Journal of Pediatric Surgery 52 (2017) 1355–1362

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

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

Basic Science Papers

Improvement of mesh recolonization in abdominal wall reconstruction with adipose vs. bone marrow mesenchymal stem cells in a rodent model

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ARTICLE INFO

Article history:
Received 5 August 2016
Received in revised form 3 November 2016
Accepted 27 November 2016

Key words:
Biological mesh recolonization
Adipose mesenchymal stem cells
Bone marrow mesenchymal stem cells
Human collagen matrix
Angiogenesis
Tissue remodeling

ABSTRACT

Background: Reconstruction of muscle defects remains a challenge. Our work assessed the potential of an engineered construct made of a human acellular collagen matrix (HACM) seeded with porcine mesenchymal stem cells (MSCs) to reconstruct abdominal wall muscle defects in a rodent model.

Methods: This study compared 2 sources of MSCs (bone-marrow, BMSCs, and adipose, ASCs) in vitro and in vivo for parietal defect reconstruction. Cellular viability and growth factor release (VEGF, FGF-Beta, HGF, IGF-1, TGF-Beta) were investigated under normoxic/hypoxic culture conditions. Processed and recellularized HACMs were mechanically assessed. The construct was tested in vivo in full thickness abdominal wall defect treated with HACM alone vs. HACM + ASCs or BMSCs ($n = 14$). Tissue remodeling was studied at day 30 for neo-angiogenesis and muscular reconstruction.

Results: A significantly lower secretion of IGF was observed with ASCs vs. BMSCs under hypoxic conditions (-97.6%, $p < 0.005$) whereas significantly higher VEGF/FGF secretions were found with ASCs ($+92\%$, $p < 0.001$ and $+72\%$, $p < 0.05$, respectively). Processing and recellularization did not impair the mechanical properties of the HACM. In vivo, angiogenesis and muscle healing were significantly improved by the HACM + ASCs in comparison to BMSCs ($p < 0.05$) at day 30.

Conclusion: A composite graft made of an HACM seeded with ASCs can improve muscle repair by specific growth factor release in hypoxic conditions and by in vivo remodeling (neo-angiogenesis/graft integration) while maintaining mechanical properties.

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Abdominal wall defects are usually treated using bioresorbable or synthetic non-resorbable meshes. In contrast with synthetic implants, biomaterials are known to reduce the risk of adhesions, decrease infection rates and hernia recurrences [1–3] because they constitute a favorable environment for cell incorporation after implantation and for tissue regeneration [2,4].

Our group previously proposed the use of a human acellular collagen matrix (HACM) for the reconstruction of abdominal wall defects. In this aspect, we demonstrated the efficacy of fascia lata in terms of improved revascularization and lower rates of hernia recurrence when compared to synthetic mesh [5].

Recently, seeding matrices with cells was proposed to improve mesh incorporation and muscle regeneration [6–9]. The efficacy of differentiated cell transplantation is known to be limited by low rates of cellular engraftment, due mainly to hypoxic stress in the early post implantation period [10,11].

Therefore, stem cells were proposed to improve muscular tissue remodeling trough a better incorporation of avascular engineered constructs [4].

Indeed, stem cells are capable of proliferation, self-renewal, myogenesis, but also do resist oxidative or hypoxic stress and release several myogenic growth factors, especially VEGF [10–13]. In addition, Mesenchymal Stem Cells (MSCs) have been shown to modulate the cellular immune and inflammatory responses in wounded tissue, recruit local stem cells at the wound site, and to reduce the amount of apoptotic cells and fibrosis by promoting neoangiogenesis [14–16]. However, the best source of stem cells remains a matter of discussion.

In the literature, some studies were conducted with biological meshes seeded with Adipose Stem Cells (ASCs) or Bone Marrow Mesenchymal Stem Cells (BMSCs). The majority of those concluded to the superiority of composite matrices in terms of neo vascularization and cell

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http://dx.doi.org/10.1016/j.jpedsurg.2016.11.041
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colonization for abdominal wall regeneration [6,9]. However, those studies focused on in vivo properties of non-differentiated MSCs from either bone marrow or adipose origin, individually.

Our work therefore compared muscle regeneration by a tissue engineered construct made of our HACM seeded with non-differentiated BMSCs versus ASCs. In vitro, cellular viability, muscular and angiogenic growth factor release under normoxic and hypoxic conditions were compared between ASCs and BMSCs. Hypoxic cultures were used to assess cell survival in conditions reproducing the harsh environment of a surgical implantation site where neoangiogenesis will not start before day 3 and blood vessels will only appear after 12 days post-implantation. In addition, the impact of processing and recellularization on the mechanical properties of the HACM were studied.

In vivo, the efficacy of the engineered construct was tested in a model of full thickness abdominal wall defect (n = 14) treated with HACM alone vs. HACM + ASCs or BMSCs.

1. Materials and methods

All materials were obtained from Lonza (Basel, Switzerland), Sigma–Aldrich (St. Louis, MO, USA), or Invitrogen (Carlsbad, CA, USA) unless otherwise specified.

The experimental steps are displayed in Fig. 1.

1.1. Animal sources of BMSCs and ASCs

Belgian Landrace Pigs (<100 kg, <6-month-old) were used as donors for BMSCs and ASCs (Segers, Lokeren, Belgium). Animals were housed according to the guidelines of the Belgian Ministry of Agriculture and Animal Care. All procedures were approved by the local Ethics Committee for Animal Care of the Université Catholique de Louvain).

1.2. MSCs isolation and characterization

The procedures were conducted as previously described [16].

Briefly, BMSCs were isolated from bone marrow aspirate (iliac crest) on a Ficol gradient. ASCs were obtained through enzymatic digestion of adipose tissue harvested from the lower abdomen of pigs. The isolated cells were seeded in a culture medium. Cells were then proliferated up to passage 4 before being induced into differentiation or seeded on HACM [16].

Fluorescence-activated cell sorting (at least 10,000 events analyzed by flow cytometry with CellquestPro software; FACScan, BD Biosciences, Franklin Lakes, NJ, USA) confirmed the mesenchymal stem cell lineage by revealing a positive shift of mean fluorescence intensity for CD44, CD73, CD90, and CD105 antibodies (BD Pharmigen, BD Biosciences)), whereas CD45 antigen expression remained negative. In contrast, peripheral blood mononucleated cells (negative control) demonstrated positive staining for CD45 and negative staining for CD44, CD73, CD90, and CD105 (data not showed). At P4, cell differentiations toward adipose, osteogenic, and chondrogenic phenotypes were confirmed by red oil, Alizarin red, and Alcian blue staining, respectively [16].

1.3. In vitro properties of BMSCs and ASCs

ASCs and BMSCs were seeded in 12-well culture plates and incubated in hypoxic chambers (Modular Incubator Chamber MIC-101, Billups-Rothenberg, Del Mar., CA, USA) following a protocol described previously [16,17]. Briefly, cells were incubated for 72 h at 0.1% and 21% O_2 levels reproducing a highly hypoxic environment and atmospheric normoxia, respectively. After the hypoxic stress period, cell culture supernatants were harvested individually, centrifuged, and stored at −20 °C for subsequent growth factor quantification. VEGF / IGF-1/ FGF-beta / HGF/ TGF-beta1 quantification was achieved by enzyme-linked immunosorbent assay (ELISA Quantikine Kit, R&D System, Minneapolis, MN, USA). All cell cultures were performed in triplicate. Growth factor quantification was performed in duplicate. Results were expressed in picograms and nanograms per milliliter and as ratios between hypoxic and normoxic conditions.

Cellular viability was also assessed under normoxic and hypoxic conditions by a MTS cell proliferation assay (MTS; Promega, Leiden, The Netherlands), as described previously [16,17].

1.4. Human acellular collagen matrix

Human Acellular Collagen Matrices (HACM) were processed from human fascia lata, as described previously [5,18]. Briefly, fascia lata were obtained from cadaveric donors. The matrices were mechanically striped from adipose tissue, muscle remnants and loose connective tissues. The fascias were then processed through a series of chemical baths, freeze-dried and sterilized by gamma-irradiation (25KGy).

Decellularization quality was assessed by DAPI and residual DNA quantification. DAPI (1 μg/ml) staining (Abbot Molecular Inc.-USA) was performed on paraformaldehyde (4%) fixed/paraffin embedded slices from native (n = 4) and treated matrices (n = 4) from different donors. Tissue sections were observed using a fluorescence microscope (Zeiss, Göttingen, Germany).

Residual DNA isolation was obtained using a QIAamp kit DNA Mini Kit (Qiagen, Hilden, Germany) on equal sample volumes of either native matrices (n = 4) or post treatment matrices (n = 4). DNA concentration was measured by fluorometry with a Qubit fluorometer (Invitrogen, Thermo Fischer Scientific, Waltham, MA, USA) with a wavelength set at 260 nm. Results were expressed in μg/ml.

1.5. Cell seeding

BMSCs and ASCs were expanded up to passage 4 in the proliferation medium (see above).

At passage 4, ASCs and BMSCs were manually seeded on HACM (2 $\times$ 10^5/cm^2). Cells were then further expanded on the HACM. Correct dissemination of MSCs on the HACM was evaluated after 30 days and confirmed by histological sample (Hemalun-eosin and DAPI staining).

1.6. Mechanical properties of the composite grafts

The impact of processing and in vitro recellularization of the HACM by MSCs was mechanically assessed. Uniaxial mechanical resistance test was performed on triplicate samples of native human fascia lata (n = 5), rehydrated freeze-dried HACM (n = 4), HACM alone (without MSCs) incubated 4 weeks in culture medium (n = 4), and recellularized HACM with MSCs (after 4 weeks of incubation; n = 4). Mechanical testing was performed using an Instron traction system with Instron bluehill software (Model 5600; Instron, Canton, MA, USA) with a load-to-failure test at an elongation rate of 4 mm $\times$ min^{-1}. Distance between the two grips was 26.5 mm for each test. Samples were cut to a constant length of 45 mm and a width of 15 mm. The thickness of each sample was recorded. The load–elongation behavior of the matrices and failure modes were recorded. The structural properties of the matrices were represented by stiffness (Newton $\times$ mm^{-1} – N/mm) and ultimate load (Newton - N). Stiffness (k) was calculated as $k = \Delta F/\Delta L$, where F is the force applied on the body and L is the displacement produced by the force along the same degree of freedom (for instance, the change in length of a stretched spring). These parameters were compared between each experimental groups.

1.7. In vivo assessment of the composite graft

1.7.1. Surgical procedure

Fourteen nude rats (Charles River Laboratories International, Wilmington, MA, USA) weighting approximately 200–300 g (5–8 weeks old) were used in this study. The rats were housed according to the

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