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DEVELOPMENT OF A SIMPLE, LOW COST, BIOCOMPATIBLE ACELLULAR MATRIX TO BLADDER RECONSTRUCTION.

Hypothesis / aims of study

There are several diseases that can affect the urogenital system leading to lower urinary tract dysfunction. Usually, cystoplasty is performed but there are several further complications related to this procedure. Therefore there is a necessity to develop alternatives such as biological and synthetic scaffolds. Biological scaffolds have characteristics that could be more appropriated for tissues and organs repair. In the present study, we developed a bladder decellularization protocol to produce a biocompatible non-immunogenic acellular matrix derived from urinary bladder.

Study design, materials and methods

Bladders were surgically removed from Wistar rats aged two to three months. The bladders were extensively washed with phosphate buffered saline (PBS) before being subjected to two decellularization protocols (R1 or R2).

In protocol R1 bladders were washed in PBS containing 10Kiu/mg aprotinin and 0,1% w/v EDTA (ethylenediaminetetraacetic acid) for 3 periods of 30 minutes with agitation. Then it was incubated with hypotonic buffer (10mM Tris, pH 8.0) containing aprotinin and EDTA for 20 hours. It was washed in PBS before it was treated with 0.1% (w/v) sodium dodecyl sulphate (SDS) in hypotonic buffer for 20 hours. The tissue was then washed in PBS and treated with a Tris buffered saline (at 37ºC) containing 50U/ml of DNase and 1U/ml of Rnase for 4 hours.

In protocol R2 the bladder was initially washed in PBS containing 10Kiu/mg aprotinin for 30min, then incubated in deionized water containing 1% (w/v) SDS for 20 hours at room temperature with agitation. This was followed by 2 periods of 15 minutes treatment of deionized water. The piece was then treated with 1% Triton-X100 in deionized water for 60 minutes.

Matrixes were paraffin embedded, prepared in 5 µm slides and evaluated by H&E, immunohistochemistry for desmin and smooth muscle actin (SMA) - to evaluate residual smooth muscle fibers (proteins) and DAPI (4',6-diamidino-2-phenylindole) to verify remaining DNA.

To determine the potential cytotoxic effect of the acellular matrix, conditioned media was prepared by adding minced acellular matrix to DMEM (Dulbecco`s modification of Eagle`s medium). Muscle derived stem cells (MDSC) growth rates were compared among cultures using conditioned media with R1 or R2 and control cultures using only DMEM as culture media.

To verify the acellular matrix immune response, four acellular matrix including: 1- R1 protocol, 2- R2 protocol, 3- synthetic scaffold of PGA (polyglycolic acid) and 4- bioscaffold SIS® were implanted in the back of a healthy male rat. The aforementioned scaffolds were harvested at 4 and 8 weeks after implanted and H&E stained for histological analysis.

Results

It was found no remaining nuclei in the scaffolds originated from either R1 and R2 protocols (fig. 1). The immunohistochemistry for SMA and desmin showed no muscle cells components remaining (fig. 1).

The cells cultivated with conditioned media derived from scaffolds of the protocols R1 or R2 showed similar growth rate as the cells in control culture without a significant difference (p=0,123) (fig. 2).

Regarding the immunological response tests, all four different implants were macroscopically visible after 4 weeks. However, after 8 weeks, only SIS implants were evident. The scaffolds derived from protocols R1 and R2 triggered similar inflammatory responses constituted by a mild lymphocytic infiltrate (fig. 3A and 3B) if compared to SIS (fig. 3C), which initiated the most intense response among all 4 implants. These responses were different from what was observed for PGA, which activated a foreign body reaction with multinucleated giant cells (fig. 3D).

Interpretation of results

The histological analysis and immunohistochemical assay show that both protocols R1 and R2 were efficient to remove cells. The R2 protocol is simpler and cheaper than R1 and offered a similar final scaffold. The immune response to the bioscaffolds derived from decellularization protocols R1 and R2 is milder than the commercial bioscaffold SIS or PGA scaffold.

Concluding message

We described the process to create a simple low cost extracellular matrix which is an option to bladder reconstruction with minimum inflammatory response, without cellular toxicity and host cellular compatibility. These findings provide a promising future for further studies with bladder acellular matrix.

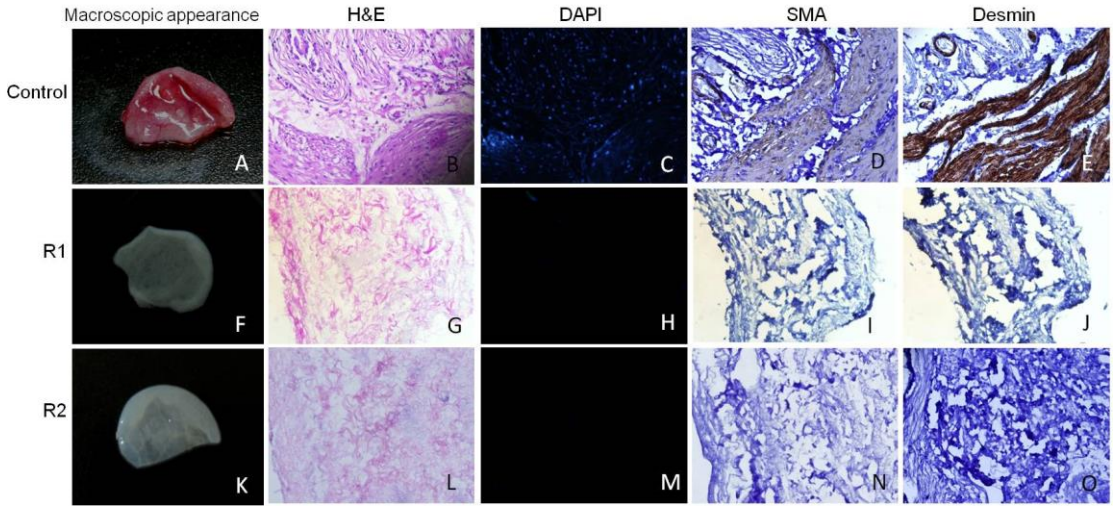

Fig. 1 – Macroscopic appearance and histological images of rat´s decellularized and control urinary bladder. Control bladder: A, B, C, D, E. Protocol R1: F, G, H, I, J. Protocol R2: K, L, M, N, O. H&E: B, G, L. DAPI: C, H, M (blue coloration indicates cells). Immunohistochemistry for SMA: D, I, N; and for desmin: E, J, O (brown coloration indicates positiveness). (Magnification of histological images: 400x)

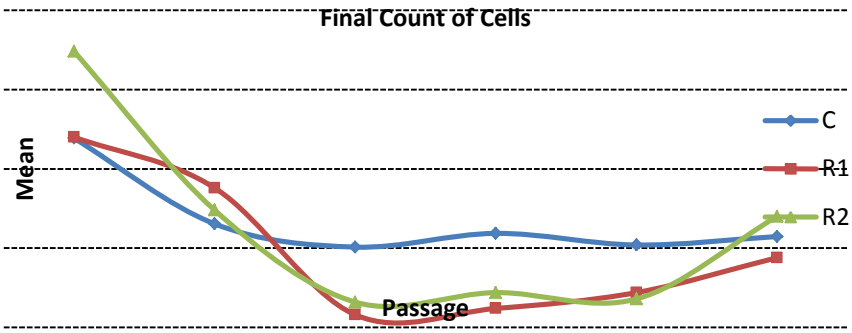

Fig. 2 – Grafic of the final number of cells at each passage for the MDSC cultures in conditioned media R1, R2 and MDSC control culture.

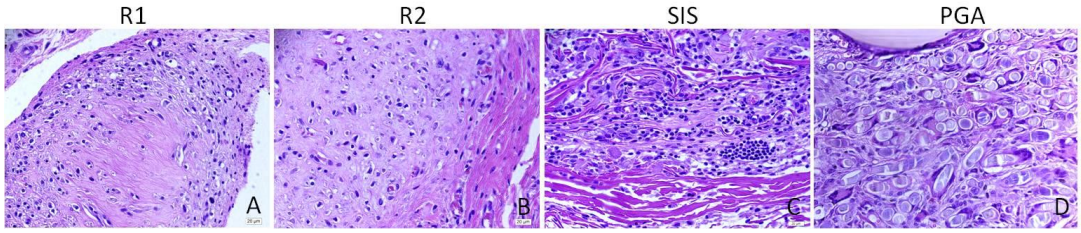

Fig. 3 - Histology (H&E) of implants after 4 weeks. Protocol R1:A, Protocol R2: B, SIS:C, PGA: D (Magnification:400x)

Disclosures
Funding: FAPESP (2009/12201-5) **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** COMITÊ DE ÉTICA EM PESQUISA da Universidade Federal de São Paulo (UNIFESP)/Hospital São Paulo