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Dry acellular oesophageal matrix prepared by supercritical carbon dioxide

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ABSTRACT

Recently, the use of acellular matrices in tissue engineering has become extremely significant as tissue substitute for organ/tissue reconstruction. In this clinical scenario, banking decellularised organs ready for transplantation would be mandatory for patients with emergency needs.

In this work a new process based on supercritical carbon dioxide ($SC\text{-}CO_2$) drying technique was investigated for obtaining a dry/preserved decellularized oesophagus. Experiments were performed coupling a detergent enzymatic treatment with two different protocols: (i) $SC\text{-}CO_2$ drying; (ii) dehydration in ethanol and a subsequent $SC\text{-}CO_2$ drying. The efficiency of the treatments was investigated by monitoring the loss of weight of the treated samples and the maintenance of the extracellular matrix (ECM) architecture, composition and mechanical properties after rehydration.

A successful dry acellular matrix was reached in a shorter time using the combined ethanol and $SC\text{-}CO_2$ treatment. Histological analysis reported the maintenance of the tissue matrix architecture and the collagen content for all the treated samples, while the preservation of ultrastructural features were confirmed by scanning electron microscopy (SEM). Tensile tests did not show significant differences in terms of fracture strength before and after the supercritical process. Furthermore, the scaffolds demonstrated good biocompatible properties in terms of cell culture viability *in vitro*. Overall, the results highlighted the potential of this novel technology to obtain a dried acellular matrix for oesophageal regeneration, preserving the extracellular matrix structure of the native tissue.

1. Introduction

Surgical oesophagus replacement may occur in many congenital [1] and acquired diseases [2]. Nowadays, the most commonly applied clinical treatment for oesophagus replacement is the substitution using the stomach (gastric pull-up), the colon (colonic interposition) or the small bowel, with high risks associated with morbidity and potential post-surgical complications [3–7]. Oesophageal tissue engineering (OTE) is an emerging alternative for the development of suitable tissue replacements for oesophageal repair. In the last decay, oesophageal engineered substitutes have been explored using several synthetic and natural derived scaffolds and materials [8]. Early studies seem to indicate that using synthetic material, such as silicone-based scaffolds lead to poor result in animal models [9,10], while naturally derived collagen scaffolds showed promising results [11,12].

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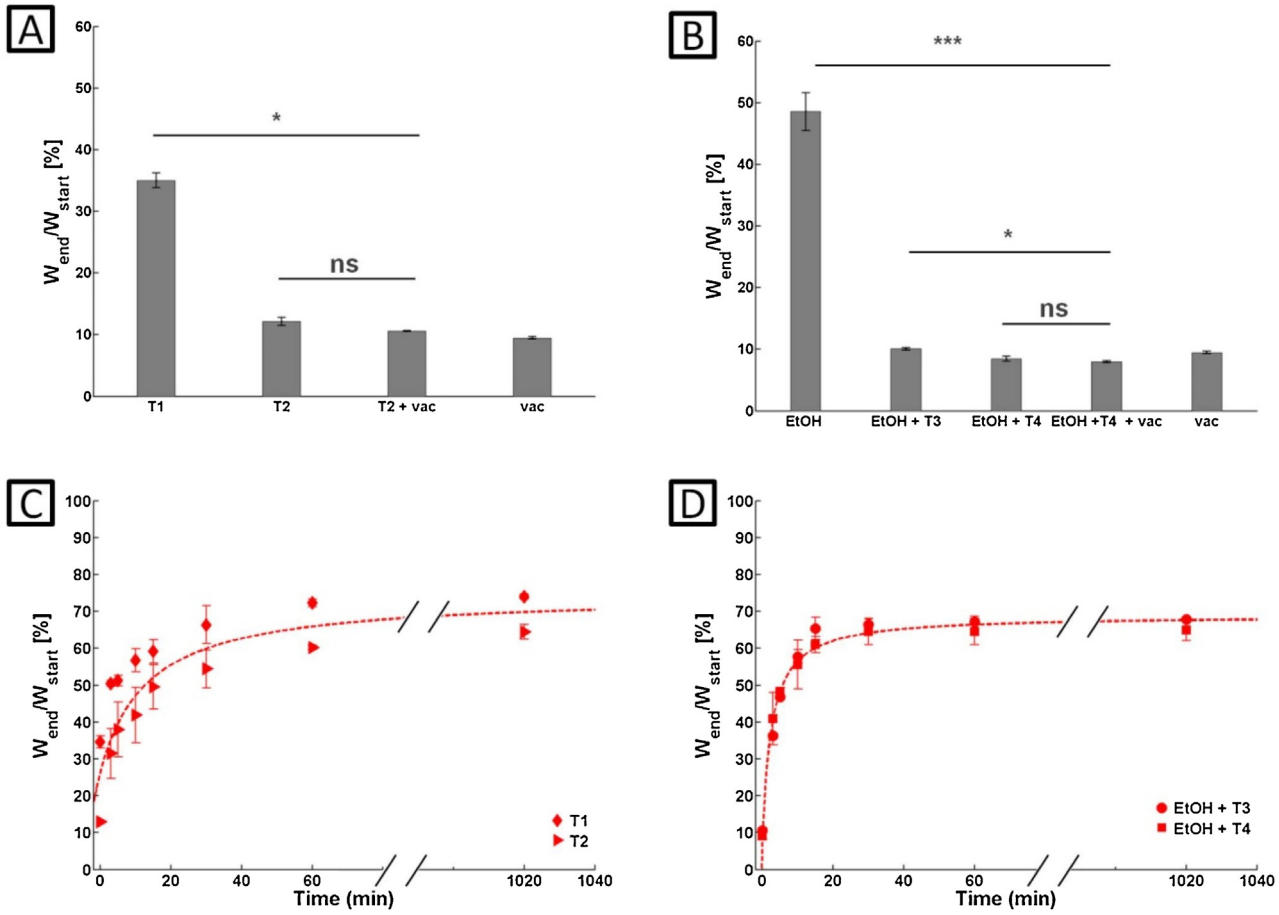

Fig. 1. (A) Loss of weight percentage of ECM after SC-CO_2 treatments (T_1 for 60 min and T_2 for 120 min), SC-CO_2 treatment for 120 min + vacuum drying for 12 h (T_2 + vacuum), and vacuum drying for 12 h as control (vacuum); (B) loss of weight percentage of ECM after EtOH dehydration (80 min), EtOH dehydration (80 min) and SC-CO_2 treatments (T_3 for 15 min and T_4 for 30 min), EtOH dehydration (80 min) + SC-CO_2 for 30 min + vacuum drying for 12 h (T_4 + vacuum), and vacuum drying for 12 h as control (vacuum); (C) rehydration kinetics of dried samples after SC-CO_2 treatment (♦, 60 min (T1); ►, 120 min (T2)); (D) and EtOH (80 min) + SC-CO_2 treatment (●, 15 min (T3); ■, 30 min (T4)).

Indeed, starting from the native tissue has already shown some value for clinical translation, and organ decellularization for tissue engineering has become more and more promising for the organ/tissue replacement with initial satisfactory clinical experience with simpler tabularised organs [37,38]. Organs that are more complex have also been engineered using decellularized materials in experimental animals such as heart [39] liver [13], kidney [14], intestine [40] and other organs/tissues [15–17]. Our group has recently demonstrated that a natural oesophageal acellular matrix can be also obtained directly from pig oesophagus, maintaining the ultrastructure and composition of the native extracellular matrix (ECM) [18]. These scaffolds could represent a valid therapeutic solution for the replacement of damaged organ, overcoming rejection and immunosuppression complication. Typically, natural acellular matrices from animal tissues are obtained through different chemical and enzymatic treatments [15–18]. Despite the safety of the procedure, detergent residua might be presented at the end of the process causing high toxicity and therefore limiting their use at clinical level [19]. Moreover, most of those processes are conducted with freshly isolated tissue that is promptly transplanted after the decellularisation/recellularization process. However, this limits the potential use in the clinical scenario where an *ad hoc* preparation may not always be possible, and banking decellularised organs ready for transplantation may offer advantage for patients with emergency needs. To date limited knowledge is available about the storage and preservation of these decellularized tissues. Regarding the preservation of the decellularized trachea, there is evidence that the maintenance of the decellularized scaffold is limited to few weeks after their production, requiring storage solutions and controlled temperatures [20]. Nowadays no efficient and inexpensive protocol is available for long-term maintenance of natural decellularized scaffolds. It is known that sterilization processes can significantly affect both the structure and the residual protein content [21] thus, there is a need for new conservation methods.

The drying process has a great potential as preserving option and it has already been largely used for the food products [22]. It is widely known that the residual water, or in other terms the water activity, is mostly in charge of microbial development and quality decay, while its removal ensures long term conservation.

A recent drying technique for long-term preservation in the food processing has been proposed by Brown et al. [23]. They applied SC-CO_2 and EtOH + SC-CO_2 drying treatment to carrots, demonstrating that samples displayed more favourable rehydrated textural properties than air-dried equivalents, with no substantial change of product microstructure.

In the same way, SC-CO_2 drying treatment could allow long-life to natural acellular scaffolds, acting like a storage treatment and avoiding their rapid degradation.

Advancements in the production and long-term preservation technology of a natural acellular matrix could be achieved by using supercritical carbon dioxide (SC-CO_2) drying process which has been already investigated for food [23] with promising applications as maintenance process. SC-CO_2 has already shown interesting application for biomaterials on the preparation of 3D porous scaf-