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DEVELOPMENT OF ACELLULAR MATRIX DERIVED FROM RAT'S URINARY BLADDER TO URINARY TRACT RECONSTRUCTION.

Hypothesis / aims of study

This study aims to develop a decellularization protocol for urinary bladder of rat and to produce an acellular matrix that can be used as a biological scaffold.

Study design, materials and methods

The urinary bladders were surgically removed from Wistar rats aged two to three months. The bladders were then extensively washed with phosphate buffered saline (PBS) and divided in two equal parts. Each half was processed with two distinct methods. One piece was washed in PBS for 15 minutes with agitation and then incubated with hypotonic buffer (10mM Tris, pH 8.0) for 24 hours at 5°C. It was treated with 0.1% (w/v) sodium dodecyl sulphate (SDS) in hypotonic buffer at room temperature for 24 hours with agitation. The tissue was then washed in PBS for 24 hours with agitation, concluding the protocol A. The second protocol (B), was used in the other half of the urinary bladder. First, it was incubated in deionized water containing 1% (w/v) SDS for 24 hours at room temperature with agitation. This was followed by 15 minutes treatment of deionized water with agitation. The piece was then treated with 1% Triton-X100 in deionized water, keeping the agitation. Finally, it was washed in PBS for 24 hours. As a control, half of another urinary bladder was only washed in PBS. The resulting matrices of both protocols and the bladder used as control were all stored within PBS at 5°C. These material were then used for histological analysis. The general histoarchitecture of the tissue were evaluated with Hematoxylin and eosin (H&E) staining. DAPI (4',6-diamidino-2-phenylindole) staining were used to verify the presence of remaining DNA. In addition to that, immunohistochemical staining was performed with anti-smooth muscle actin (SMA) to spot residual smooth muscle fibers. In order to verify the immune response, the matrices were implanted in rabbit's urinary bladder. One matrix of each protocol were implanted under the detrusor muscle of different sides of the same bladder. The animal was killed four weeks after the implant. Hhistological analysis such as H&E, DAPI, Masson's trichrome staining and immunohistochemistry for anti-SMA were made on the urinary bladder.

Results

The resulting matrixes were similar for both decellularization protocols as shown in figure 1. The histology of them showed no nuclei remaining on the matrix (fig. 2f, 2i), however the immunohistochemistry indicated residue of cell components (fig. 2e, 2h). The analysis of the immune response suggest that both matrices trigger inflammatory reaction, however the one derived from protocol B showed less reaction (fig.3).

Fig. 1 – Resulting matrix of the decellularization protocol.

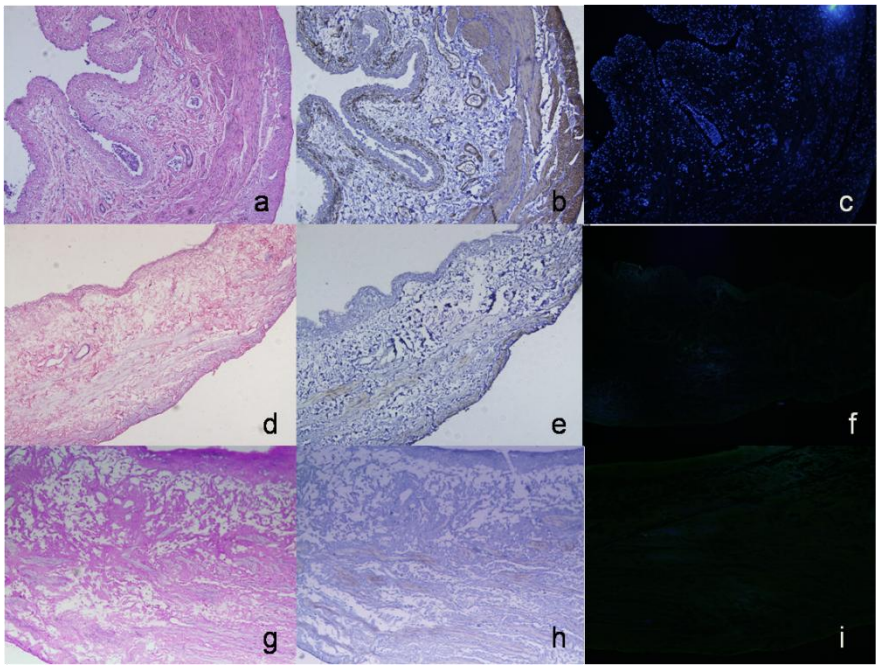

Fig. 2 – Histology: control (a,b,c); protocol A (d,e,f); protocol B (g,h,i). H&E (a,d,g). immunohistochemistry (b,e,h). DAPI (c,f,i). (magnification: 100x)

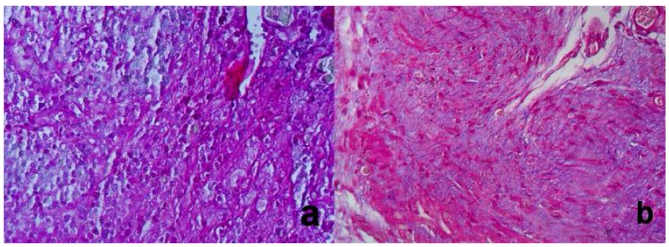

Fig. 3 – Histology: Masson's trichrome. Protocol A (a); protocol B (b). (magnification:400x)

Interpretation of results

When comparing both protocols, it can be noticed a tenuous difference concerning the amount of cell components left. Protocol B seems to remove more cell components than protocol A, which can explain the more accentuated immune response regarding the matrix resultant from the first protocol.

Concluding message

Since both protocols could not remove entirely the cell components, it is still necessary to improve them by adding reagents like detergents and some reagents that can protect the matrix structure.

Specify source of funding or grant	**FAPESP**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Research Ethics Committee of Universidade Federal de São Paulo**