

KERATINOCYTES HaCat PROLIFERATION ON 3D-PRINTED CHITOSAN-COLLAGEN SCAFFOLDS

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Despite the wide advances in biomaterials for tissue engineering, autografts are today the only artifact to promote epidermis regeneration in chronic wounds and in acute wounds greater than 4 cm in diameter. This feature remarks the importance of autologous epidermal cells in the healing process, thus the importance of developing biomaterials for seeding these cells. Hydrogels have largely proved to be suitable for cells proliferation, and 3D bioprinting has become an innovative technique for setting biomaterials and cells according to a controlled digital design. We aimed to inquire in the collagen (Col) and chitosan (Chit) blends to this type of application.

Two printable hydrogel precursors were selected, in % w/v, Chit 1.00:Col 0.36 and Chit 0.50:Col 0.54, both at pH=4.50 because of the polymer solubilities. They were analyzed rheologically, particularly considering the strain rate of a 3D-bioprinter (30-60 1/s) made in Argentina. Viscosities during the extrusion of the hydrogel were in the interval 0.5-0.8 Pa.s. In order to make the inks suitable for cell life, a method for misting NaHCO₃ 0.8 M was developed, obtaining pH ~ 6 homogenous inks storable during 5 days with negligible changes in stability, viscosity and printing fidelity, when compared to their non-misted counterparts.

To achieve proper mechanical properties, activators EDC/ NHS in powder was added to the hydrogels immediately before printing. The final products were crosslinked manipulable scaffolds, non cytotoxic according to the ISO rule 10993-5 using NH-3T3 fibroblasts. In order to assess the epidermis regeneration, keratinocytes proliferation was tested by seeding 10⁵ HaCat cells/well, in a 24-wells plate with a printed scaffold in each well. Cells showed excellent adhesion and capability to proliferate on the hydrogels after 5 days in both scaffolds. Window time available for printing without gelation remains to be defined, as well as scaffold swelling and stability, both features partially dimmable by crosslinkers concentration.