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## Brief Report

# Corneal Regeneration Following Implantation of a Biomimetic Tissue-Engineered Substitute

Per Fagerholm<sup>1\*</sup>, Neil S Lagali<sup>1\*</sup>, David J Carlsson<sup>2,3</sup>, Kimberley Merrett<sup>3</sup> and May Griffith<sup>3,4</sup>

<sup>1</sup>Department of Ophthalmology, Linköping University Hospital, 58185 Linköping, Sweden

<sup>2</sup>National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada

<sup>3</sup>University of Ottawa Eye Institute, Ottawa, Ontario K1H 8L6, Canada

<sup>4</sup>Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada

Correspondence should be addressed to Per Fagerholm (perfa@inr.liu.se).

\*denotes equal contributions

## Introduction

Loss of vision due to corneal disease or trauma affects over 10 million individuals worldwide. For many, corneal transplantation is the only treatment but the supply of good quality donor tissue cannot meet the demand, especially in the developing world. To address this need, several corneal substitutes have been proposed,<sup>1,2</sup> although to date, the only substitutes clinically tested in humans have been fully synthetic prostheses (keratoprotheses).<sup>3,4</sup> Although improving, complications with corneal prostheses including retroprosthetic membrane formation, calcification, infection and glaucoma,<sup>2,4,5</sup> have limited their use to cases where human donor tissue fails,<sup>1,2</sup> so prostheses do not alleviate the primary need for human donor corneas.

An alternative approach is to support the inherent regenerative capacity of the human cornea to promote the in-vivo reconstruction of healthy, viable corneal tissue. In this regard, tissue-engineered biomimetic materials designed to mimic the corneal extracellular matrix (ECM) have been proposed as scaffolds for endogenous tissue regeneration.<sup>6</sup> We have previously reported on a biomimetic corneal substitute composed of porcine atelocollagen cross-linked by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to give robust, implantable, cornea-shaped scaffolds.<sup>7</sup> Subsequently we adapted this cross-linking strategy to recombinantly-produced human collagen, either type I or III (RHCI or RHCIII), that gave similar, stable host-graft integration and regeneration of corneal cells and nerves in pigs<sup>8</sup>. Although both recombinant collagens gave similar results, RHCIII implants had superior optical properties.<sup>8</sup> Recombinant human collagen promises a source of safe, predictable and chemically-defined material for tissue engineering<sup>9</sup>, while minimizing the risk of disease transmission or immune reaction which could result after implantation of animal-source collagen.<sup>8</sup>

Following the successful implantation of biomimetic corneas in animal models, we report here early results from the first clinical study to surgically implant a biomimetic corneal substitute in human subjects.

## Methods

Following approvals from the Swedish Medical Products Agency and the Regional Ethical Review Board in Linköping, Sweden, and following the tenets of the Declaration of Helsinki with informed consent given by each patient, we conducted a Phase I clinical study of 10 patients with vision loss from keratoconus or scarring as indications for corneal transplantation. The cohort comprised eight males and two females, aged  $43.6 \pm 18$ y (mean  $\pm$  SD, range: 18 to 75y) at the time of surgery. RHCIII (13.7% wt/wt) was crosslinked with EDC-NHS and fabricated into substitutes with the dimensions of a human cornea.<sup>8</sup> Using anterior lamellar keratoplasty (ALK)<sup>10</sup> under general or local anesthesia, a button (6.0 – 6.5mm dia., 370 – 400 $\mu$ m thick) of host corneal tissue was manually excised, leaving a recipient bed of posterior stromal tissue and an intact endothelium. Patients received biomimetic substitutes (6.25 – 6.75mm dia., 500  $\mu$ m thick) anchored with 3 to 4 overlying 10-0 nylon sutures and covered with a bandage lens. Antibiotic eye drops (chloramphenicol 0.5%, MINIMS® Chauvin Pharmaceuticals, London England) and topical steroid drops (dexamethasone 0.1% without preservatives, Opnol®, Clean Chemical Sweden, Borlänge, Sweden) were given postoperatively and tapered off over one month. Bandage lenses and sutures were typically removed 5 weeks after surgery.

## Results

After six to seven months, the corneal substitutes were well-integrated in all ten recipients, without adverse effects or complications such as neovascularization, inflammation, or rejection. Remodeling of the thick substitutes occurred, as previously observed in animal studies, resulting in restoration of a seamless host-graft interface and a corneal surface becoming progressively smoother over time (Fig 1A,B). Visually, the operated eye was indistinguishable from the patient's unoperated eye. Mean central corneal thickness after 6 – 7 months was  $420 \pm 99\mu$ m (mean  $\pm$  SD, range: 307 to 581 $\mu$ m) as determined by ultrasound pachymetry. Tear film production was normal in seven operated eyes as measured by Schirmer's test (>15mm in 5min) while the remaining operated eyes exhibited moderate wetting (9 – 12mm in 5min).

Regeneration of host corneal epithelium to cover the implant surface was observed in all patients, with the time to full epithelial cover ranging from 1 – 3 months in nine patients and 5 months postoperatively in one patient (who received an amniotic membrane patch after 3 months to facilitate epithelialization). In-vivo confocal microscopy (IVCM) showed that the regenerated epithelium exhibited a normal, stratified morphology with superficial, wing, and basal epithelial cells (Fig 1C), that has remained stable at seven months. Fine sub-epithelial nerves were observed (Fig. 1D), and corresponded to a partial return of sensitivity as determined by Choquet-Bonnet aesthesiometry (20 – 50mm in the centre of all operated corneas; normal 60mm). Varying degrees of stromal cell migration and activity (Figs. 1E,F) were observed within the

implanted region by IVC. The residual posterior stromal bed and endothelium in each patient's operated cornea maintained a normal morphology (Figs. 1G,H). Vision continues to change as the stromal wound healing activity subsides and the ocular surface quality improves, however, even at this early stage best-spectacle-corrected visual acuity improved from preoperative values in 4 patients, remained unchanged in 4 patients, and decreased slightly in 2 patients.

## **Discussion**

The results of this study are encouraging. To our knowledge, this is the first report of the regeneration of corneal epithelium, stroma, and nerves in humans implanted with a corneal substitute. The clinical data supports the hypothesis that a cell-free corneal substitute mimicking the ECM can support endogenous regeneration of corneal tissue without rejection, inflammation, or neovascularization. The absence of adverse reactions in the first small patient group provides initial clinical evidence of the safety of RHC and the cross-linking method used. Unlike most cross-linkers that become chemically bound into the final collagen product with a subsequent propensity for breakdown products causing in-vivo toxicity, neither EDC nor NHS are incorporated into the final product,<sup>7</sup> circumventing the possibility of toxic substance release from crosslink breakdown.

## **Conclusion**

Visual acuity, ocular surface quality and corneal sensitivity are continuously improving in the first patients to receive a biomimetic corneal substitute. Such substitutes could find use as temporary or emergency corneal replacements where human tissue is unavailable and prostheses are undesirable. The prospect for biomimetic substitutes to serve as longer-term corneal replacements, and therefore as an alternative to the use of human donor corneas for transplantation – awaits a longer-term follow-up in a large patient population.

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## **Figure**

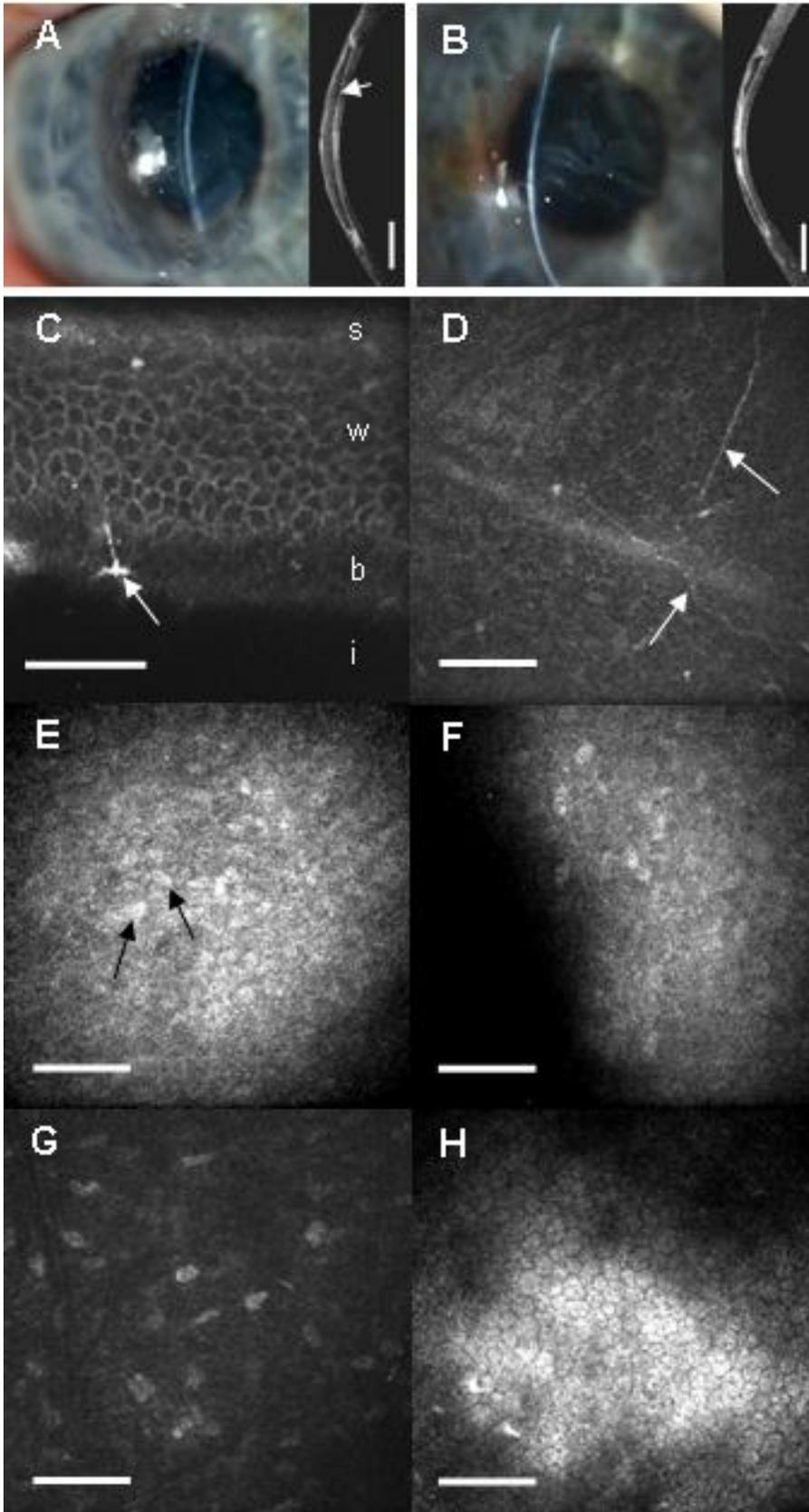


Figure 1.

Representative slit-lamp photographs, optical coherence tomography images (ASOCT, Visante, Carl Zeiss Meditec, Jena, Germany), and in-vivo confocal microscopy images (HRT3, Heidelberg Engineering, Heidelberg, Germany) of corneas 7 months after lamellar keratoplasty. **(A)** Operated cornea of a 36-year-old male, which is transparent with a smooth corneal surface. In the OCT image, the boundary of the biomimetic substitute (arrow) is distinct, with initial remodeling evident (bright areas) at the peripheral host-to-graft interface (OCT image width = 9.2mm, scale bar = 1.5mm). **(B)** Operated cornea of a 57-year-old male with a slight central haze. The OCT image indicates remodeling of the central region of the implanted tissue by wound-healing stromal fibroblasts (bright areas). Detailed in-vivo micro-morphology **(C-H)** of corneas at 7 months postoperative, with the depth of each image from the corneal surface given in parentheses. **(C)** Oblique section showing regenerated stratified epithelium with superficial (s), wing (w), and basal (b) epithelial cells, overlying the implant (i). New, sprouting nerves were observed at the basal epithelium (arrow), scale bar = 30 $\mu$ m. **(D)** Fine, regenerating nerves (arrows) at the regenerated basal epithelium-implant boundary (depth 43 $\mu$ m), scale bar = 100 $\mu$ m. **(E)** Nuclei of stromal cells (arrows) that have migrated into the implant from the host's cornea. The bright background represents endogenous wound-healing fibroblast activity in the anterior part of the implanted tissue (depth 76 $\mu$ m), scale bar = 100 $\mu$ m. **(F)** Advancing front of endogenous stromal cells deep within the implant, with as yet unpopulated, cell-free areas appearing dark (depth 192 $\mu$ m), scale bar = 100 $\mu$ m. **(G)** Host keratocyte nuclei within the host's preserved posterior stroma (depth 322 $\mu$ m), scale bar = 100 $\mu$ m. **(H)** Preserved host endothelium (depth 493 $\mu$ m), scale bar = 100 $\mu$ m.