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Comparison of clinical & research decellularization protocols

Julie Manon[1, 2, 3], Louis Maistriaux[1], Lies Fievé[1], Delphine Magnin[4], Robin Evrard[2, 3], Thomas Schubert[2, 3], Benoît Lengelé[1], Catherine Behets[1], Olivier Cornu[2, 3]

[1]IREC, UCLouvain, Morphology lab (MORF), Brussels, Belgium, [2]IREC, UCLouvain,Neuromusculoskeletal Lab (NMSK), Brussels, Belgium, [3]Cliniques universitaires Saint-Luc, Centre de Thérapie Cellulaire et Tissulaire locomoteur, Brussels, Belgium, [4]Institute of Condensed Matter and Nanosciences – Bio & Soft Matter (IMCN/BSMA), Louvain-la-Neuve, Belgium

INTRODUCTION

Whereas tissue engineering research has hugely grown up, few decellularization methods are used to decrease cell immunogenicity[1, 2]. However, differences between treatments have not yet been evaluated.

METHODS

Analysis of human periosteum (HP) and fascia lata (HFL) (n=4) allowed to confront 4 research procotols (with soap) with a clinical one (without soap). Cellular clearance, extracellular matrix (ECM) preservation and immunogenicity were examined by histology, immunohistochemistry (IHC) for type 1 collagen and MHC-1, DAPI as well as matrix components quantifications. The 3D ECM ultrastructure was visualized by scanning electron microscopy (SEM). Finally, acellular patches (n=3, for each protocol) were sterilized by γ-irradiation (25 kGy) and seeded with $5x10^5$ human fibroblasts to highlight matrix cytotoxicity and cellular viability.

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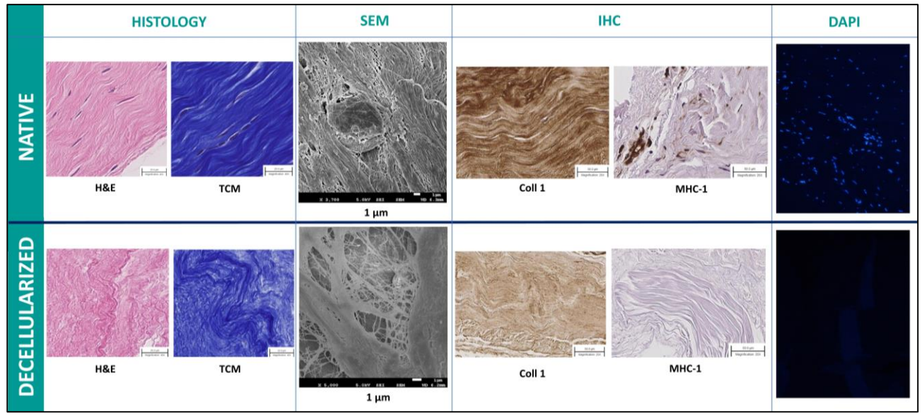
CONTACT

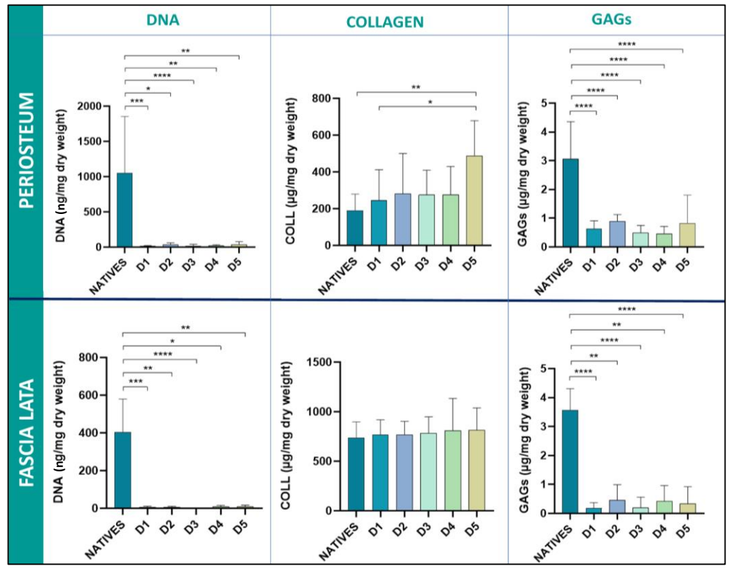
Julie MANON, MD, PhD Student
Email : julie.manon@uclouvain.be

RESULTS

DECELLULARIZATION



Histology and IHC brought out similarities (type I collagen fibers, layer organization) and differences (density and compaction of fibers) between native and decellularized tissues, as confirmed by the SEM. For all 5 protocols, histology and DAPI showed a successful decellularization with a "no man's land" concerning nuclei and a disappearance of immunogenicity derived from membrane components.

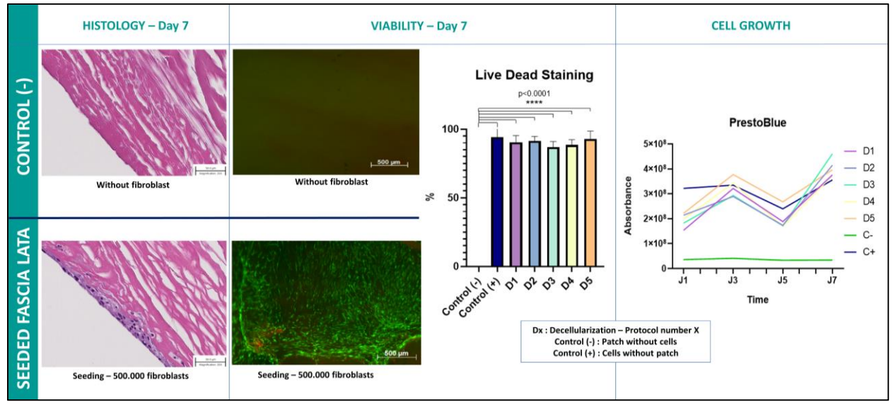


DNA mean content always fell below the critical threshold (50ng/mg dry weight) without statistical difference between protocols. Only for HP tissue, collagen content relatively increased after decellularization with a statistical difference only for the 5th protocol (190.8 vs 486.2 µg/mg dry weight, p=0.0004). GAGs amount decreased statistically after decellularization no matter the protocol or tissue (p<0.0001).

SCAN ME

RECELLULARIZATION

For both tissues, the PrestoBlue test revealed an increase in the amount of living cells after 7 days in all patches in the same way as the positive controls. By the way, the cellular proliferation slope was better for cells seeded on treated patches than those in empty wells.



CONCLUSION

All protocols successfully decellularized both collagen membranes and offered biocompatible scaffolds with some slight micromorphological modifications. The relative collagen rise can be explained by the cellular compartment disappearance allowing an increase in the percentage of space occupied by the collagen fibers compared to the whole tissue dry weight. Both HP and HFL ECM appeared as a suitable environment to allow cellular growth. None of these analyses could underscore the superiority of one specific protocol although the clinical one stands out from the others because of its virus and prions inactivation permitting safe clinical applications.

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