 3, 4.5, 5, and 24 h, then biaxial mechanical testing was performed. After 5 h of digestion with collagenase-I, mechanical compliance under 1 MPa peak stress was increased by 7% in the circumferential direction, compared to control SIS. These findings suggest that the release of MMP-I in response to initial seeding on SIS and subsequent breakdown of collagen fibers is the mechanism responsible for an increase in mechanical compliance.

Keywords: Muscle-derived stem; SIS; Biaxial mechanical testing; Bladder wall; Tissue engineering

1. Introduction

The clinical need for urologic tissue reconstruction may arise from several pathologies such as stress urinary incontinence, diabetes, neurogenic disorders, or chronic inflammation. Biomaterials that have been used as urologic replacement tissues include gastrointestinal segments, autologous fascia, synthetic biomaterials [1], or naturally derived scaffolds [2]; each graft material having its particular advantages and disadvantages. Natural scaffolds derived from ECM, however, are non-immunogenic, promote cell integration and angiogenesis, and have been used in over 200,000 human patients [3].

Porcine small intestinal submucosa (SIS) is one such scaffold, and has been shown to be resorbable [4] and support vascularization in vivo [5]. Many studies have shown that SIS is useful for several types of urologic surgery applications where reinforcement of native structure or new tissue is needed [6,7]. It has been pointed out, however, that SIS initially exhibits substantially higher mechanical stiffness than the native bladder and results in incomplete, disorganized muscle regeneration in the urinary bladder [8].

In order to promote muscle regeneration in urologic tissues, muscle-derived stem cells (MDSC) may be injected directly into the tissue. Previous studies demonstrated that the introduction of MDSC into the rat urethral and

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bladder wall was feasible and resulted in formation of myotubes in the smooth muscle layers of the lower urinary tract [9]. MDSC injection alone, however, does not provide the immediate mechanical support necessary for complete tissue regeneration.

Prior studies have suggested that seeding SIS with autologous cells may be advantageous in regenerating functional and mechanically sound tissue replacements. Autologous skeletal muscle cells suspended in a collagen gel seeded onto SIS showed higher mechanical tensile strength than unseeded SIS when implanted into the abdominal muscles of Lewis rats [10]. In addition, using a de-cellularized bladder matrix seeded with canine autologous urothelial and bladder smooth muscle cells for bladder augmentation resulted in an increase in bladder capacity [11] and a more complete retention of implanted diameter [12] compared to unseeded matrix. These studies provide evidence that a tissue engineering approach, utilizing autologous cells and a 3-D, biological scaffold, toward urologic tract repair and replacement may provide better long-term successes. Previously our laboratory demonstrated in vitro, MDSC seeded on SIS formed a calcium-dependent contractile muscle-like tissue after 4 weeks in culture [13]. Additionally, we found that MDSC-seeded SIS after 10 and 20 days in culture is more compliant when compared to unseeded SIS as demonstrated through biaxial mechanical testing, but there was no change in mechanical compliance between 10 and 20 days of seeding [14]. To date, however, the mechanisms by which the MDSC remodel the SIS construct have yet to be elucidated.

One possible mechanism vital to the initial remodeling event is the release of matrix metalloproteinases (MMPs). MMPs are a family of zinc-dependent proteolytic enzymes that function mainly in the ECM, where they contribute to the development, functioning, and pathology of a wide range of tissues [15]. Since collagen type-I is the main component of SIS, it is possible that MMP-I plays a role in the interaction of MDSC with SIS during the initial remodeling process. Other studies have shown that MMP-2 and -9 may play a role in the break down of collagen type-I [16]. However, MMP-2 and -9 are active in degrading gelatin, which is the byproduct of collagen-I cleavage by MMP-I [15,17]. Since MMP-I acts on fibrillar collagen-I before MMP-2 and -9 are activated, we chose to examine MMP-I as our potential mechanism responsible for the initial remodeling event marked by an increase in mechanical compliance. We hypothesize that the release of MMPs and the resultant breakdown of collagen fibers by MDSC seeded onto SIS is a potential mechanism responsible for the initial event in the remodeling process, signaled by an increase in mechanical compliance observed in our previous study [14]. In the present study, we examined the role of MMP-I in the initial remodeling events, specifically studying the activity of MDSC seeded onto SIS as well as the impact of MMP-I on the mechanical properties of SIS.

2. Methods

2.1. Cell culture

Mc13 cells, clonal murine MDSC, transfected with a plasmid encoding for the β-galactosidase, minidystrophin, and neomicin resistance genes (gift from Dr. Johnny Huard, University of Pittsburgh), were expanded in culture passages 8–10 in Dulbecco's modified Eagle medium (DMEM, Invitrogen) supplemented with 10% FBS (Invitrogen), 1% penicillin streptomycin (Invitrogen), and 500 μg/mL G418 (Invitrogen). These cells were cultured under standard cell culture conditions (37 °C, humidified, 5% CO_2/95% air environment.

2.2. Cell seeding on SIS

Ninety samples of SIS (Cook Biotech) were cut into circles and attached with a 1 in. o-ring to a modified costar (24 mm) transwell cell culture insert (Fisher Scientific), which provided a cell growth area on the SIS of 4.7 cm^2. Prior to seeding, the constructs were placed in six-well tissue culture dishes, and each well was then soaked in 70% EtOH under a laminar flow hood until the EtOH had evaporated. The cell culture constructs were then soaked in 5 mL of Hank's balanced salt solution (HBSS) for 2 h to remove residual EtOH. Mc13 cells were seeded at 1×10^6 cells/insert in 5.5 mL of DMEM supplemented with 20% FBS, 1% penicillin streptomycin, and 500 μg/mL G418. Media was changed every 48 h and supernatant were collected at time points of 1, 3, 5, 7, and 10 days for assays. Controls were medium alone and SIS soaked in medium in the incubator for the same time points of 1, 3, 5, 7, and 10 days.

2.3. DNA quantification assay

In order to examine MDSC proliferation, DNA was quantified by a technique adapted from Kim et al. [18]. Each sample was cut into fourths and weighed (average wet weight 0.0691 g) prior to extraction. Samples were placed in a microcentrifuge tube and extracted in 1 mL of 0.125 mg/mL papain solution for 10 h in a 60 °C water bath. Digested samples were analyzed with a PicoGreen dsDNA quantitation kit (Molecular Probes, Eugene, OR, USA) as per the manufacturer's instructions and using the blue channel of a TBS-380 Mini-Fluorometer (Turner Biosystems, Sunnyvale, CA, USA). There was a small amount of DNA found in the unseeded SIS scaffold; this amount was subtracted from the DNA found in each sample. The total DNA quantitation was done in triplicate with $n = 3$ per group.

2.4. Total protein assay

Total protein was determined from supernatant culture media collected at 1, 3, 5, 7, and 10 days using a Coomassie Blue total protein kit (Fisher, Cat #P1-23236) as per the manufacturer's instructions and using a spectrophotometer set at 595 nm. Protein concentration was calculated using a standard curve with known amounts of Bovine Serum Albumin (Fisher). The total protein assay was done in triplicate with $n = 3$ per group.

2.5. MMP-I assay

MMP-I activity was determined from supernatant at 1, 3, 5, 7, and 10 days using a collagenase activity kit (Chondrex Inc.) as per the manufacturer's instructions. Briefly, samples were read on a Tecan Spectrafluorometer at 485 nm excitation and 535 nm emission. MMP-I activity (units/mL sample) was calculated from the fluorescent intensity of FITC labeled collagen after it has been denatured by the activated sample. The MMP-I activity was then normalized (units of activity/mg protein) by the amount of total protein in each sample. The MMP-I assay was done in triplicate with $n = 3$ per group.