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A low-cost 3D-printed sample-holder for stirring-based decellularization of biological tissues

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Abstract—An innovative, low-cost, 3D-printed sample-holder is proposed for reproducible and effective stirring-based decellularization of biological tissues. The sample-holder was designed to be low-cost, easy to use with conventional laboratory equipment, and manufacturable through 3D printing. During stirring-based decellularization, the sample holder exposes the samples to convective flow, enhancing the reagent transport while protecting the samples from disruptive forces. Computational fluid dynamics analyses were carried out to elucidate the developing hydrodynamics. Explanatory tests, performed on human cardiac tissue samples, demonstrated the effectiveness of the presented device.

Keywords – Stirring-based decellularization, biological tissues, 3D printing, computational fluid dynamics.

I. INTRODUCTION

DUE to their biochemical cues and three-dimensional (3D) microarchitecture, decellularized tissues represent promising biological scaffolds to support tissue regeneration [1]. Decellularization processes aim to remove the cellular content while preserving the native 3D extracellular matrix (ECM), and are commonly based on chemical reagents combined with dynamic methods, such as stirring in a beaker or perfusion [2]. Compared to static approach, these methods exploit convective flow for enhancing reagent transport through the samples, assuring faster and more effective processes [3]. However, in case of soft tissues, the stirring can lead to the entrapment of the samples around the magnetic bar, with consequent sample damage. To protect the samples, embedding cassettes could be used, nevertheless, the random suspension of the cassettes within the beaker can cause irregular and uncontrolled solution transport through the samples, leading to low process reproducibility. On the other hand, perfusion needs native vasculature, not always available, and complex, expensive pumping and control systems [4]. Here we propose a novel, low-cost, 3D-printed sample-holder (patent pending) to overcome the limitations of the stirring-based decellularization of biological soft tissues. The sample-holder, inserted in a beaker placed on a magnetic stirrer and containing the reagent solution, maintains up to four samples, enclosed in embedding cassettes, immersed in a steady position that prevents random floating. Equipped with a cross-shaped magnetic bar, the sample holder rotates and enables to homogeneously expose the samples to convective fluid while protecting them from disruptive forces generating within the

beaker, eventually reducing process duration and increasing reproducibility and efficiency.

II. METHODS

The design of the sample-holder was guided by the need to satisfy specific requirements in terms of Good Laboratory Practices (GLP), ease of use with conventional laboratory equipment, performance, and 3D-printing. The design was performed with a computer aided design software (Solidworks). The manufacturing was carried out with a 3D printer with double nozzle (Stratasys uPrint SE Plus), based on fused deposition modeling (FDM) technology combined with ABS and a soluble support material. The sample-holder is composed by a cross-shaped base, with a central square shaft, and four rectangular frames, designed to be bi-directionally hookable to the shaft for cyclically exposing both sides of the samples to the same flow fields. The modular structure permits to manage each sample independently. For stirring-based decellularization, the samples are loaded in commercial embedding cassettes stuck within the frames, and the sample-holder base is equipped with a cross-shaped magnetic bar at the bottom (Fig. 1). Once assembled, the sample-holder is immersed within a beaker placed on a magnetic stirrer and filled with reagent solution. The stirring velocity is then gradually increased, inducing the rotation of the sample-holder. Multiphase computational fluid dynamics (CFD) simulations were performed to model the hydrodynamics developing during the process. A finite volume method (Ansys Fluent) and the standard $k-\epsilon$ turbulence model were applied to a dynamic mesh to model the sample-holder rotation, simulated at 50, 100 and 150 rpm. Samples were modelled as porous media, governed by the Ergun equation [5]. A bidimensional section was considered for computational convenience. Explanatory tests were carried out on human cardiac tissue samples, adapting a previously developed decellularization protocol [6]. Native and decellularized samples were fixed and processed for Hematoxylin and Eosin (HE) staining or snap-frozen for DNA quantification. Patients provided written, informed consent and specimens were collected following protocols approved by the Hospital and in conformity with the principles outlined in the Declaration of Helsinki.

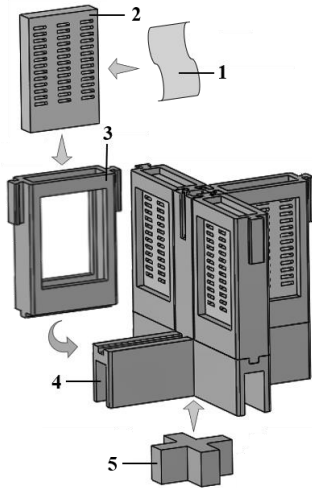


Fig. I. Components and assembling procedure of the sample-holder: 1) Sample; 2) Embedding cassette; 3) Frame; 4) Cross-shaped base with central square shaft; 5) Cross-shaped magnetic bar.

III. RESULTS AND DISCUSSION

The sample-holder components were 3D-printed separately, taking a global working time of about 12 h with an overall cost of approximately 18 €, providing design flexibility and time and cost efficiency. Ease of assembling and correct couplings between components were confirmed, despite the surface roughness characteristic of the applied FDM technology. In-house tests performed with the assembled sample-holder immersed in the beaker, filled with water and positioned on the stirrer, proved the operating performance, showing that its rotation is stable up to 150 rpm (Fig. IIA). As regards the CFD outcomes, Figures IIB and IIC show the total pressure and velocity magnitude contours in the samples and in the solution for a rotation velocity of 150 rpm, respectively. The CFD revealed that the sample-holder rotation induces a pressure drop across the samples, increasing with the radius, that promotes reagent transport, showing the significance of cyclically switching the samples to expose them to the fluid transit direction. Pressure and velocity contours obtained for the three simulated rotation velocities differed only for their absolute values. Moreover, the CFD approach enabled an *a priori* definition of the operating conditions, with time and cost advantages.

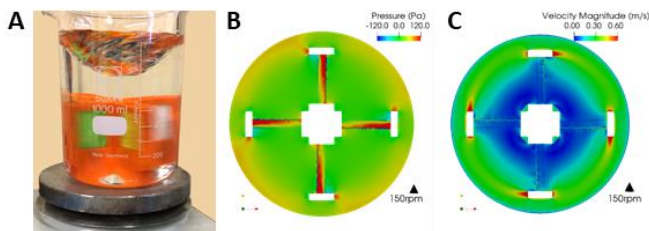


Fig. II. A) In-house test of the sample-holder. CFD results for a rotation velocity of 150 rpm counter clockwise: B) Pressure contours in the samples and in the solution that allow to appreciate the pressure drop across the samples; C) Velocity magnitude contours.

The effectiveness of the sample-holder for decellularization processes was tested in a cell culture laboratory. After 24 h of treatment, the human cardiac tissue samples exhibited the characteristic color change from brown-reddish to

translucent-white (Fig. IIIA), typical of a thorough decellularization. This qualitative result was confirmed by the quantitative assessment of residual double stranded DNA content (4.7 ± 0.71 ng/mg of tissue in decellularized samples), well below the accepted standards of 50 ng/mg of tissue [7] (Fig. IIIB). HE staining confirmed that, compared to native tissue (Fig. IIIC), treated tissue did not present any nuclei (Fig. IIID) and the 3D structure of the decellularized ECM was not affected by the process. Compared to previously performed decellularization procedures based on samples enclosed in embedding cassettes freely floating in the solution, the use of the sample-holder led to a 30-50% reduction in treatment time.

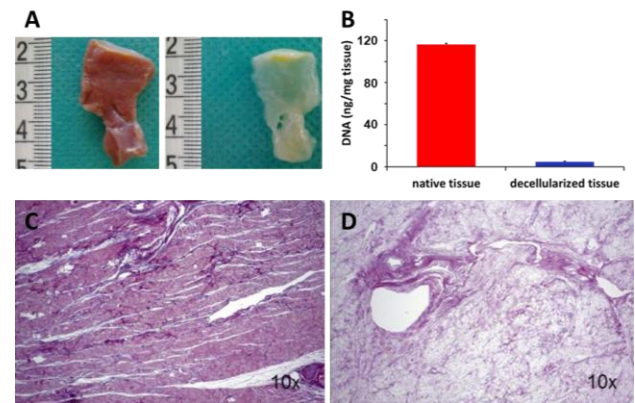


Fig. III. A) Human cardiac tissue samples before and after decellularization; B) Residual double stranded DNA content; C) HE staining of native tissue; D) HE staining of decellularized tissue.

IV. CONCLUSION

The novel 3D-printed sample-holder, proposed for stirring-based decellularization, met successfully the requirements of cost-efficiency, ease of use, and functionality. In detail, CFD investigation and biological explanatory tests demonstrated that the sample-holder promotes the transport of the reagent solution through the samples while protecting them, leading to fast, reproducible and efficient decellularization processes.

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