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Humanized mouse model for assessing the human immune response to xenogeneic and allogeneic decellularized biomaterials

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ABSTRACT

Current assessment of biomaterial biocompatibility is typically implemented in wild type rodent models. Unfortunately, different characteristics of the immune systems in rodents versus humans limit the capability of these models to mimic the human immune response to naturally derived biomaterials. Here we investigated the utility of humanized mice as an improved model for testing naturally derived biomaterials. Two injectable hydrogels derived from decellularized porcine or human cadaveric myocardium were compared. Three days and one week after subcutaneous injection, the hydrogels were analyzed for early and mid-phase immune responses, respectively. Immune cells in the humanized mouse model, particularly T-helper cells, responded distinctly between the xenogeneic and allogeneic biomaterials. The allogeneic extracellular matrix derived hydrogels elicited significantly reduced total, human specific, and $CD4^+$ T-helper cell infiltration in humanized mice compared to xenogeneic extracellular matrix hydrogels, which was not recapitulated in wild type mice. T-helper cells, in response to the allogeneic hydrogel material, were also less polarized towards a pro-remodeling Th2 phenotype compared to xenogeneic extracellular matrix hydrogels in humanized mice. In both models, both biomaterials induced the infiltration of macrophages polarized towards a M2 phenotype and T-helper cells polarized towards a Th2 phenotype. In conclusion, these studies showed the importance of testing naturally derived biomaterials in immune competent animals and the potential of utilizing this humanized mouse model for further studying human immune cell responses to biomaterials in an *in vivo* environment.

1. Introduction

The field of decellularized extracellular matrix (ECM) based biomaterials is rapidly growing and has developed therapies for numerous applications including wound healing, hernia repair, skeletal muscle defect repair, and myocardial infarction [1–4]. Decellularized ECM biomaterials are an attractive platform for biomaterial therapies since tissue derived ECM can promote tissue remodeling by influencing cellular metabolism, proliferation, migration, maturation, and differentiation [5]. In fact, these biomaterials, derived from xenogeneic and allogeneic tissue sources [6,7], have been successfully implanted into millions of patients [8]. Xenogeneic materials, from porcine tissue for example, are readily available and can be produced from younger tissue sources, which is desirable for regenerative medicine therapies [9]. However, xenogeneic materials can have potential immunogenic issues, regulatory hurdles and xenogeneic disease transfer. Allogeneic materials avoid some concerns associated with xenogeneic materials, but are typically from older and more limited cadaveric sources, and can have larger batch variability.

While xenogeneic and allogeneic sources for decellularized ECM

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have been widely used to date, preclinical understanding of these scaffolds is mostly based off immune responses to these matrices in rodents and a few large animals [1,10,11]. Given difficulties with obtaining sequential patient biopsies, no one has thoroughly monitored or understood the human immune response to these materials. Although connected evolutionarily, rodents typically used for biocompatibility testing provide limited representation of the human immune response. Differences in immune cell receptors, cytokine expression and response to various stimuli highlight how responses in rodents might not correlate with outcomes in humans [12]. Even non-human hominids have various biomedical differences from humans [13]. This combined with our incomplete understanding of the human immune system has led to the removal of several well characterized materials from the market [14,15].

One method to address these shortcomings is the use of a humanized mouse (Hu-mice) model for preclinical assessment of the human immune response. Over the last 20 years, significant improvements have transformed Hu-mice into a valuable model for mimicking the human immune response [16–18]. In particular, Hu-mice developed by implantation of human fetal thymus tissue and injection of human $CD34^+$ fetal liver cells into immune compromised NSG mice have been shown to be robust and contain human T-cells, B-cells, and dendritic cells, allowing the ability to reject xenogeneic tissue [19]. This model has been used extensively for studying autoimmune disease, virus infections, xenogeneic transplantation, and more recently allogeneic stem cell transplantation [20]. However, it has yet to be exploited in the biomaterials field. In this study, we utilized this Hu-mouse model to assess the human immune response to decellularized ECM biomaterials, specifically injectable hydrogels derived from porcine or human myocardium, which were initially developed to treat the heart post-myocardial infarction [11,21–23]. Our goal with this study was to evaluate the utility of the Hu-mice for evaluating biocompatibility and studying the human immune response to biomaterials prior to clinical translation. We hypothesized that this model would demonstrate different immune responses to human versus xenogeneic ECM, unlike a wild type rodent model.

2. Methods and materials

All experiments in this study were performed in accordance with the guidelines established by the committee on Animal Research at the University of California, San Diego, and the American Association for Accreditation of Laboratory Animal Care.

2.1. Fabrication of PMM, HMM, and NDM

Both the porcine myocardial matrix (PMM) and human myocardial matrix (HMM) were developed and characterized according to established protocols [21,23]. Human hearts were obtained from donor patients whose hearts could not be used for transplantation under an institutionally approved protocol. In brief, left ventricular tissue (porcine or human) was isolated and chopped into small pieces. The tissue was spun in phosphate buffered saline (PBS) containing 1% (wt/vol) sodium dodecyl sulfate (SDS) (Fischer Scientific, Fair Lawn, NJ) with 0.5% penicillin streptomycin (PS) (Gibco, Life Technologies, Grand Island, NY) of 10,000 U/mL until fully decellularized. The human tissue was treated with additional lipid and DNA/RNA removal steps that were needed to fully decellularize the tissue [23]. Once decellularized, the remaining ECM was lyophilized, milled, and partially digested with pepsin into a liquid form as previously described [21,23]. Non-decellularized myocardial matrix (NDM) was also produced from porcine ventricular tissue as a control. The tissue was simply rinsed in the PBS and PS solution with no SDS for one day. Then, the non-decellularized porcine tissue was processed into an injectable form using the same methods as the decellularized myocardial matrix. Finally, the materials were lyophilized and stored at −80°C until re-suspending with sterile water prior to injection.

2.2. Hydrogel characterization

Porcine and human myocardial matrix hydrogels were imaged for nano-scale topography and fiber formation with scanning electron microscopy (SEM) as previously described [23,24]. In brief, samples were gelled for 24 h at 37 °C and then fixed in a solution of 4% paraformaldehyde and 4% glutaraldehyde for 24 h. Next, the gels were dehydrated with a series of gradated ethanol rinses. Then, fixed and dehydrated hydrogels were processed in an automated critical point drier (Leica EM CPD300, Leica, Vienna). Mounted samples were subsequently sputter coated (Leica SCD500, Leica, Vienna) with platinum while being rotated. The samples were then imaged on a FE-SEM (Sigma VP, Zeiss Ltd, Cambridge, UK) at 0.6 kV using the in-lens SE1 detector.

Immunohistochemistry was performed on the porcine derived material to assess removal of the alpha-gal epitope. Freshly isolated porcine left ventricular tissue, decellularized porcine myocardium, and porcine myocardial matrix hydrogels were fresh frozen in OTC for cyrosectioning. Sections (20 μm) were mounted onto glass slides, fixed in 4% paraformaldehyde for 10 min and permeabilized in acetone for 1.5 min. Slide samples were either stained with hematoxylin and eosin (H&E) or prepared for immunohistochemistry. Samples were blocked with a buffered solution containing bovine serum albumin (BSA) and stained for at least 12 h at 4°C with M86 anti-alpha-gal (1:10, Enzo Life Sciences, Framingdale, NY) followed by incubation for 30 min with secondary anti-mouse Alexa Fluor 488 antibody (1:100, Life technologies, Carlsbad, CA) [25]. Hoechst 33342 was used to stain nuclei. Slides were imaged with a Carl Zeiss Observer D1 and Zeiss AxioVision SE64 software (Carl Zeiss, Jena, Germany).

2.3. Humanized mouse model

NOD.Cg-Prkdc^{scid}Il2rg^{tm1wjl}/SzJ (NSG) (The Jackson Laboratory) mice of 6–10 weeks of age after conditioning with sublethal (2.25 Gy) total body irradiation underwent the following procedure, as previously described, to create the humanized mouse model (Hu-mice) [17]. First, the mice were transplanted under the kidney capsule with a piece of human fetal thymic tissue of about 1 mm^3 that had been previously frozen. Next, the animals were transfused intravenously with 1–5 x 10^5 human $CD34^+$ fetal liver cells from the same patient donor. Human fetal tissue, from Advanced Bioscience Resource, with gestational ages of 17–20 weeks was utilized.

2.4. Biomaterial injection and harvesting

Animals were briefly put under anesthesia using either 2.5% isoflurane or via injection with ketamine and xylazine. Each mouse was injected with only one type of biomaterial and received four 250 μL evenly spaced subcutaneous injections in the dorsal region. Each injection was premixed with 0.5 μL of sterile india ink to visually label the matrices for ease of identification upon harvesting. The injections, along with neighboring dermal tissue and spleens, were harvested three days and 1-week later for analysis with histology and immunohistochemistry ($n = 8–16$), flow cytometry ($n = 4$), or qRT-PCR ($n = 8–12$). Along with the Hu-mice, both male NSG and male Balb/c (Jackson Laboratories and Harlan Laboratories, respectively) of the same ages were used for immune