Biological and mechanical characterization of a decellularized porcine esophageal biological matrix

Romane Lesieur, Cécile Monfoulet, Samantha Roques, Martine Renard, Agnès Drochon, Denis Collet, Laurence Bordenave, Marlène Durand

Biological and mechanical characterization of a decellularized porcine esophageal biological matrix

Romane LESIEUR[1,2], Cécile MONFOULET[2], Samantha ROQUES[2], Martine RENARD[2], Agnès DROCHON[3], Denis COLLET[4], Laurence BORDENAVE[1,2,5,] Marlène DURAND[1,2]

[1]BIOTIS U1026 INSERM, Université de Bordeaux, France
[2]CIC1401 INSERM, CHU de Bordeaux, France
[3]Institut de mécanique et d'ingénierie, UMR CNRS 5295, Campus Talence, France
[4]Service de Chirurgie Digestive, CHU de Bordeaux, France
[5]Service de Médecine Nucléaire, CHU de Bordeaux, France
Mail: romane.lesieur.stag@chu-bordeaux.fr

INTRODUCTION

The restoration of digestive continuity after the ablation of a portion of the esophagus is currently ensured by the interposition of a colon segment or by the tubulation of the stomach. The development of a tissue-engineered esophageal substitute is part of the improvement of the surgical treatment of esophageal pathologies. Previous studies[1,2] have focused on the development of a decellularized porcine biological matrix, using a chemical and enzymatic detergent perfusion method[3]. These protocols have been upgraded to ensure sterility and thus avoiding deleterious Gamma rays irradiation. The objective of this study is to characterize both the biological and mechanical properties of this decellularized matrix (DM).

EXPERIMENTAL METHODS

DM design and decellularization process

Esophagi were collected from pigs of 30 kg to 50 kg at the IHU Liryc of Pessac. The design of a DM is done according to 3 steps: a decontamination process with antibiotics/antimycotics solutions, a decellularization process with the successive perfusion of chemical and enzymatic solutions, and a detoxification process using a resin that absorbed chemical residues from the decontamination and decellularization processes.

The decellularization process was performed by perfusing the organs with a controlled flow and rotation in the SYNTHECON system. The flow characterization of the perfusion/rotation system confirms its use in our application. The detersions solutions ensured the removal of the cells and their contents. These phases were separated by rinsing phases.

Biological characterization

Histological analysis

Samples of DM (n=20) were embedded in paraffin, cross-sectioned and stained with hematoxylin eosin saffron (HES). The efficacy of the decellularization process and the histological structure were then analysed.

Cytotoxicity analysis

BALB/3 T3 cells were seeded in direct contact with the DM (n=6) following ISO 10993-5 standard. Viable cells were counted after neutral red staining.

Mass spectrometry analysis

The identification of the proteins contained in the MD is performed by nanoLC-ESI-MS/MS Fusion Lumos coupling, at the Functional Genomics Center of Bordeaux. The DM were chemically treated in order to be analyzed (n=3). Data are collected via Proteome Discoverer 2.4 / PEAKS and compared according to an adapted database.

Mechanical characterization

Longitudinal traction

Native esophagus patches are prepared (n=10) as well as DM patches (n=30). The samples were clamped in jaws of a tensile testing machine and pulled at a constant speed until rupture.

Suturability

Native esophagus patches are prepared (n=10) as well as DM patches (n=10). The suture thread and the samples are maintained in clamps that are extended with increasing tensile force until rupture is reached.

Burst pressure

The burst pressure test was performed on the Bose Biodynamic Test©. Increasing endoluminal pressures are applied to the DM until burst (n=7).

RESULTS AND DISCUSSION

A total of 40 porcine esophagi were decellularized using our in-house upgraded method. No remaining cells were observed within the stained cross-sections of the matrix confirming the efficiency of the decellularization process. No delamination of the tissue layers was observed showing no deleterious effect of this process. More than 70% of cells were viable when in contact with the DM showing the preservative effect of the detoxification step. The main collagens and glycoproteins were identified showing the complex and rich composition of the DM. Mechanical tests demonstrated that the DM is compatible with an implantation as an esophageal substitute.

CONCLUSION

We characterized biologically and mechanically a DM, with which we can expect to improve clinical outcomes after implantation in comparison to Luc *et al.* and Arakelian *et al.*

REFERENCES

1. Luc G. *et al.* Biomaterials. 175:1-18, 2018.
2. Arakelian L. *et al.* Tissue Engineering and Regenerative Medicine. 13:2191-2203, 2019.
3. Zambon P.J. *et al.* Methods. 171:3-10, 2020.