Sterilization and storage issues in the development of a biomaterial for esophagus substitution

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Introduction

Each year in Europe, thousands of patients undergo an esophagectomy for tumor resection or trauma treatment. Frequent occurrence of severe postoperative complications demonstrate that surgical management with surrounding tissues is not satisfactory (Chirica et al. 2010 and Poghosyan et al. 2011). Surgeons have been appealing for a substitute for decades and the specifications of their ideal biomaterial are particularly demanding: **biocompatible, with a tubular shape and appropriate mechanical properties, cell friendly, remodellable, easy to handle, suturable, sterile and off-the-shelf.** Above all, it must heal avoiding the surgeons' nightmare: fistulae and stenosis. After extensive review of the literature (Luc et al. 2014), the team of Bordeaux University Hospital chose developing an **Extra Cellular Matrix (ECM) based biological biomaterial** fitting most of these specifications (Fig. 1). They anticipated the need for a proof of concept in an animal model by producing a porcine-based decellularized matrix (DM) (Luc et al. 2018). The last issues remaining are avoiding destructive sterilization process and selecting **the best storage conditions to guarantee the preservation of the mechanical and biological properties of this off-the-shelf biomaterial.**

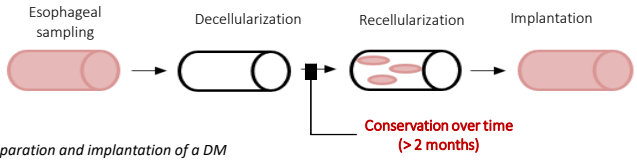


Fig. 1: Preparation and implantation of a DM

Objectives

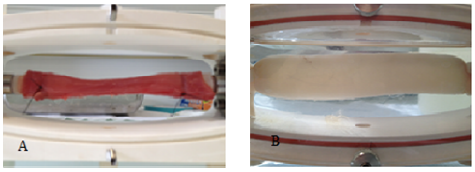
To develop **optimum storage conditions** over time and to assess their impact after a short duration (7 days) on mechanical and biological properties of the DM.

Studied storage conditions: 1. PBS+1% PSA 2. Lyophilization 3. Cryopreservation

Materials & Methods

Decellularization

Eight porcine esophagus were decellularized (Luc et al. 2018; Marzaro et al. 2006).



(A) Native esophagus (NE) on the bench *(B) DM after decellularization*

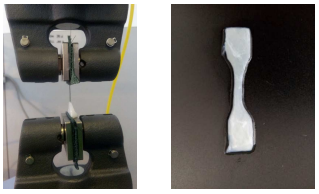
Storage conditions

Different conditions of storage were tested:
- in PBS buffer + 1% antibiotics at 4°C (pDM, n=6),
- lyophilisation during 14 hours (lyoDM, n=4),
- cryopreservation in DMEM f:12 with 10% DMSO (cpDM, n=3) (Urbani et al. 2017).

Characterization

Mechanical tests

After 7 days, the mechanical properties of the DM were measured:
- Suturability tests: study of the force required to pull out a suture located 2mm from the edge of the sample.
- Longitudinal tensile tests on a flat specimen (7.5x38.63mm): the maximal strength is measured.



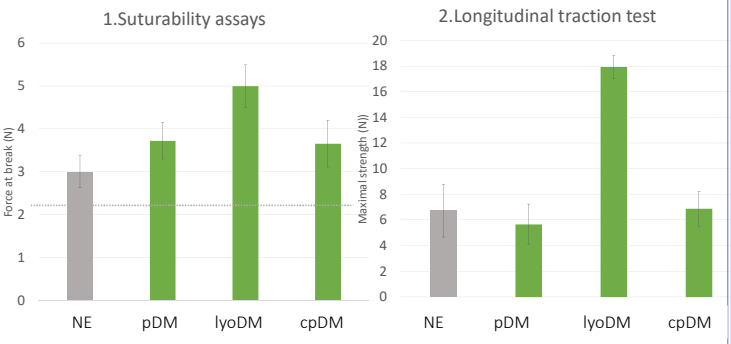
(A) Suturability assay *(B) Sample item for traction tests*

Study of the organization of proteins in the matrix

After 7 days, histological (Hematoxyline Eosine Safron (HES) staining) and immuno-histo-chemistry analyses (with laminin, elastin and fibronectin antibodies) were performed to assess the conservation of the esophagus structure. Elastin is a protein essential for the mechanical strength of the esophagus.

We observed the organization of the proteins on the surface of the submucosa, the most elastic layer found in MDs, with the Scanning Electronic Microscopy (SEM) technique. These samples were compared to native esophagus.

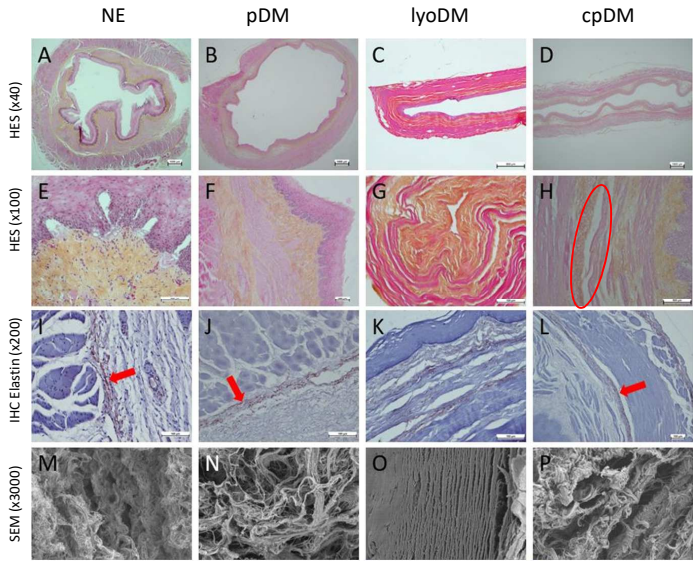
Results



NE (n= 6), pDM (n=6), lyoDM (n=4), cpDM (n=3) 4 assays per DM

NE (n=3), pDM (n=4), lyoDM (n=2), cpDM (n=3) 4 assays per DM

Under all storage conditions, we obtain suturability at least as good as for native esophagi (1). However, in tension, the lyoMD have a rigidity 3 times higher than the native ones ($p<0.05$) (2).



Histological observation with HES (A-H) staining, with IHC labeling of elastin (I-L) and observation of submucosal proteins by SEM (M-P) of native esophagus, pDM, lyoDM and cpDM. Elastin is marked with a red arrow and gaps in the matrix with a red oval.

Tissue structure and cohesion of the DM were similar to the native tissue (NE) for any kind of treatment except for the lyophilisation, lyoDM showing crushing of the layers that makes them indistinguishable even though it had been hydrated 24 hours in PBS buffer before the embedding.

The surface of the lyoDM observed with SEM was an homogeneous and smooth structure on the contrary to the DM obtained with other treatments. The SEM observations clearly explain the results of the mechanical tests. LyoDM resists a higher tensile force than other DM due to the agglomeration of elastic fibers. However, the elasticity of the structure is severely affected. pDM and cpDM have a tensile strength comparable to that of NE. Circumferential mechanical tests would have been necessary to verify if the DMs retain their elasticity. Indeed, an elasticity or a resistance in circumferential too low could make the DM unusable for a clinical application. However, results obtained in previous studies suggest that the treatments affect the mechanical characteristics in circumferential and in tension in the same way (Luc et al., 2018).

Conclusion

These preliminary data confirm that **the storage conditions of a biological matrix are critical for the preservation of its tissue integrity and mechanical properties.** Even though lyophilisation is currently used for other biological biomaterials, it does not seem to be adapted in our conditions. We will have to confirm our results for a longer time. Providing an off-the-shell biomaterial, we also have to consider ensuring the sterility and virus inactivation of the porcine-based ECM **to comply with regulatory requirements**. We are currently evaluating different conditions of sterilization compared within the same DM using recommended protocols for medical devices: gamma irradiation, ethylene oxide and supercritical CO_2.

Acknowledgements:

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