

Multilayered Chitosan – Alginate Hollow tubes for cardiovascular tissue engineering

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Statement of Purpose: In this work we develop a method for the preparation of nanostructured hollow multilayered tubes combining layer-by-layer (LbL) technique and template leaching. The use of LbL allows the production of nanometer-sized polymer films of particular interest for tissue engineering and regenerative medicine as they allow a precise control of physical and biological cues as well as the recreation of the natural complexity of ECM. Our aim was to produce hollow tubes for cardiovascular tissue engineering applications. Tubes of chitosan and alginate were prepared with or without crosslinking and their physico-chemical characterization was performed using different techniques. The permeability of the membranes towards glucose and oxygen was evaluated. We further evaluate the biological performance in terms of cell adhesion, viability and proliferation. The results suggested the potential of these structures to boost the development of innovative tubular structures for tissue engineering approaches.

Methods:

Hollow multilayer tubes of chitosan and alginate were prepared from prepared combining LbL with leaching of sacrificial templates. The templates were produced by dip-coating glass tubes (\varnothing 1 mm) in molten paraffin (Jojoba Desert Whale, USA). Upon solidification an homogeneous coating of paraffin (thickness \approx 600 μ m) was produced around the glass tube. Polyelectrolyte solutions were alternately deposited on the tubular template, using an in-house developed dipping robot to form 100 dL of ALG and CHT.

To evaluate the biological performance of ALG/CHIT and crosslinked ALG/CHIT tubes, cell culture studies were performed with L929. DAPI& Phalloidin and SEM were used to assess the morphology and the viability was measured with MTS.

Permeability measurements were conducted using a glass Franz-type diffusion cell (PermeGear). The membranes, prepared using the same LbL procedure as the tubes were previously equilibrated in a PBS solution for 1h, placed between the two compartments and hold with a stainless steel clamp. The donor compartment was filled a glucose solution with 1mg/mL and aliquots of 100 μ L were withdrawn from the receptor compartment at predetermined time periods and replaced by fresh PBS. The experiments were carried out at 37 $^{\circ}$ C with stirring. The kinetics of oxygen diffusion was also evaluated using a CellOx 325 probe.

Results: In this work it has been hypothesized that a hollow tube could be prepared combining LbL with leaching of sacrificial templates. Basically tubular

templates of paraffin were coated with alternate layers of ALG and CHIT. Afterwards, the sacrificial template was completely leached out. The hollow tube entirely composed of ALG/CHIT was dried using supercritical fluid technology, in order to avoid the collapse of the structure and to ensure the complete removal of the organic solvent (DCM). To increase the mechanical strength and consequently the stability, the tubes were crosslinked using genipin. Regarding the cell viability assessment, in general, the results reveal that cells are able to remain viable in the tubes up to 7 days in culture. MTS results show a statistically significant enhancement of total cell viability for crosslinked tubes for 3 and 7 days of culture when compared with native tubes. The cells on crosslinked films are able to grow and spread along the surface. Figure 1 presents an optical microscopy image of the native and crosslinked CHT/ALG multilayer tubes and a confocal microscopy of the tubes after 3 days cell culture.

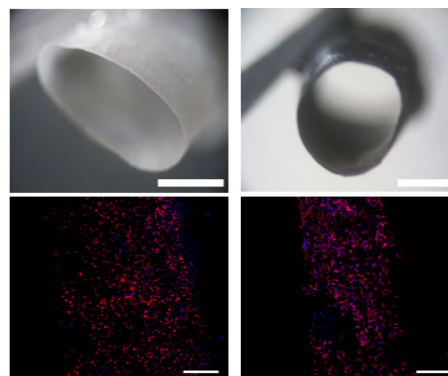


Figure 1: Optical microscopy image of the native and crosslinked CHT/ALG multilayer tubes (scale bar is 1 mm) and a confocal microscopy of the tubes after 3 days cell culture (scale bar is 200 μ m).

Permeability studies carried out up to 24 hours show that the membranes are permeable to glucose and oxygen, confirming their potential to be used in cardiovascular tissue engineering.

Conclusions:

The results obtained with the crosslinked films have demonstrated that these were more suitable structures for cell adhesion and spreading on polymeric films that are otherwise non-cell adhesive.

References: (J.M. Silva, A.R.C. Duarte, C.A. Custódio, P. Sher, A.I. Neto, A.C.M. Pinho, J. Fonseca, R.L. Reis, J.F. Mano, *Advanced Healthcare Materials* 2013, DOI: 10.1002/adhm.201300265)