3D-printed biomimetic scaffolds as promising therapeutic approach for corneal melting *in vivo*

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INTRODUCTION

Corneal melting represents one of the most severe ocular surface disorders, characterized by corneal thinning, loss of transparency and in advanced stages, severe visual impairment and risk of perforation. Corneal transplantation currently remains the main treatment for restoring vision, but the shortage of donor tissue leaves millions of people without effective options. Alternative biological materials such as amniotic membrane (AM) or decellularized corneal stroma still depend on donated tissue, so the shortage persists. Advances in 3D-bioprinting open new possibilities for creating standardizable, biomimetic scaffolds that can restore corneal tissue and stimulate regeneration.

OBJECTIVE

We propose 3D bioprinted type I collagen scaffolds subsequently dehydrated and crosslinked to be applied superficially in a rat model of corneal melting with the aim of evaluating their structural viability, transparency, and *in vivo* integration in comparison with the standard AM transplant, as a preliminary step toward a fully biofabricated and clinically translatable therapeutic alternative.

METHODS

A chemical burn was performed on the corneal epithelium with heptanol for 4 minutes and manual scraping with a crescent knife. Ten rats (5 male and 5 female) were randomly assigned to two experimental groups: one treated with a 3D bioprinted collagen I scaffold (n=7) and the other one with AM (n=3). In addition, two control groups of the same rats were used: the healthy eye control group (n=4) and the lesion control group (n=8), the latter established at the time of sacrifice for ethical reasons. Fifteen days post-surgery, corneal re-epithelialization was evaluated macroscopically using visible and UVA light with fluorescein staining. *Ex vivo* corneal thickness was measured by optical coherence tomography (OCT), and histological analysis to evaluate epithelial morphology.

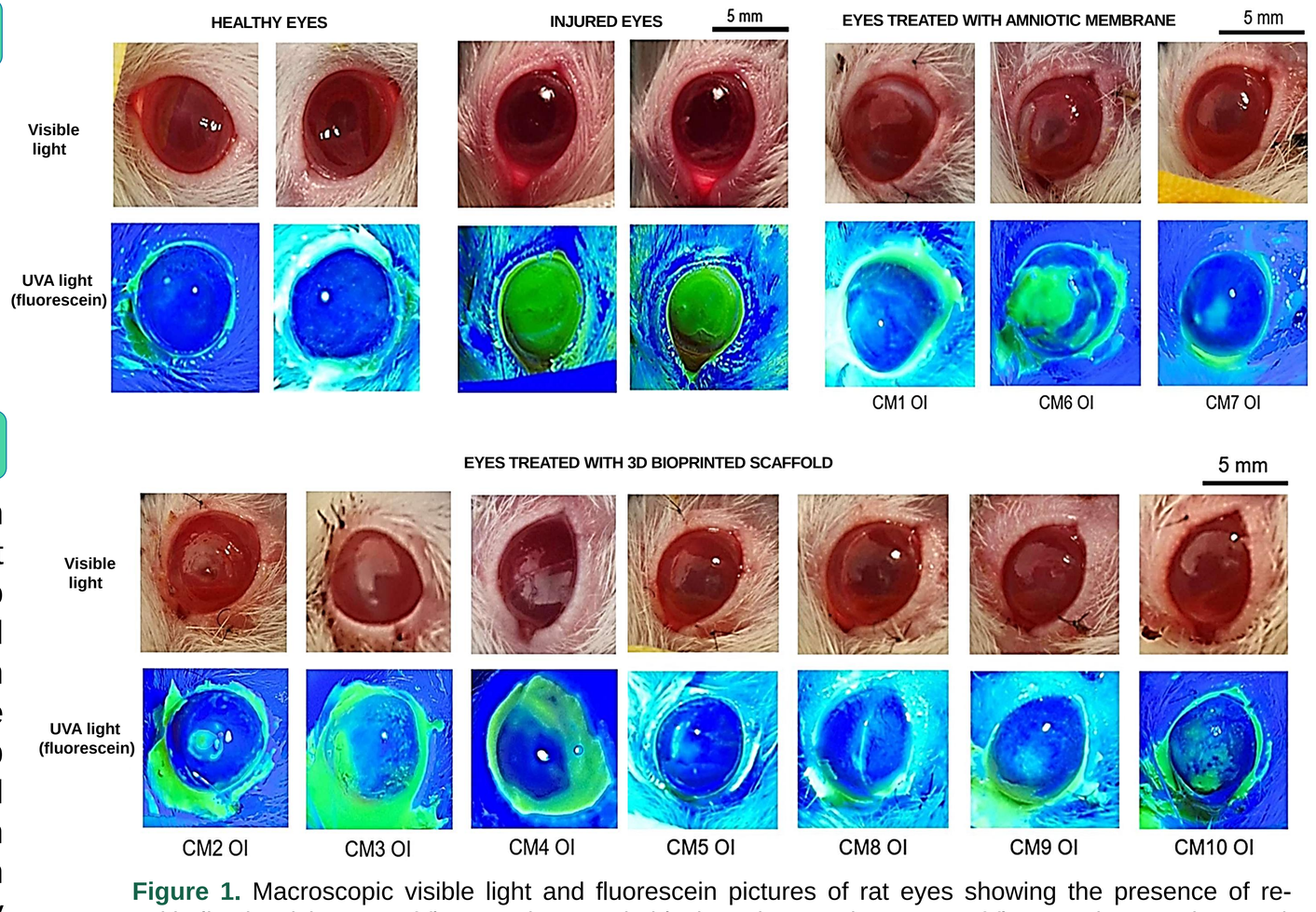


Figure 1. Macroscopic visible light and fluorescein pictures of rat eyes showing the presence of re-epithelization (absence of fluorescein, seen in blue) or absence (presence of fluorescein, seen in green) of the wound after AM or 3D-printed collagen transplantation.

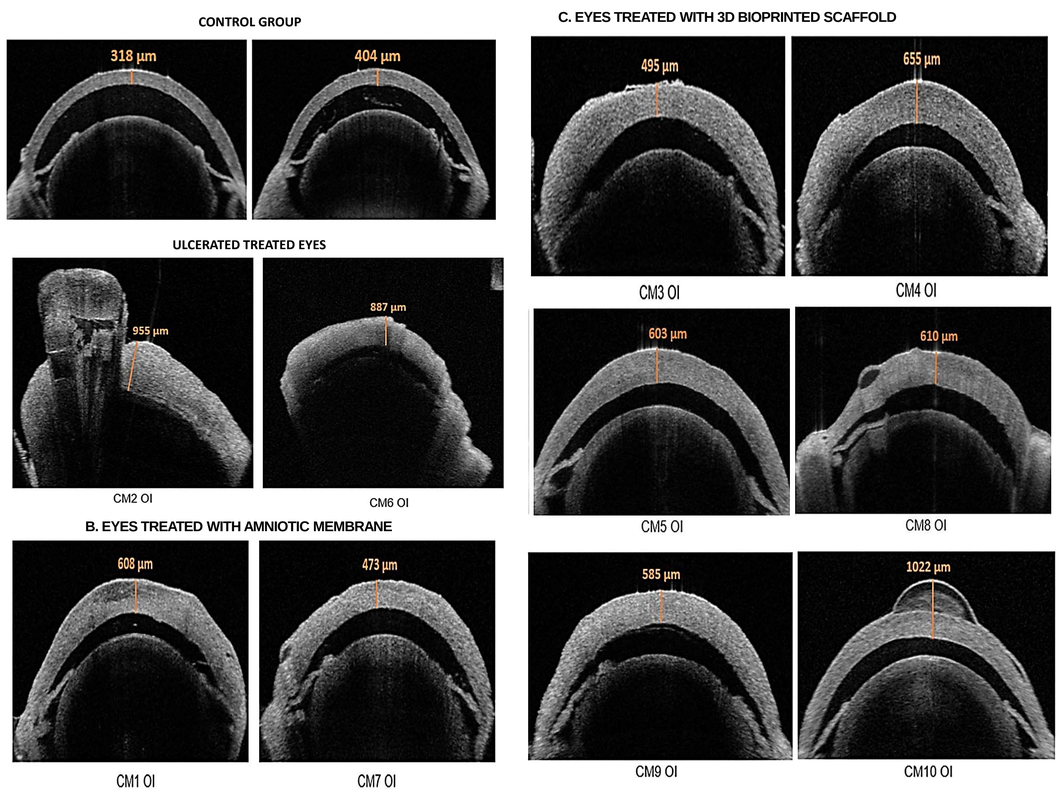


Figure 2. Optical coherence tomography (OCT) images of the corneas from each experimental group. CM2 (3D-treated female rat) and CM6 (AM-treated male rat) showed alterations consistent with corneal ulcers.

RESULTS

All treated eyes showed complete corneal transparency and comparable re-epithelialization between 3D bioprinted collagen and AM. OCT showed increased corneal thickness and good optical integration, and histological analysis confirmed biocompatibility and effective epithelial regeneration. Exceptions (CM2, CM3, CM6, CM10) were attributed to surgical factors, with ulcers in CM2 and CM6 likely due to more aggressive surgical debridement. In CM10 and probably in CM3, a fibrin deposit on the implant caused an abnormal elevation. Overall, 3D bioprinted collagen demonstrated lower inflammation compared to AM. Taken together, 3D bioprinted collagen showed more advanced tissue maturation than AM, with improved basement membrane organization, epithelial structure.

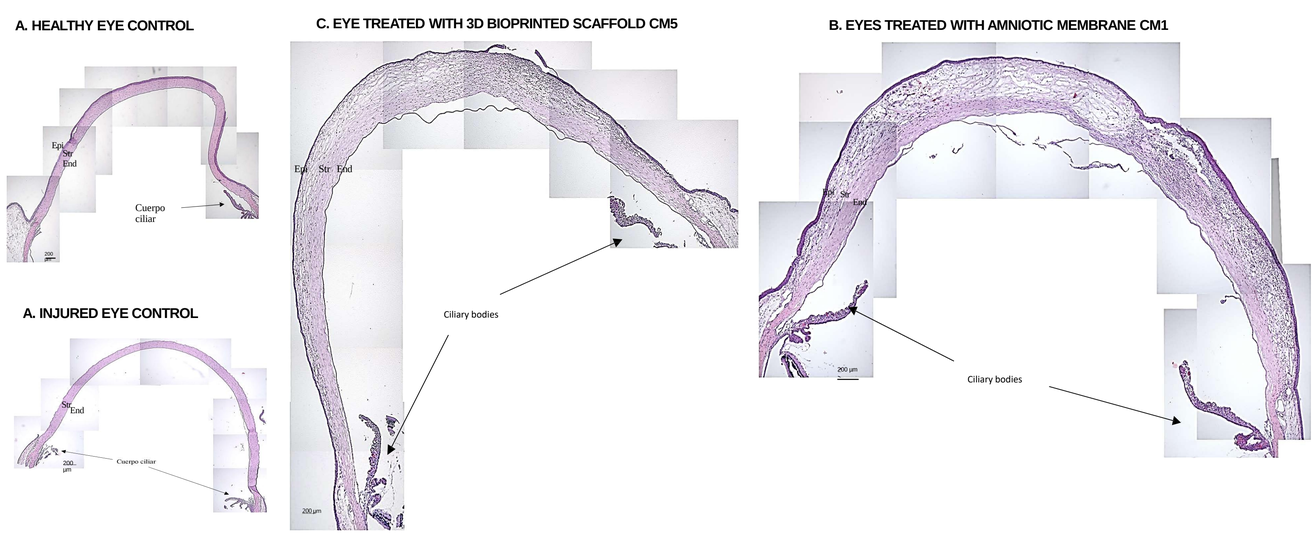


Figure 3. Representative H&E corneal sections from each experimental group. Epi: corneal epithelium, Str: corneal stroma, End: corneal endothelium. Scale bars: 200 µm.

CONCLUSIONS

Our results highlight the potential of these 3D-printed collagen scaffolds with high optical transparency and good mechanical properties, demonstrating biocompatibility and *in vivo* safety in a preclinical animal model.

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