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Preparation and characterization of a biologic scaffold for esophageal tissue engineering

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Abstract – The aim of this work is to obtain a suitable matrix for tissue engineering of the esophagus. Swine esophagi are decellularized following a new dynamic process. In vitro biocompatibility is evaluated and histological analyses and circumferential and longitudinal traction tests are performed. The results are encouraging, showing good decellularization and maintaining tissue structure, no toxicity of the final tubular matrix and mechanical properties compatible with the specifications of an esophagus substitute.

I. INTRODUCTION

Biologic scaffolds composed of extracellular matrix (ECM) are commonly used to facilitate a constructive remodelling response in different types of tissue, including esophagus [1]. Surgical procedure of the esophagus is often complicated by infectious complications and stricture in long-term [2]. Preclinical studies have shown that the use of an ECM scaffold can mitigate stricture and promote a constructive outcome after resection of full circumference esophagus. The objective of the present study was to prepare a decellularized porcine esophagus and characterize it with biological and biomechanical tests.

II. MATERIALS AND METHODS

Esophagi were harvested from (weight 30-40 kg) swine. All esophagi were then subjected to a series of treatment with dynamic conditions as follows: sodium azid for 16 hours at room temperature, 4% deoxycholate for 24 hours, 100 U / ml DNase for 12 hours. In-vitro cytotoxicity using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test on L929 cells was performed on the final rinced water. All ECM were sterilized with Gamma irradiation at 25kGys. DNA content in ECM was assessed using spectrophotometry (n = 14). Native esophagus and ECM were stained with H&E and Masson trichrome, and immunolabeling was performed, for a qualitative histomorphologic analysis that evaluated cell infiltration, connective tissue and ECM structure. Sulfate glycosaminoglycan (GAG) in ECM samples was determined by histology analysis with Blue Alcian coloration. Scanning electron micrographs were taken to examine the surface topology of ECM (at 1000x and 2000x magnification). Biomechanical testing was performed in longitudinal (n=10) and circumferential direction (n=10), and burst pressure (n=4) for both native esophagus and the ECM.

III. RESULTS

III.1 Biological characterization

The concentration of remnant DNA in ECM samples (247 ± 125 ng / mg) was less (p = 0.001) than that in native esophageal tissue (854 ± 580 ng / mg). H&E and Masson trichrome (Fig. 1) and immunolabeling showed no intact nuclei but remaining structure (collagen IV and laminin, fibronectine). GAGs were visible by Alcian blue staining in the native esophagus and disappeared in ECM samples. SEM images of the luminal and external surface of ECM showed a smooth surface on the luminal area of the ECM. The external surface, however, had a more textured and fibrous structure. After the assessment of non-attendance of bacteriological contamination in the final rinsed water, no cytotoxicity with the colorimetric MTT assay was observed.

III.1 Mechanical characterization

The biaxial stress response of the native esophagus showed anisotropic behavior with a maximum strain of 107±16 % and 87±23 % in the circumferential and longitudinal direction, respectively. The ECM showed similar anisotropy, with a maximum strain of 554±140 % and 50±5 % in the circumferential and longitudinal direction, respectively. Statistical differences were observed between native esophagus and ECM regarding maximum stress in kPa ($p<0.05$), tensile modulus in kPa ($p<0.001$), maximal strain in % ($p<0.001$) and maximal strength in N for longitudinal direction ($p<0.05$), but only for maximal strain in % ($p<0.01$) and tensile modulus in kPa ($p<0.001$) for circumferential direction. Maximal pressure obtained for the burst pressure test was similar in both groups but burst never occurred for ECM.

IV. DISCUSSION-CONCLUSION

Porcine esophagus was effectively decellularized with the use of a detergent-based protocol. The ECM scaffold maintained structural protein and an ultrastructure consistency. Since our ECM reached the specifications for an esophagus substitute with acceptable mechanical properties in circumferential direction, esophageal replacement in swine has been performed.

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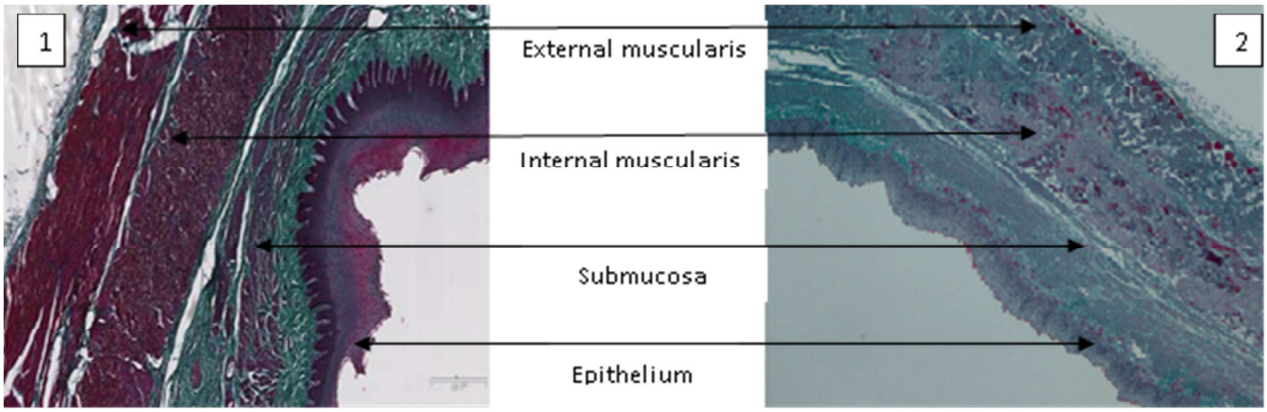

Fig. 1 Masson trichrome staining on a native esophagus (1) and on an ECM (2) x10. Scale bar --- : 100 µm