Biocompatibility of PGG-Stabilized Collagen Scaffolds used for Heart Valve Tissue Engineering

Mary E. Tedder, Dan T.Simionescu
Clemson University, Clemson, SC.

Statement of Purpose: Pericardium has been widely researched as a biomaterial, including examination of its mechanical and fiber orientation properties by mapping techniques [1], isolation of pericardial, fibroblasts and analysis of their biosynthetic abilities [2, 3] and detailed analysis of its collagen and proteoglycan components [2, 3]. Only recently has decellularized pericardium attracted attention as a scaffold for tissue engineering [4,5].
Our working premise is that the ideal scaffold must function immediately after implantation, but also need to tolerate cell infiltration and gradual remodeling. We hypothesized that moderately cross-linked collagen scaffolds prepared from acellular pericardium would fulfill these requirements.

Methods: To test this hypothesis, collagen scaffolds were prepared from porcine pericardium by decellularization and cross-linked with pentagalloyl glucose (PGG) and then tested for in vivo biocompatibility and remodeling. Their properties were compared to Glut-fixed scaffolds. For rat subdermal implantation, a small transverse incision was made on the backs of the rats and two subdermal pouches (one superior and one inferior to the incision) were created. Control (no cross-linking), Glut, and PGG-treated scaffold samples (6 mm diameter circles) were implanted into the subdermal pouches (a total of 8 implants in 4 different rats per group per time point). The rats were euthanized by CO_2 asphyxiation at 1, 3, and 6 weeks and samples were retrieved. Each circular explant was cut in half and arbitrarily labeled for histology, immunohistochemistry (IHC) for vimentin, proline hydroxylase, and macrophages, phenol stain, DNA content analysis by extraction and agarose gelatin electrophoresis , calcium content by atomic absorbance spectrophotometry, matrix metalloproteinase (MMP) activity by gelatin zymography, and GAG content analysis by DMMB assay. Capsules were maintained for those samples delineated for histological evaluation.

Results: H&E staining revealed a time dependent increase in cell infiltration in control (untreated) scaffolds associated with visible collagen fiber degeneration, confirming the fact that the collagen scaffold is degradable in vivo. PGG-treated scaffolds exhibited clear signs of collagen fiber degradation, visibly less than control, untreated collagen and more than Glut-fixed collagen. Similarly, larger numbers of infiltrating cells were found in PGG-treated collagen as compared to Glut-fixed collagen, but less than in untreated controls.
Cell infiltration in PGG treated scaffolds increased with time, clearly showing that PGG treatment is not cytotoxic. IHC revealed a large majority of cells infiltrating collagen scaffolds were vimentin-positive cells resembling fibroblasts. Some infiltrating cells were also positive for proline hydroxylase, an enzyme involved in collagen synthesis. Macrophage infiltration was very scarce in all implants at all time points. Phenol staining showed tight binding of PGG to collagen which appeared to be maintained even after 3 weeks of subdermal implantation, suggesting stable interactions of PGG with collagen. Extraction and analysis of DNA content confirmed histology results- data showed significant DNA present at all time points in most tissues, with less DNA present in Glut-fixed tissues at 3 weeks ($p<0.05$). Control, untreated collagen scaffold samples, as well as PGG-treated scaffolds did not accumulate any significant amounts of calcium (1.2 ± 0.3 and 1.5 ± 0.5 µg / mg respectively at 3 weeks), indicating that despite effective collagen stabilization, PGG treatment may not induce collagen calcification. Furthermore, the total MMP enzyme activity distribution resembled that of the DNA content. Two major proteases were identified in tissue extracts, namely MMP-9 (migrating at around 90-95 kDa) and MMP-2 (65-80 kDa). In addition, GAG levels within all implant groups showed a mean overall value of 175 +/- 30 ug per mg extracted protein, but without showing statistical significance among treatment groups and time points ($p>0.05$). Overall results confirm the presence of cells actively involved in matrix remodeling.

Conclusions: In the current study we demonstrated that PGG partially stabilizes collagen and that PGG treated collagen did not calcify in vivo, suggesting that the nature of the cross-linker may determine the outcome of collagenous implants. Host cells infiltrated implants relatively rapidly indicating that PGG-treated collagen is not cytotoxic and may support scaffold repopulation upon implantation. Moreover these fibroblast-like cells secreted MMPs, expressed proline hydroxylase and secreted GAGs and thus may exhibit true potential for remodeling. This indicates that PGG is a most promising collagen stabilization process and that these scaffolds are biocompatible. Ongoing research will investigate the use of PGG-stabilized collagen scaffold for heart valve tissue engineering.

References:

1. Simionescu D, et al. J Biomed Mater Res 1993;27:697-704.
2. Simionescu D, et al. Matrix 1989;9:301-10.
3. Simionescu DT, et al. Exp Cell Res 1991;195:171-6.
4. Oswal D, et al.. J Heart Valve Dis 2007;16:165-74.
5. Mirsadraee S, et al.J Surg Res 2007.

Acknowledgements: Agneta Simionescu, all other BTRL members, Linda Jenkins, Jun Liao from Mississippi State University, NIH Grants P20 RR-016461 and HL084194, Clemson University Department of Bioengineering.