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PP224
Chondrosia reniformis marine-sponge collagen membranes for skin re-epithelialization

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Introduction: Chondrosia reniformis collagen has been identified as mainly of type IV. Being collagen IV the main component of the epidermal basal layer [1], *C. reniformis* represents a valuable source to be explored in the skin regeneration field. This work envisaged the production of *C. reniformis* collagen membranes for the selection of rapidly adherent epidermal cells, like the commercial collagen coatings, and for their subsequent culture. This approach would permit a single system for culturing and carrying basal epidermal cells aimed at re-epithelialize skin wounds.

Materials and methods: The collagen of *C. reniformis* marine-sponge was extracted with 100 mM Tris-HCl, 10 mM EDTA, 8 M urea and 100 mM 2-mercaptoethanol. To define the best re-solubilization conditions, the obtained precipitate was dissolved in five different solutions: Solution A: 100 mM Tris-HCl+8 M Urea+10 mM EDTA (pH 9.5); Solution B: 50 mM Tris-HCl+1 M NaCl (pH 7.4); Solution C: 100 mM Tris-HCl (pH 7.4); Solution D: 0.5% H_2O_2 (v/v) (pH 11) and Solution E: 100 mM Tris-HCl (pH 9.5). Solutions of 1% collagen were prepared and cross-linking was performed with HMDI, genipin and EDC/NHS at different concentrations. The membranes were obtained by solvent-casting and/or freeze-drying, and their stability was tested both in PBS and culture medium, for at least 7 days. Morphological characterization of the membranes was carried out by scanning electron microscopy (SEM). Cytotoxicity, based on metabolic activity (MTS assay) and cell proliferation (DNA quantification) analysis of the 100 mM Tris-HCl (pH 9.5) and 8 mM EDC/NHS cross-linked collagen membranes, was assessed with L929 cells. Results were analyzed by IBM SPSS Statistics Version 20 using one-way ANOVA and Kruskall-Wallis test. Significance was set for $p < 0.05$.

Results: The re-solubilization of the collagen was achieved with solutions A, D and E. Nonetheless, although it was possible to obtain the 1% collagen membranes, those prepared from A and D solutions were not stable and collapsed during the tests. Stable membranes were obtained after re-solubilization of the collagen in solution E and upon cross-linking by immersion in 8 mM and 25 mM EDC/NHS. Additionally, the freeze-dried membranes were analyzed by SEM (Fig. 1), revealing no porosity but some roughness on their surface.

The *in vitro* tests showed a reduced cytotoxicity, confirming the capability of the membranes to support cell adhesion and proliferation.

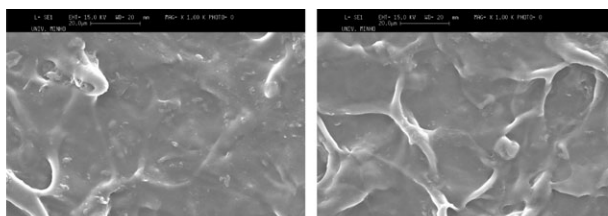

Figure 1. Freeze-dried 8 mM (left) and 25 mM (right) EDC/NHS cross-linked membranes.