# Towards the development of a biomimetic corneal stroma by 3D printing: comparison of collagen I biomaterials with clinical applicability

**Marta Cadenas-Martín , Ana I. Martín-González , Malgorzata A. Marć, Lucía Pardinas and Maria P. De Miguel**

Cell Engineering Research Group. The Health Research Institute of La Paz University Hospital, IdiPAZ, Madrid, Spain

## INTRODUCTION

The cornea is an avascular tissue that performs the first step in vision formation. About 12.5 million people suffer vision loss due to corneal disease or injury, with 2 million new cases diagnosed every year. Moreover, it is estimated that in developed countries there is 1 cornea accessible for transplantation for every 7 patients, and 1 cornea every 70 worldwide.

To solve this problem, several approaches aimed at developing an artificial cornea are being investigated, among which there is 3D-bioprinting. In tissue engineering, collagen is one of the most widely used biomaterials, since it is biocompatible as it is a protein abundant in the extracellular matrix, it has a high affinity for adherent cells, and is biodegradable, aiding tissue regeneration. Moreover, collagen type I is the main component of the cornea as well.

## MATERIALS AND METHODS

We compared extrusion-based 3D-printing and different type I collagen bioinks to evaluate their possible clinical application in corneal fabrication. Collagens of different origins have been used: rat collagen, porcine collagen and human collagen. Printing tests were carried out at different concentrations and controlled dehydration times, and then transparency, thickness, and degradation by corneal keratocytes was assessed by photography, optical coherence tomography (OCT) and culture with activated keratocytes respectively.

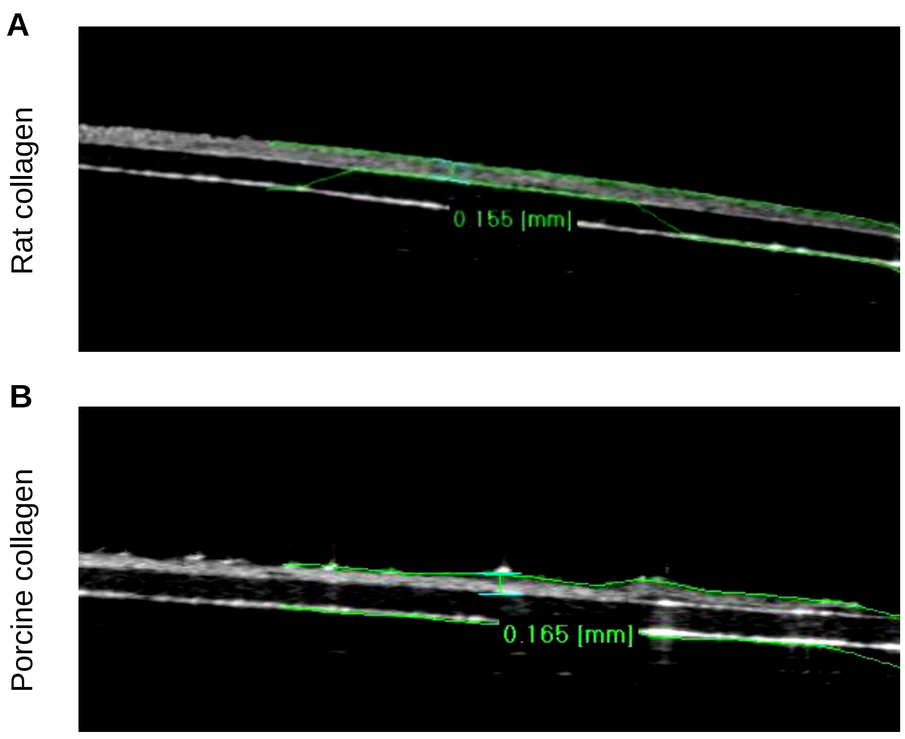



**Figure 2:** Representative OCT images of cross sections of the rat collagen printed scaffold (A), and porcine printed scaffold (B). Corneal measurements in green.

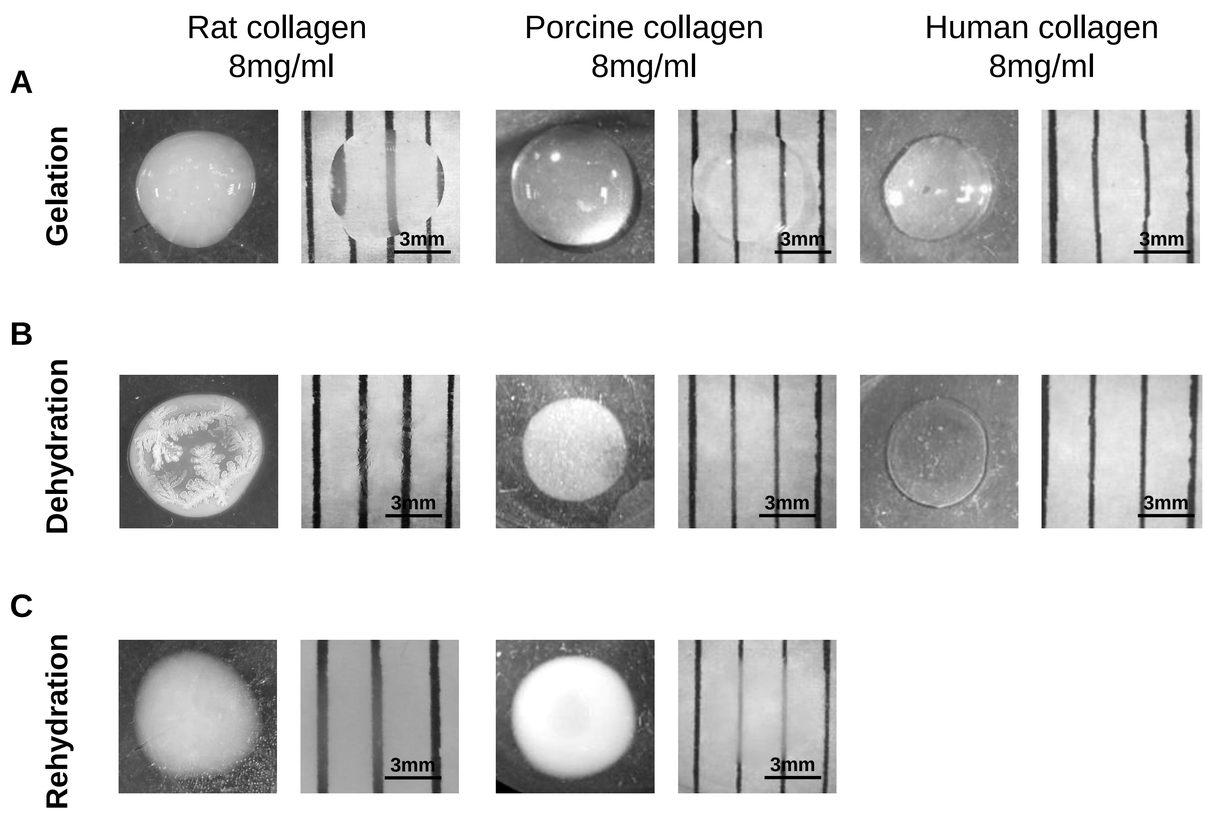



**Figure 1:** Representative images of rat, porcine, and human type I collagens printed scaffolds at 8 mg/mL after gelation (A), dehydration (B), and rehydration (C) on a black background or over a graph background showing different degrees of transparency. Scale bar 3 mm.

## RESULTS

Transparency, thickness and degradation times were affected by the species of origin, the method of collagen extraction, and dehydration time, with a wide margin for improvement.

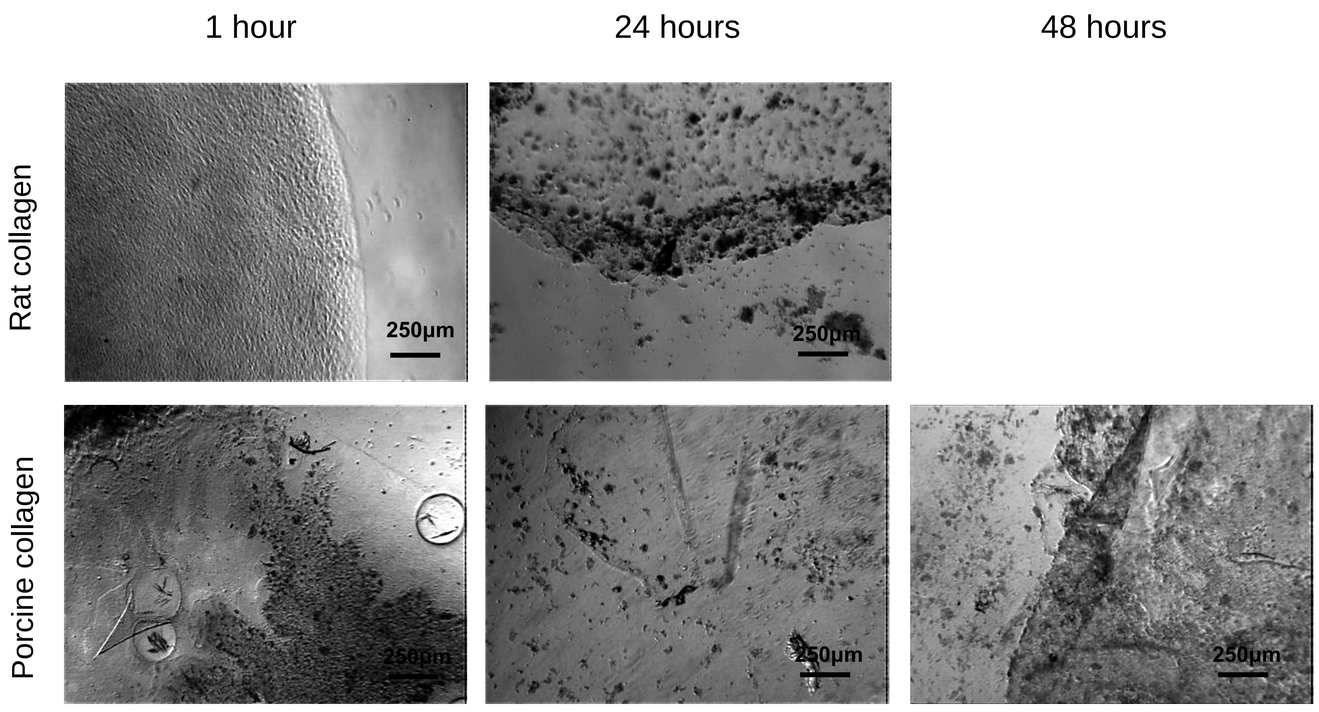



**Figure 3:** Representative phase contrast images of the follow-up of degradation by activated keratocytes. Rat collagen scaffold at 1 and 24 hours, and porcine collagen scaffold at 1, 24 and 48 hours. Scale bar 250µm.

## CONCLUSIONS

All tested 3D-printed scaffolds were rapidly degraded by activated keratocytes, so they would not be useful for the treatment of corneal ulcers. In contrast, usage of these 3D-printed scaffolds for transplantation in the stromal layer could be useful and will be further investigated.

**Supported by:** The Ministry of Science and Innovation, Strategic Health Action, Instituto de Salud Carlos III (ISCIII), Proyectos de I+D+I en Salud (PI23/00207) and co-funded by the European Union and Proyectos Luis Alvarez from FIBHULP.