Synergistic Acceleration of Stem Cell Mediated Heart Valve Tissue Formation by Cyclic Flexure and Laminar Flow

George C. Engelmayr, Jr [1], Virna L. Sales [2], John E. Mayer, Jr. [2], and Michael S. Sacks [1]
[1] Department of Bioengineering and McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA
[2] Department of Cardiac Surgery, Children's Hospital Boston, Harvard Medical School, Boston, MA

Statement of Purpose: Bone marrow-derived mesenchymal stem cells (BMSC) represent a potentially attractive cell source for use in cardiovascular tissue engineering. BMSC can be isolated from adult patients relatively non-invasively, and BMSC have a pluripotent differentiation potential. Sutherland et al. [1] recently demonstrated that BMSC isolated from juvenile sheep can be used to fabricate tissue engineered heart valves (TEHV), and that these TEHV can successfully function in the pulmonary outflow tract of sheep for at least 8 months. We previously demonstrated that cyclic flexure can independently stimulate engineered heart valve tissue formation by vascular smooth muscle cells (SMC). Toward developing optimized bioreactor conditioning regimens for BMSC-seeded TEHV, in the current study we investigated the independent and coupled effects of cyclic flexure and laminar flow on BMSC-seeded nonwoven scaffolds.

Methods: Ovine BMSC were isolated from juvenile sheep by the method of Pittenger et al. [2], expanded in vitro in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic (Gibco). BMSC were seeded onto nonwoven 50:50 blend poly(glycolic acid) and poly(L-lactic acid) scaffolds (PGA/PLLA) (Albany International, Mansfield, MA) at a density of ~$17x10^6$ cells/cm^2 as described previously [2]. Following 30 hours seeding, BMSC-seeded scaffolds were maintained in static culture for 4 days and then loaded into our novel flex-stretch-flow (FSF) bioreactor. The FSF bioreactor accommodates 24 rectangular tissue specimens (~ 25 x 7.5 x 1 mm), with the ability to apply cyclic flexure and stretch by a linear actuator and laminar flow by a magnetically-coupled paddlewheel. A total of 48 BMSC-seeded scaffold specimens were prepared for evaluation in this study. Two separate runs of the bioreactor were required to test all of the specimens. In the first run, BMSC-seeded scaffolds were loaded into the bioreactor under aseptic conditions and incubated under static (n=12) or cyclic flexure (n=12) conditions. Cyclic flexure was applied at a frequency of 1 Hz and change-in-curvature of 0.554 mm^{-1}. In the second run, BMSC-seeded scaffolds were incubated under laminar flow (n=12) or laminar flow/cyclic flexure (n=12) conditions. Laminar flow was applied by setting the paddlewheel rotational velocity to the maximum value of 2000 RPM, which was shown to yield an average fluid shear stress of 1.1505 dyne/cm^2. Specimens from each mechanical loading group were removed following 1 (n=6) and 3 (n=6) weeks. Following removal from the FSF bioreactor, specimens were characterized by effective stiffness (E) testing, DNA and extracellular matrix (ECM) assays, histology, immunohistochemistry, and scanning electron microscopy (SEM).

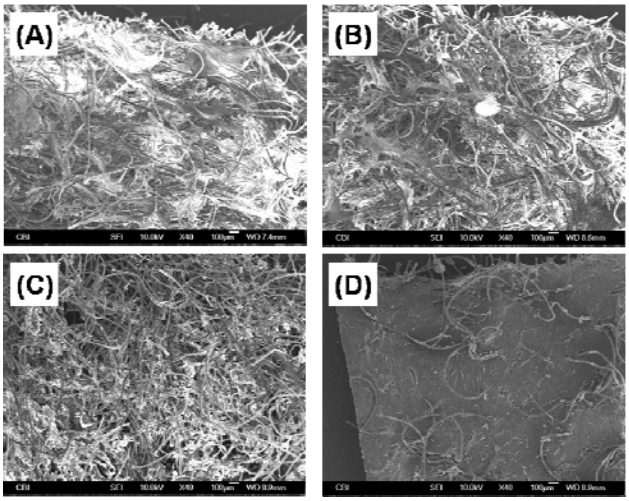

Figure 1 – Representative SEM micrographs of BMSC-seeded scaffolds incubated for 3 weeks under static (A), cyclic flexure (B), flow (C), and flex-flow (D) conditions.

Results / Discussion: By 3 weeks, the average DNA concentrations had decreased from 1 week values to 3.1 ± 0.5 (-89%; $p<0.001$), 3.8 ± 0.6 (-75%; $p<0.001$), 35.1 ± 1.8 (-23%; $p<0.05$), and 47.0 ± 4.8 (-21%; N.S.) μg/g wet weight in the static, flex, flow, and flex-flow groups, respectively. By 3 weeks, the average S-GAG concentrations had increased from 1 week values to 3636 ± 345 (+21%; N.S.), 5637 ± 897 (+102%; $p<0.01$), and 2268 ± 67 (+1%; N.S.) μg/g wet weight in the static, flex, and flow groups, respectively, and had decreased to 1919 ± 54 (-23%; $p<0.05$) μg/g wet weight in the flex-flow group. Collagen was not detected biochemically at 1 week in any specimen group. By 3 weeks, the average collagen concentrations measured were 422 ± 98, 530 ± 106, 498 ± 95 and 844 ± 278 μg/g wet weight in the static, flex, flow, and flex-flow groups, respectively. SEM provided dramatic evidence for accelerated tissue formation in the flex-flow group (**Fig. 2**) corroborated by histology.

Conclusions: The primary mechanical stimuli experienced by TEHV in pulsatile flow bioreactors, cyclic flexure and laminar flow, synergistically accelerated BMSC-mediated tissue formation. The results of this study provide guidance for optimizing bioreactor conditioning regimens for BMSC-seeded TEHV.

Acknowledgments: NIH HL-68816 (MSS) and AHA fellowship 0415406U (GCE; PA-DE affiliate).

References:

[1] Sutherland et al. (2005) Circulation **111**(21):2783-91.
[2] Engelmayr et al. (2005) Biomaterials **26**(2):175-87.
[3] Pittenger et al. (1999) Science **284**(5411):143-7.