Development of a Decellularised Bladder Matrix for Functional Tissue Engineering

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Statement of Purpose: Surgical reconstruction of the urinary bladder is performed on patients with a wide range of conditions, including congenital abnormalities and cancer. A number of approaches are currently being developed to find practical and functional substitutes for native bladder tissue. As cell phenotype is strongly influenced by the composition and structure of the extracellular matrix (ECM) it is considered that naturally-derived ECM scaffolds have many advantages over synthetic scaffold materials [1]. The aim of this study was to produce a natural, decellularised matrix from porcine bladder tissue for use in developing a functional tissue-engineered bladder replacement. A natural bladder tissue substitute would also have potential applications as a research tool to study host cell-matrix interactions, the effect of urine on the properties of the matrix and the role of mechanical forces on tissue functionality. This last feature is of particular importance, as despite sustaining considerable structural deformation during its normal function, the bladder has received little attention regarding the role of mechanical forces on tissue structure, function and maturation [2].

Methods: Full thickness porcine bladder was decellularised through sequential incubation with hypotonic buffers containing 0.1% (w/v) SDS and nuclease enzymes (method adapted from [3]). The matrix was sterilized using 0.1% (v/v) peracetic acid. Histological staining was used to determine the cell and DNA content and histoarchitecture of the resultant matrix. Immunohistochemistry was used to characterise the expression of major structural proteins such as collagen type I; the basement membrane components collagen type IV and laminin and the cytoskeletal proteins, smooth muscle actin, desmin and vimentin. Biochemical analysis was performed on hydrolysed samples of fresh and decellularised tissue to determine the glycosaminoglycan, hydroxyproline and denatured hydroxyproline content of the matrices. Tissue strips dissected from the wall of fresh and decellularised bladders were subjected to low strain-rate uniaxial tensile loading to failure and the stress-strain behaviour was analysed by means of six parameters: the elastin and collagen phase slopes, transition stress and strain, ultimate tensile strength and failure strain. In addition suture-retention and burst testing was performed. Cytotoxicity of the matrix was assessed by contact inhibition and conditioned-medium assays with porcine bladder smooth muscle (PSM) cells. Preliminary cell seeding experiments of the matrix were performed with PSM cells.

Results / Discussion: Histological analysis confirmed that the treated porcine bladder matrix was completely acellular and that the general histoarchitecture of native bladder had been retained. Whilst the decellularisation process resulted in the removal of the basement membrane from the bladder lumen, there was residual expression of some poorly soluble cytoskeletal components of the smooth muscle. The amount of DNA per mg dry weight of porcine bladder before and after decellularisation was 2.8 (+/- 0.1) $\mu g.mg^{-1}$ and 0.1 (+/- 0.1) $\mu g.mg^{-1}$ respectively. Compared to fresh bladder tissue, the proportion of hydroxyproline and GAGs in the decellularised tissue samples increased, probably due to the relative loss of other soluble proteins and cell components. The proportion of denatured hydroxyproline decreased, indicating that the decellularisation process did not adversely affect the collagen content of the tissue. Biomechanical testing demonstrated that decellularisation resulted in an increased collagen phase slope and decreased extensibility. The ultimate tensile strength of the tissue, however, was unchanged. There was no difference in the burst pressure or ability of fresh and decellularised bladder tissue to retain sutures under force. Cytotoxicity assays showed that PSM cells grew well both in direct contact with the matrix and in matrix-conditioned growth medium, indicating that the washing procedure was efficient and had removed any residual chemical and biological agents. Sterilisation with peracetic acid did not affect biocompatibility as smooth muscle cells had begun to repopulate the matrix after 21 days in culture.

Conclusions: A natural acellular matrix comprising the major structural components of the urinary bladder has been successfully developed. This matrix has potential for use in investigating bladder tissue structure and function in vitro and as a functional, tissue-engineered construct in vivo.

References:

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