

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

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We aimed to produce **3D-bioprinted Chitosan-Collagen hydrogel scaffolds**, suitable for keratinocytes seeding and proliferation. Our research is framed on the possibility to advance towards epidermal cell bioinks. Despite the advances in biomaterials for tissue engineering, autologous skin grafts are today the only artifact to reliably promote epidermis regeneration in several healing process, remarking the importance of autologous epidermal cells

The **three-dimensional (3D) bioprinting** strategy is under intense research to address the limitations of conventional fabrication methods to create clinically applicable tissue constructs¹. **Collagen and Chitosan** blends were scarcely tested in the assessment of inks for 3D-bioprinting². The low pH of these inks, according to polymer solubilities, could be a drawback for cell life in a direct 3D-bioprinting approach.

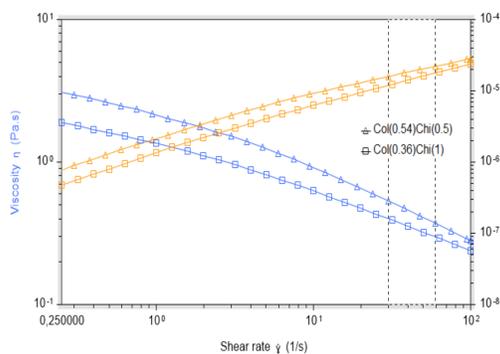
In our lab, we have been working in preliminary evaluations, interested in solutions which could include these biomaterials. Two printable hydrogel precursors were selected, in %w/v, both with pH \approx 4.50:

- Chit 1.00:Col 0.36
- Chit 0.50:Col 0.54



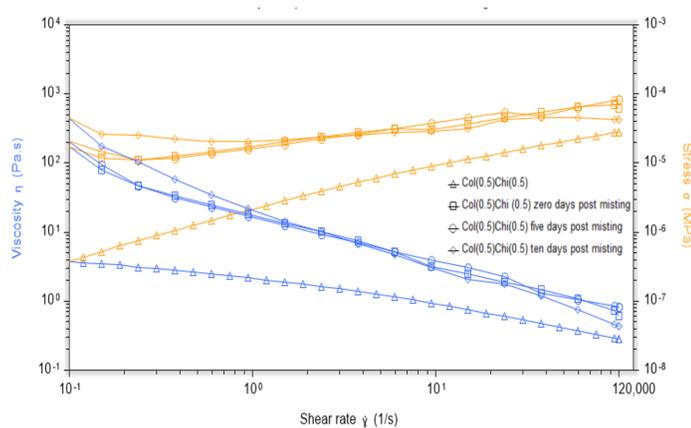
Collagen was extracted from rat tails³ and low-molecular weight Chitosan was used.

1. Rheological analysis



Flow sweeps showed similar shear-thinning behavior for both inks. Between bars, bioprinter shear rate frame (Viscosities 0.50-0.80 Pa.s.)

By misting with NaHCO₃ before printing homogenous inks with pH \approx 6 were obtained. Changes in viscosity did not affect their stability at 4°C for at least ten days, nor their ability to be printed.



Viscosity and Stress as a function of Shear rate comparison before and after 10 days of misting with NaHCO₃ showed for Col:Chi 0.54:0.50

¹ J. T. Huh *et al.*. Three-dimensional bioprinting for tissue engineering. INC, 2020.

² A. C. Heidenreich *et al.* Collagen and chitosan blends for 3D bioprinting: A rheological and printability approach, Polym. Test., vol. 82, 2020.

³ J. Habermehl *et al.* Preparation of ready-to-use, stockable and reconstituted collagen, Macromol. Biosci. 5, 2005.



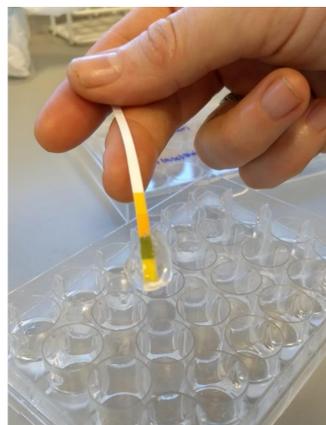
Left: 3D bioprinter used to print scaffolds, LifeSI (Argentina) with syringe in position. Right: Ink-loaded syringe being placed into printer cartridge.

2. The scaffolds were printed in a piston-based, locally developed low-cost 3D bioprinter.

Each round scaffold for a well of 24-well plates had a volume of 500 μ l. Crosslinkers EDC/NHS in powder form were added just before printing, in concentrations of 0.5 mg of NHS and 2.8 mg of EDC per 1 ml of Col-Chi ink



3. Hydrogel gelation was achieved by incubation in a 37 °C bath for 30 min, while collagen was gelling and crosslinkers activators were acting, obtaining manipulable structures:

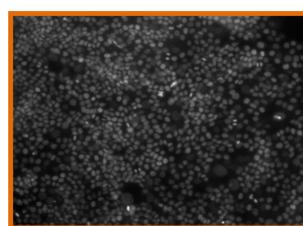


Obtained crosslinked scaffolds

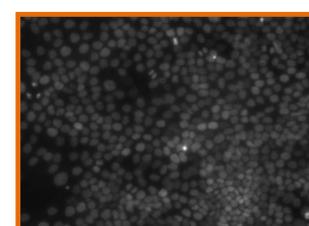


The time taken to print each scaffold is dependant on the set printing parameters. For standard printing settings, a two layers well-sized cylinder takes a five minutes on average.

4. HaCaT keratinocytes were seeded on the printed scaffolds (10⁵ cells/well), showing excellent adhesion and proliferation after 5 days.



Col:Chi 0.54:0.50



Col:Chi 0.36:1.00

Keratinocyte proliferation on Chitosan-Collagen scaffolds
Pictures taken with Microscope Nikon TE2000, magnification 60x

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