473

Corcos J[1], Loutochin O[1], Mahfouz W[1], L. Coutu D[2], Galipeau J[2]
1. *Department of Urology, Jewish General Hospital, McGill University, Montreal, Quebec, Canada,* ***2.*** *Lady Davis Institute for medical research, Jewish General Hospital,McGill, Montreal, Quebec*

COMPARATIVE DECELLULARIZATION PROTOCOLS OF RAT, RABBIT AND PORCINE BLADDERS FOR MESENCHYMAL STEM CELL-BASED TISSUE ENGINEERING

Hypothesis / aims of study
A variety of congenital and acquired conditions result in compromised bladder capacity. Intestine is extensively used as a substitute, but it has several deleterious complications. Therefore, artificial bladder tissue is a constant need in reconstructive surgery.
Our objective is to reach an optimized decellularization protocol. These decellularized patches could be seeded with autologous bone marrow mesenchymal stem cells (MSCs) for bladder replacement/augmentation by tissue engineering. We also report the adherence and integration of MSCs on decellularized scaffolds in vivo rat model.

Study design, materials and methods
This study compared four decellularization protocols for bladders of three species: rat, rabbit and porcine. This was done using different detergents (1% SDS or 1% TritonX in hypotonic Tris-HCl) to identify which was more efficient at removing cytoplasmic debris while preserving the structural anatomy. This was followed by the use of deoxyribonuclease (DNase) for digestion of nuclear debris. Analysis of the decellularized extracellular matrices for evidence of preserved matrix proteins was preformed by conventional histology, immuno-fluorescence staining and confocal microscopy. We then tested the ability of MSCs to adhere to the decellularized scaffolds both in vitro and in vivo animal rat model.

Protocol A	**Protocol B**	**Protocol C**	**Protocol D**
PBS (3 days)	PBS (3 days)	PBS (3 days)	PBS (3 days)
1% SDS in Tris-HCl(3 days)	1% Triton-X in Tris –HCl (6 days)	1% SDS in Tris-HCl(6 days)	1% Triton-X in Tris –HCl (6 days)
PBS (3 days)	PBS (3 days)	PBS (5 days)	PBS (5 days)
DNAse (1 day)	DNAse (1 day)	DNAse (1 day)	DNAse (1 day)
PBS (5 days)	PBS (5 days)	PBS (5 days)	PBS (5 days)

Results
Both detergents were equally efficient at removing cytoplasmic debris. The duration of detergent treatment proved to be critical here. Triton X appears to preserve the extracellular matrix better. DNase digestion was always necessary for complete removal of nuclear debris. MSCs adhered well on the scaffolds.

Interpretation of results
The use of Triton X followed by DNAse is efficient for production of decellularized bladder tissue. This method preserves the structural anatomy, extracellular matrix proteins, and growth factors within bladder tissue. Moreover, MSCs adhere well on these scaffolds without rejection.

Concluding message
1. An optimized protocol for decellularization can be applied to bladders of various species (Protocol D).
2. Detergents and DNase treatments are required for efficient decellularization
3. Extracellular matrix (ECM) integrity is preserved using optimized protocol.
4. MSCs adhere to and colonize decellularized bladder tissue.
5. MSC-seeded decellularized xenogenic bladders are not rejected upon transplantation and integrate with host bladder tissue.

Specify source of funding or grant	**None**
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	**McGill University Animal Care Committee**