Bioactive Tissue Scaffolds from decellularized ascidian tunic

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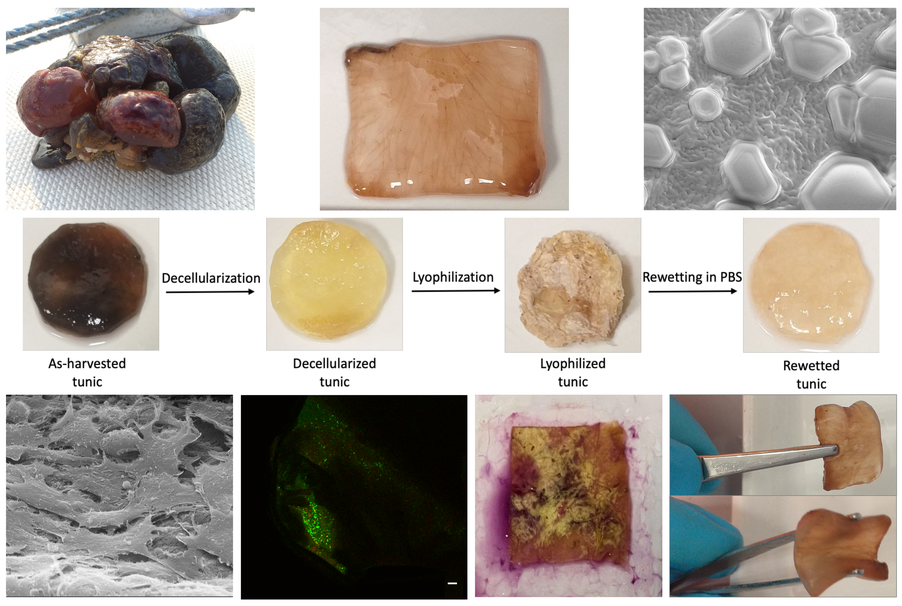
Introduction

Urochordates are the closest invertebrate relative to humans and commonly referred to as tunicates, a name ascribed to their leathery outer tunic. Several ecological, economical, and public health hazards are associated with tunicates. Tunicates are 'invaders' that travel from one region or port to other by attaching to the ship bottoms. As a consequence of their filtration dominant survival techniques, tunicates compete for food with other filter feeders such as clams, mussels and scallops. However, due to their high reproduction rate and temperature and salinity tolerance they can quickly replace such native species by overgrowing and taking over an area, making them a major threat to biodiversity. Invasive or fouling tunicates pose a great threat to the indigenous marine ecosystem and governments spend several hundred thousand dollars for tunicate management, considering the huge adverse economic impact it has on the shipping and fishing industries. Harvesting the invasive tunicates for extraction of useful biomaterials offers a potential solution. The tunic, which is the thick external skin from which the organism derives its name, is mainly composed of tunicin, a cellulose polysaccharide, in addition to some collagen and elastin which act as a skeletal support structure, as well as the tunicate's first line of defense. Tunicate-derived cellulose and nano-cellulose has been explored as a potential polymeric material. However, the yield is only 5%, leaving the rest as waste. We have attempted to use the native tunicate extra-cellular matrix, after decellularization and lyophilization, as a bioactive tissue engineering scaffold in this work.



Graphical Abstract

Materials and Methods

Materials: Tunicates were collected from the Zayed Port, Abu Dhabi, UAE. The species was identified as Polyclinum Constellatum and submitted to NCBI (Accession # MW990087). *Decellularization and Lyophilization*: The outer rough layer was removed using surgical knife and the whole hydrogel-like tunic tissue was separated from the freshly harvested tunicate. The tunic tissue pieces are stirred well in decellularization buffer (10mM Tris, 1mM of ethylenediamine tetra acetic acid (EDTA), 0.2%V/V of Triton X-100, and 1.5% of sodium dodecyl sulfate (SDS); pH 7.5 all from Sigma-Aldrich, USA) for 48 hours. The buffer was changed every 2 hours. The decellularized tissue pieces were frozen in -80ºC overnight and lyophilized (Christ Alpha 1-2 LD Lyophilizer) for 48 hrs. The lyophilized scaffolds were sterilized with ethanol and UV radiation for further characterization and analysis. Characterization: Extensive characterization studies using SEM, Raman, FT-IR, XRD, and AFM has been done. *Cell culture*: In vitro cell culture studies using Mouse Embryonic Fibroblasts (MEFs) was performed and the cell viability was assessed using various assays including AlamarBlue, Picogreen DNA quantification, BCA protein quantification and Live/Dead.

Results and Discussion

We found the tunicate species with various color morphs including brown, red, green, and honey-colored – all belonging to the same species. This species has a jelly-like tunic consistency externally and more so internally. Predominantly made of cellulose, the cellulose fibrils are visible on slicing the top layers of the tunic. The presence of cellulose was confirmed by the material characterization experiments. The SEM images revealed that the lyophilized tunic has a rough multi-layered networked structure, with the presence of micro/nano-fibrils and crystals. It is interesting to note that the nano-fibrillar cellulose networks remain intact even after the process of decellularization and lyophilization. Tunic, being a natural ECM material, has a modulus of 4 MPa, which is much higher than other hydrogels reported so far in the literature. The tunic, derived in this work, combines both excellent biocompatibility and mechanical strength, overcoming the limitations of most of the hydrogel-based constructs.

Results and Discussion (Cont…)

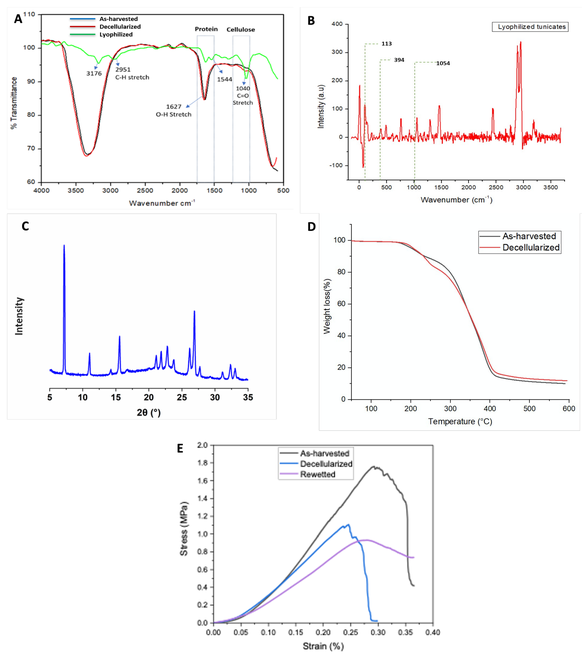


Figure 1. A) FT-IR spectra of as-harvested, decellularized, and lyophilized tunic of *Polyclinum constellatum*. B) Raman spectra of the (decellularized) lyophilized tunic. C) X-ray diffractogram of the (decellularized) lyophilized tunic, showing sharp peaks, indicating the presence of cellulose crystals. D) TGA thermogram of the (as-harvested) lyophilized and (decellularized) lyophilized tunic. E) Stress-strain curve (tensile) of as-harvested, decellularized, and lyophilized tunic of *Polyclinum constellatum*.

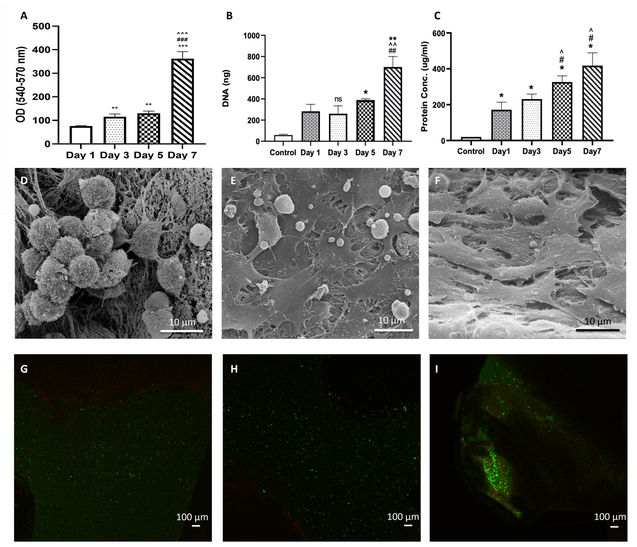


Figure 2. A) Metabolic activity of MEFs seeded on lyophilized tunics assessed using Alamar Blue assay. B) DNA quatification of MEFs seeded on lyophilized tunics assessed using Pico green-dsDNA quantification assay. C) Protein quantification of MEFs seeded on lyophilized tunics assessed using BCA protein quantification assay (n=9, *, #, and ^ indicate significant differences compared to day 1, 3, and 5 respectively and *, #, ^ represents p≤0.05, **, ##, ^^ represents p≤0.005, and ***, ###, ^^^ represents p≤0.0005). D-F) SEM image of MEFs seeded on Day 1, 5 and 7 respectively. G-I) Live/dead image of MEFs seeded on Day 1, 2 and 3 respectively.

Conclusion

In this work, we have successfully identified the environmentally destructive colonizing tunicate species of *Polyclinum constellatum* in the coast of Abu Dhabi and propose a method of using it as scaffolds for tissue engineering and regenerative medicine applications. Detailed morphological analysis revealed the intricate 3D nanofibrous cellulosic networks that remain intact even after the multi-step process of decellularization and lyophilization. The fact that the lyophilized tunics are dry, can be easily transported compared to other 3D culture systems such as hydrogels and on rewetting, the 3D tunic structure is regained, is a huge advantage for labs around the world trying to establish sustainable 3D culture systems. The tunic showed excellent biocompatibility, high mechanical properties (a modulus of 3.85±0.93 MPa compared to ~0.1 – 1 MPa of hydrogels) and exhibited high fluid-absorption capability. Experiments with camel blood plasma as wound exudate and alginate-based artificial wound proved the superiority of the tunic over the other commercially available wound-dressing materials, with a capacity of absorbing 20 times its weight in the dry state.

References

Govindharaj, M., Soman, S.S., Al Hashemi, N.S., **Vijayavenkataraman, S.** (2021). Bioprinting of bioactive tissue scaffolds from ecologically-destructive fouling tunicates. *Journal of Cleaner Production* (2021), 129923. https://doi.org/10.1016/j.jclepro.2021.129923

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