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Xenogeneic cardiac extracellular matrix scaffolds with or without seeded mesenchymal stem cells exhibit distinct *in vivo* immunosuppressive and regenerative properties

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ABSTRACT

Cardiac extracellular matrix (cECM) scaffolds are promising biomaterials for reconstructive surgery applications since they possess the structure/function properties of native tissue. Production of cECM scaffolds has been achieved using decellularization approaches, which commonly employ denaturing detergents, such as sodium dodecyl sulfate (SDS). Our antigen removal (AR) method has been shown to remove cellular and nonmyocyte components, while preserving cECM scaffold structure/function relationships. Here, we demonstrate that more human mesenchymal stem cells (MSCs) invaded AR scaffolds compared to SDS controls. Additionally, AR scaffolds stimulated a constructive remodeling response similar to allograft controls, and were transformed to adipose tissue in a xenogeneic rat to mouse subpannicular *in vivo* model. Conversely, SDS scaffolds showed a chronic inflammatory response that worsened throughout the 12-wk time course preventing constructive remodeling and mirroring the response seen towards xenogeneic tissue. AR scaffolds and xenogeneic controls recellularized with murine MSCs (mMSCs) were also implanted to assess whether mMSCs would offer any additive benefit in overcoming residual scaffold-specific immune responses. Paradoxically, recellularization resulted in chronic inflammatory response in AR-recellularized scaffolds. We conclude that AR cECM scaffolds represent a promising biomaterial, which is accepted by the recipient as self in origin and fosters implantation site appropriate regenerative responses.

Statement of Significance

We demonstrated that an antigen-removal (AR) approach utilizing principles of differential solubility for production of a xenogeneic rat cardiac extracellular matrix scaffold results in improved recellularization efficiency with human and mouse mesenchymal stem cells (MSCs) *in vitro*. Furthermore, we tested the immune response to AR scaffolds versus allograft and xenograft controls with or without MSC recellularization using a rat to mouse subcutaneous model. We showed that AR scaffolds and allograft controls resulted in significant adipose tissue transformation after 12 weeks. Paradoxically, MSCs had a positive impact in the immune response to xenografts, but had the opposite effect in AR scaffolds, resulting in chronic inflammatory response, which might be attributed to a change of their phenotype following recellularization into scaffolds.

1. Introduction

Various surgical techniques utilizing synthetic or chemically cross-linked biological tissue patches have been developed for cardiovascular reconstructive surgery applications [1].

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Endoventricular patch repair (Dor procedure) to treat post infarction left ventricular (LV) aneurysms for example is thought to be acutely beneficial because it reestablishes LV wall geometry [1]. However, current patch materials have several major disadvantages, including their relative noncompliance compared to surrounding myocardium and lack of regenerative capacity, since they fail to support constructive endogenous cellular repopulation [2]. Additionally, current patches have been shown to induce a recipient inflammatory foreign body-type response with resultant fibrotic encapsulation [2,3]. Xenogeneic cardiac tissue-derived extracellular matrix (cECM) scaffolds have potential to overcome the deficiencies of current patch materials, since they possess appropriate matrix architecture, biochemical composition and mechanical properties, which provides an ideal environment for cell differentiation and orientation to occur [4–7].

Decellularization methods aim to reduce *in vivo* immune response towards xenogeneic tissues while maintaining the native ECM to serve as an informative biomaterial capable of fostering recipient regenerative processes [7,8]. The most widely used approaches include use of ionic detergents, such as sodium dodecyl sulfate (SDS) [7,8]. However, several reports have indicated that use of such harsh detergents might result in inability to fully remove the detergent from scaffolds after the procedure [9], denaturation of ECM macromolecules [10,11], depletion of growth factors [12], and altered ECM scaffold mechanical properties [8,13,14], any of which could negatively impact recellularization efforts and/or *in vivo* outcomes upon implantation [10,15]. We have developed a novel antigen removal (AR) process to remove cardiomyocyte and nonmyocyte antigenic components and cellular elements from cardiac muscle in a stepwise manner without using denaturing detergents [14]. Unlike SDS-decellularized controls, our approach produced a cECM scaffold that is completely acellular, with 95% reduction in hydrophilic and 85% reduction in lipophilic xenoantigens, while preserving native tissue structural, biochemical and biomechanical properties [14]. However, the extent to which the AR approach is able to reduce cECM scaffold-specific *in vivo* immune response, while fostering constructive regenerative responses remains unknown.

The acceptable level of residual antigenicity which will confer immune compatibility in an immunocompetent recipient has yet to be determined. In theory, even low levels of residual antigenicity could potentially stimulate detrimental graft-specific immune responses *in vivo*. Provision of an immunomodulatory cellular component to a cECM scaffold therefore has potential to further modulate and/or reduce any potential residual recipient graft-specific immune responses. Mesenchymal stem cells (MSCs) exhibit immunomodulatory properties through direct cell-cell contact and secretion of soluble factors [16]. These properties have led to their use in veterinary clinical practice and in a wide range of human clinical trials [16,17], including severe cases of acute graft-versus-host disease [18]. However, the potentially advantageous properties of MSCs in modulating residual *in vivo* graft-specific immune response towards xenogeneic ECM scaffolds in immunocompetent recipients have not been explored.

The current investigation had four objectives: (a) evaluate the compatibility of cECM scaffolds produced using different decellularization/AR approaches with *in vitro* MSC recellularization, (b) characterize the acute (2 wk) and chronic (up to 12 wk) inflammatory and immune responses to various cECM scaffolds in a subpannicular mouse model; (c) assess whether MSCs complement the AR process in reducing graft-specific inflammatory and immune responses; and (d) determine whether a cECM scaffold produced using the AR approach, with or without MSC seeding, is capable of fostering constructive tissue remodeling.

2. Materials and methods

2.1. Myocardial patch (MP) isolation and antigen removal (AR) procedure for cECM scaffold production

All chemicals were from Sigma-Aldrich (St Louis, MO) unless otherwise stated. MPs were isolated as previously described [14]. Briefly, whole hearts were isolated from 12 wk old male Sprague Dawley rats (CD®,Charles Liver Laboratories, Kingston, NY) and stored in storage solution containing Dulbecco's Modified Eagle's Medium (DMEM) with 15% dimethyl sulfoxide (DMSO) (v/v) at −80 °C. Hearts were defrosted and blood removed by antegrade coronary perfusion with 4 °C heparinized filtered ice-cold PBS (10 IU/ml). The left ventricle (LV) was isolated and planarized. A 3.5 mm biopsy punch (Integra, Miltex, PA) was utilized to cut adjacent cylindrical pieces of LV tissue from endocardium to epicardium, herein designated as myocardial patches (MP). Wet weights were recorded and only MPs that were 20 ± 2 mg were utilized for cECM scaffold production.

AR procedure was performed as previously described [14]. All solutions were made fresh and filtered. All steps were performed in 6-well plates, at 125 rpm, in 3 ml working volume at RT with solutions changed every 12 h unless otherwise stated. Samples undergoing AR were initially incubated in ice-cold permeabilizing/relaxing solution comprised of 2% amidosulfobetaine-14 (ASB-14), 120 mM potassium chloride (KCl), 4 mM magnesium chloride ($MgCl_2$), 4 mM ethylenediaminetetraacetic acid (EDTA), 5.88 mM sodium adenosine triphosphate (Na-ATP), 10 mM 2,3-butanedione monoxime (BDM), 0.5 mM Pefabloc SC (Roche Applied Science, Indianapolis, IN) and 1% antibiotic antimycotic solution (AAS) in 10 mM Tris-HCl (pH 7.6), two times for 30 min each at 125 rpm, at RT. MPs were then incubated in lipophilic AR buffer comprising of 2% ASB-14 in myocardium-optimized standard antigen removal buffer (mSARB; 0.5 mM Pefabloc SC, 1% AAS in 10 mM Tris-HCl, pH 8.0, 100 mM dithiothreitol (DTT), 2 mM $MgCl_2$, 600 mM KCl) for 2 d at RT. Samples were then washed twice in wash buffer (0.5 mM Pefabloc, 1% v/v AAS in 10 mM Tris-HCl (pH 8.0)) for 15 min. Sarcomeric components were extracted by incubating for 2 h at RT in 4 ml 50 nM Latrunculin B (Cayman Chemical, Ann Arbor, MI) in wash buffer, washing twice in wash buffer for 15 min, incubating for 2 h in 0.6 M KCl in wash buffer, washing twice in wash buffer for 15 min, incubating for 2 h in 1.0 M potassium iodide (KI) in wash buffer, followed by washing in wash buffer at RT, overnight. The following day KCl and KI incubations were repeated. Residual sarcomeric and nonmyocyte proteins were removed by placing MPs in 2% ASB-14 in mSARB for 48 h, followed by mSARB alone for 24 h at RT. MPs were then incubated for 24 h at 4 °C with nuclease solution (10 Kunitz units/mL DNase I, 7.5 Kunitz units/mL RNase A, 0.5 mM Pefabloc SC, 1% AAS, 0.15 M NaCl, 5 mM $MgCl_2$–$6H_2O$ in 10 mM Tris-HCl, pH 7.6), followed by washout for 48 h in wash buffer with two changes/day at 4 °C and stored at −80 °C in storage solution.

Rat MP native tissue controls (“Xeno” group) and planarized whole mice hearts (“Allo” group) were placed in wash buffer for 10 d to control for time in solution. SDS-decellularization control samples were incubated in 1% SDS (Bio-Rad, Hercules, CA) w/v in wash buffer for 24 h at RT, washed 2 × 15 min in 1% v/v Triton-X 100 in wash buffer and incubated in wash buffer until day 10 to control for time in solution [14]. Following processing all groups were stored at −80 °C in storage solution. Prior to *in vitro* or *in vivo* studies, MP samples were defrosted, washed for 2 d in wash buffer at 4 °C (2 changes/day), followed by overnight incubation in cell medium.