Effects of Scaffold Material Used In Cardiovascular Surgery on Mesenchymal Stem Cells and Cardiac Progenitor Cells

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Background. **Polytetrafluoroethylene (PTFE) and porcine small intestinal submucosa (pSIS) are patch materials used in congenital heart surgery. Porcine SIS is an extracellular-matrix scaffold that may interact with stem or progenitor cells. To evaluate this, we determined the in vitro effects of pSIS and PTFE on human bone marrow mesenchymal stromal cells (MSCs) and cardiac progenitor cells (CPCs) in 3 areas; cell proliferation, angiogenic growth-factor production, and differentiation.**

Methods. **Human MSCs and CPCs were seeded onto pSIS and PTFE patches. Cell-seeded patches were cultured and then assessed for cell viability and proliferation and supernatant vascular endothelial growth factor A (VEGFA) levels. Cell proliferation was quantified by MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). Quantitative real-time polymerase chain reaction was performed on cell-seeded scaffolds to determine relative changes in gene expression related to angiogenesis and cardiogenesis.**

Results. **The MSCs and CPCs were able to attach and proliferate on pSIS and PTFE. The proliferation rate of each cell type was similar on pSIS. Total RNA isolation was only possible from the cell-seeded pSIS patches. The MSC VEGFA production was increased by pSIS. Porcine SIS promoted an angiogenic gene profile in MSCs and an early cardiogenic profile in CPCs.**

Conclusions. **Both PTFE and pSIS allow for varying degrees of cell proliferation. Porcine SIS elicits different phenotypical responses in MSCs as compared with CPCs, which indicates that pSIS may be a bioactive scaffold that modulates stem cell activation and proliferation. These findings highlight the differences in scaffold material strategies and suggest potential advantages of bioactive approaches.**

(Ann Thorac Surg 2014;■:■–■)

Reconstruction of congenital cardiac defects often requires synthetic materials such as polytetrafluoroethylene (PTFE) when native tissue is not adequate. Although PTFE is durable and hemostatic, it does not grow and incites the formation of intimal hyperplasia. This may lead to reoperation for replacement and therefore increases the patient's risk of morbidity and mortality from repeat cardiac operations [1].

Decellularized porcine small intestinal submucosa (pSIS) patches has been advocated as an alternative to synthetic materials. Porcine SIS has been described in cardiac and vascular reconstructions, abdominal wall reconstruction, tendon repair, dural grafting, and lower urinary tract reconstruction [2–8]. Porcine SIS has been shown in animal studies to allow ingrowth of native tissue, as well as improved vascularization with minimal calcification or scarring [9, 10]. Porcine SIS is biodegradable and is replaced by native tissue or scar. It is unclear as to what mechanisms promote tissue ingrowth and neoformation versus scar formation. We hypothesized that patch materials may have varying effects on resident and remote progenitor or stem cells that populate the scaffold after implantation.

Mesenchymal stem cells (MSC) are being evaluated as a therapeutic agent after myocardial infarction in preclinical and clinical trials [11]. While it remains unclear the extent to which MSCs are able to differentiate into cardiomyocytes in vivo [12], numerous studies have shown that MSCs improve myocardial function through paracrine effects that improve compliance, increase angiogenesis, alter remodeling, increase cellular migration, and increase cardiomyocyte contractility [13–15]. The clinical effectiveness of MSC therapy in infarcted myocardial tissue has been limited as immediate cell retention after injection is minimal. This is believed to be due to a suboptimal delivery of cells as well as the inhospitable post-infarcted environment [16]. Research into the effectiveness of MSC seeded scaffolds as delivery agents is currently ongoing.

It was previously believed that cardiac tissue was terminally differentiated and cardiac regeneration in the

Accepted for publication Aug 15, 2014.

Presented at the Forty-ninth Annual Meeting of The Society of Thoracic Surgeons, Los Angeles, CA, Jan 26–30, 2013.

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Published by Elsevier

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2014.08.071

mammalian heart was not possible from local cell populations. In 2003, Beltrami and colleagues [17] described adult cardiac progenitor cells (CPCs) that were self-renewing as well as multipotent, with the ability to differentiate into cardiomyocytes as well as smooth muscle and endothelial cells. This discovery has fueled intensive research efforts into the application of CPCs in infarcts with both significant and negligible results [18, 19]. Delivery of CPCs on scaffolds has not been investigated, and the effects of scaffold material on CPCs have to be first delineated.

In our study, we sought to compare the effect of pSIS on the proliferation, gene expression profile, and activation in MSCs and CPCs to determine which material would provide a superior niche for stem cell populations and provide further insight into their potential for cardiac regeneration.

Material and Methods

Scaffolds

Sterile PTFE (0.4 mm thickness; W. L. Gore & Associates, Inc, Newark, DE) and pSIS (CorMatrix Cardiovascular, Inc, Alpharetta, GA) were used in this study. Patch materials were soaked in phosphate buffered saline (PBS) for 10 minutes. For the pSIS patches, the 4-ply material was separated into single layers to allow for microscopic visualization of seeded cells and facilitation of RNA isolation for quantitative real time polymerase chain reaction (qPCR) analysis.

Cell Culture

Bone marrow MSCs were purchased from Lonza (Basel, Switzerland). The MSCs were cultured in MSC medium (Dulbecco's modified Eagle medium, with high-glucose concentration, GLUTAMAX I, 10% heat inactivated adult bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin, all from Life Technologies, Carlsbad, CA), incubated at 37°C and 5% carbon dioxide (CO_2), and allowed to achieve 80% confluence prior to use.

The CPCs were isolated from neonates undergoing open heart surgery and characterized as described previously under an approved Institutional Review Board protocol from the Children's Memorial Hospital [20, 21]. Briefly, right atrial tissue were minced and partially digested with 0.05% trypsin/ethylenediaminetetraacetic acid (EDTA) and collagenase type II (Worthington Biomedical Corp, Lakewood, NJ). Explants were plated on fibronectin in CPC medium (Iscove modified Dulbecco medium with 20% fetal bovine serum). When there appeared to be an adequate number of phase-bright cells (usually 2 weeks after plating), they were removed and plated at low density (3×10^4 cells/mL) in cardiosphere-growing medium. Cardiospheres were removed and plated on fibronectin in human cardiac stem cell expansion medium (HCSCEM; Celprogen, San Pedro, CA). Phase-bright cells were harvested from individual explant cultures every 3 to 5 days up to 4 consecutive times. The CPCs were then expanded on fibronectin-coated plates with CPC media. Detailed characterization of these CPCs has been provided elsewhere [20]. Media was changed every 2 days for all cell types. All experiments were performed with cells from passages 3 to 8.

Scaffold Seeding and Stem Cell Proliferation

Single-layer pSIS and PTFE were cut into 1×1 cm^2 patches and placed in the wells of a 24-well (flat bottom) plate such that the scaffolds covered the bottom surface of the wells. Preliminary experiments indicated that MSC and CPC proliferation on scaffolds were significantly reduced as compared with that obtained on tissue culture treated polystyrene (control surface). Therefore control wells were seeded with 5×10^4 MSCs or CPCs while scaffolds were seeded with 1.5×10^5 MSCs or CPCs in 100 μL of MSC media or CPC media, respectively, on the surface of the scaffolds. Cells were allowed to adhere to scaffolds for 2 hours prior to the addition of more media.

Relative cell proliferation was assessed by MTT assay as previously described after a 7 day culture period [22]. Briefly, a stock solution of 3-(4,5-Dimethylthiazo1-2-y)-2,5-diphenyltetrazolium bromide (MTT, Life Technologies) was diluted with PBS as per manufacturer directions added to each well with patch material and cells and agitated for 3 hours. Dimethyl sulfoxide was then added to each well to lyse the adherent cells; the scaffolds were then removed and absorbance of each solution was measured at 560 nm.

Effects of Scaffold Material on Gene Expression

The effects of scaffold material on CPC and MSC gene expression were determined. We focused on human genes related to angiogenesis, cell proliferation, and cardiogenesis (Table 1). Cells were plated in 100 μL onto tissue culture treated polystyrene (control), PTFE, or pSIS in a 24-well plate as described above. After 2 hours, 400 μL of CPC medium was added. Media was changed daily until harvest for nucleic acid isolation after 14 days of incubation.

Nucleic Acid Isolation and Amplification

The RNA was isolated using Trizol-Chloroform separation (Life Technologies) followed by the Qiagen RNeasy minikit (Qiagen, Inc, Valencia, CA) according to the manufacturer's protocol. Control CPCs and MSCs were incubated with Trizol for 5 minutes and collected with a cell scraper. The CPCs and MSCs plated on scaffolds were submerged in 500 μL Trizol and vortexed for 1 minute. Phenol:Chloroform:Isoamyl-alcohol was mixed into the samples at a 1:5 dilution, then centrifuged at 13,400 RPM at 4°C for 15 minutes. Samples were then processed through the standard Qiagen RNeasy protocol using the aqueous layer of the centrifugation product in place of RLT buffer (Qiagen). Isolated products were quantified using a Nanodrop2000 (Nanodrop, Wilmington, DE). Complementary (c)DNA was generated from 0.5 μg of RNA in 20 μL reactions according to the protocol for Invitrogen High-Capacity cDNA Reverse Transcription kit (Life Technologies).