Lattice Architecture of the Cholecyst Derived Extracellular Matrix dictates an Integrative Tissue Response

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Introduction: Our research group has identified an intact extracellular matrix derived from cholecyst. The cholecyst derived extracellular matrix (CEM) has a mesh-like micro-architecture, with anisotropic mechanical properties and a nanoscale topography ideal for cell infiltration, tissue growth and regeneration[1]. However, before this matrix can be developed for any clinical application, it is essential to evaluate its in vivo tissue response and regenerative ability. In this study, we report the in vivo tissue response elicited by CEM and maximal crosslinked CEM in a rat subcutaneous implantation model.

Methods: CEM, 0.625 % (w/w) glutaraldehyde (GA) crosslinked CEM (GAxCEM) and GA crosslinked porcine heart valve (GAxHV) implants measuring 1 cm^2 were implanted subcutaneously in male Sprague Dawley rats by blunt dissection. All three samples were implanted, in duplicates, symmetrically into the back of the same animal on either side of the dorsal midline. The animals were sacrificed at 21 and 63 days (n=4 per time point). The implant and its surrounding tissue samples were harvested and processed for histology. 5 µ thick histology sections were stained with hematoxylin and eosin (H&E), and Masson's trichrome (MT). All histological preparations were assessed by a pathologist, who was not informed of the identity of the implant in each slide. Histomorphometrical assessments of the cell types, collagen remodeling and the zone of inflammation were conducted by stereological image analysis.

Results and Discussion: Tissue response studies were designed to evaluate the long-term fate of CEM when implanted subcutaneously in rats. The tissue response elicited by CEM was compared with its crosslinked form, GAxCEM. GAxHV was used as positive control. At 21 days, CEM demonstrated an integrative tissue response with active fibroblasts, macrophages and capillary infiltration (Fig 1a). Focal aggregation of lymphocytes surrounding the blood capillaries was also observed. Native CEM showed a remarkable response, with uniform distribution of host cells over the entire implant area – probably facilitated by its nanoscale lattice-like architecture. The nature and type of host cell infiltration, in the case of GAxCEM (Fig 1c) was similar to that observed with CEM, except that the infiltration was incomplete with the formation of giant cells. The presence of giant cells has been attributed to the glutaraldehyde crosslinking[2]. Glutaraldehyde crosslinking completely eliminated the infiltration of host cells into the GAxHV implant matrix (Fig 1 e). The host cells were restricted to the periphery along with giant cell formation. By 63 days, the native CEM samples were completely degraded and the implant area was returning to the original architecture (Fig 1b). No degradation was seen in GAxCEM and the nature of integration with host tissue was similar to that observed at 21 days in native CEM (Fig 1d). In the case of GAxHV (Fig 1f), the penetration of host cells into the matrix was lower and the core of the implant was intact. A thin and highly vascular collagen deposit of 8 to 20 µm thickness, containing active fibroblasts was observed in all implants at all time points. None of the implants showed any signs of rejection such as swelling, ulceration, discharge of pus, scarring, tissue necrosis or avascular fibrous capsule formation at both time points.

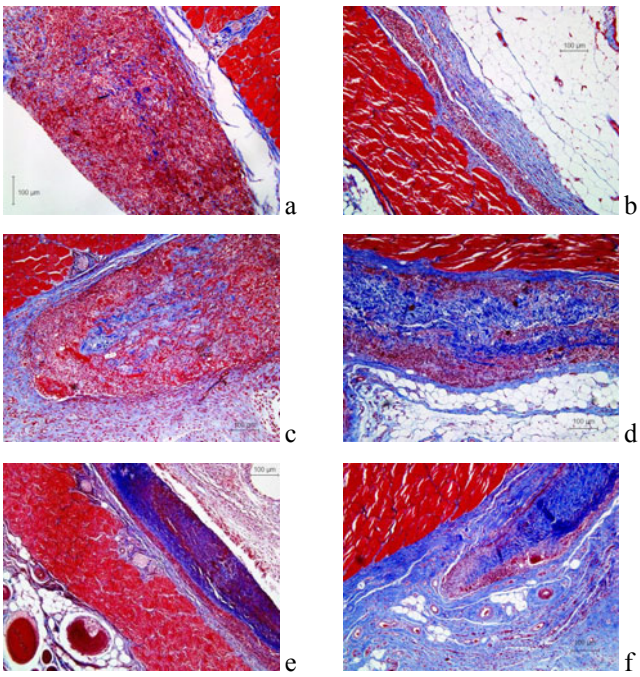

Figure 1: Tissue response elicited by CEM (a, b), GAxCEM (c, d) and GAxHV (e, f) at 21 days (a, c, e) and 63 days (b, d, f) of subcutaneous implantation in rat (MT).

Conclusions: CEM demonstrated an integrative tissue response, which is facilitated by its porous lattice-like network structure. Native CEM was completely degraded after 63 days of implantation whereas crosslinking with glutaraldehyde reduced the rate of degradation of CEM. The clinical applications of CEM may be tailored where a lattice like structure is required.

References

1. Burugapalli K. et al. Proceedings of 8th Annual Meeting of TESI, Shanghai, Oct 22-25, 2005.
2. Courtman, D.W. et al. J Biomed. Mater. Res. 55, 576, 2001.

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