Characterization of corneal wound healing process using an in-vitro 3D corneal model and optical coherence elastography

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INTRODUCTION

- The tissues of the cornea are arranged in five different layers. Among them, the **epithelium** measures slightly less than **10%** of the thickness of the overall cornea, and the **stroma** is approximately **90%** of the thickness of the entire cornea, where **collagen** is the major mechanical and structural protein in the extracellular matrix (ECM).

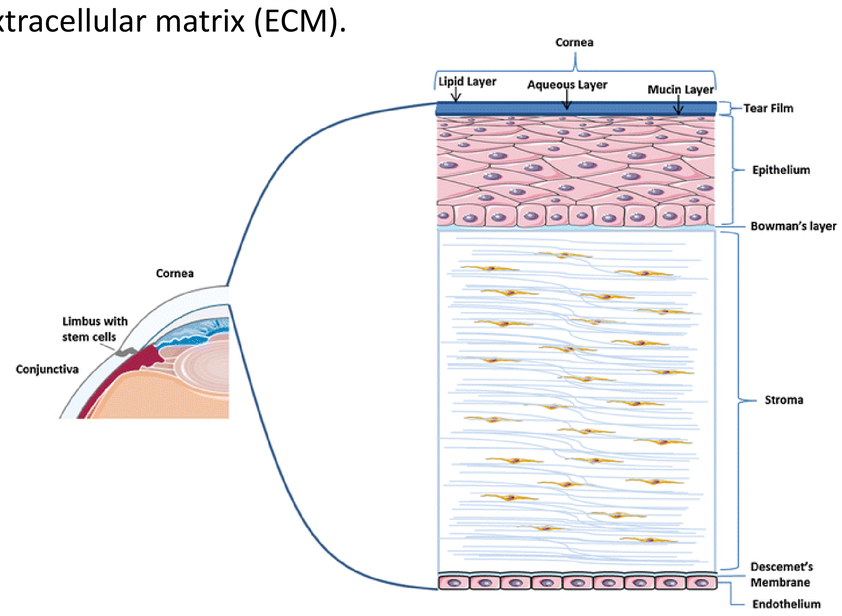


Figure 1. A sketch of the layer structure of the cornea [1].

- However, the cornea is also exposed to damages, such as scratches, laser surgery, radiation exposure and chemical reagents. Anti-inflammatory drugs and antibiotics have been used to reduce the infection and regulate the immune system to promote the **wound healing process**. To avoid the use of animal experiments, *reconstructed in vitro models* [2-3] proved to suit better for the rapid testing of the uptake or metabolism of drugs in corneal cells based on tissue engineering techniques.

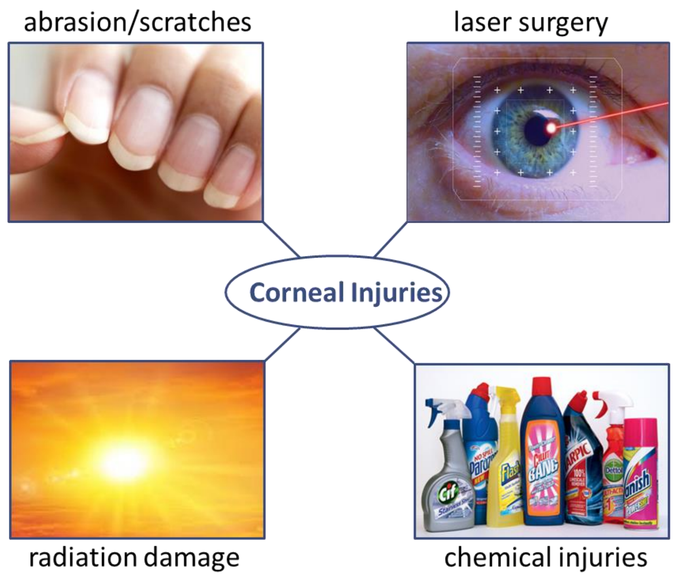


Figure 2. The most common corneal injuries.

- Optical Coherence Tomography (OCT) is a real-time, in vivo and non-invasive imaging technology with a ***high resolution*** and a high acquisition speed.

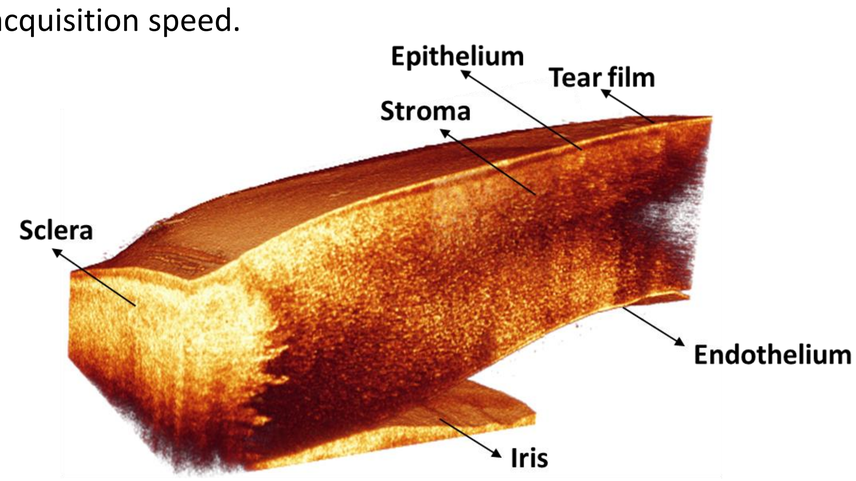
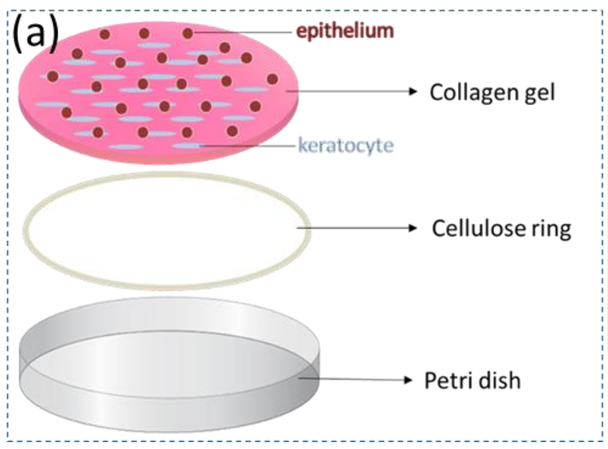


Figure 3. High- resolution OCT 3D structure image of a porcine eye with a size of 2 x 3 x 6 mm (depth x lateral x length).

- The use of OCT to perform elastography is a technique known as optical coherence elastography (OCE). Measuring elastic properties of biological tissues has become a popular research topic these days [4-7].
- In this study, a novel vibrational OCE method was applied to characterise the corneal wound healing process on an *in vitro* corneal 3D model.

MATERIALS AND METHODS

- The in-vitro corneal model was developed using three main components, namely epithelium, keratocyte, and type I collagen (3mg/ml) as illustrated in the figure below. Corneal lesion was mimicked with Ferric Chloride ($FeCl_3$) solution and a small piece of paper to control the size.



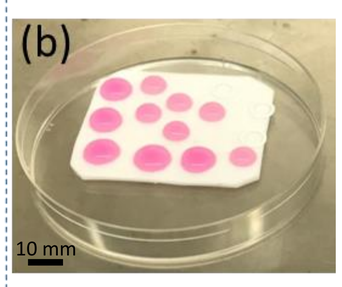


Figure 4. (a) The components of in-vitro corneal model and (b) the established stromal equivalent.

- During the OCT/OCE scanning, the corneal samples were kept in 35-mm petri dish with no more than 50µl culture medium to keep the samples hydrated during imaging without causing reflection and image distortion.

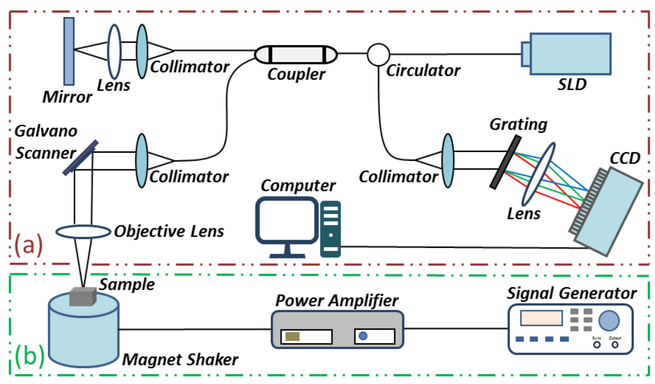


Figure 5. The system set up of phase-sensitive OCE consisted of (a) OCT and (b) vibration generation.

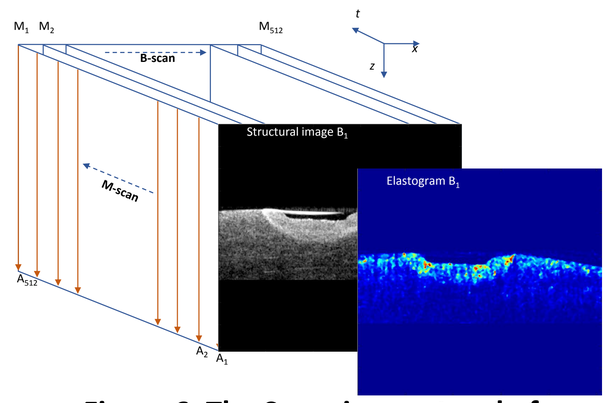


Figure 6. The Scanning protocol of corneal models using OCE .

RESULTS

- After the lesion created on top of the equivalent, the resulting structure change can be depicted by OCT as presented in Figure 7 (a). A great amount of bundled collagen fibres are noticed horizontally cross the sample area in Figure 7 (b).

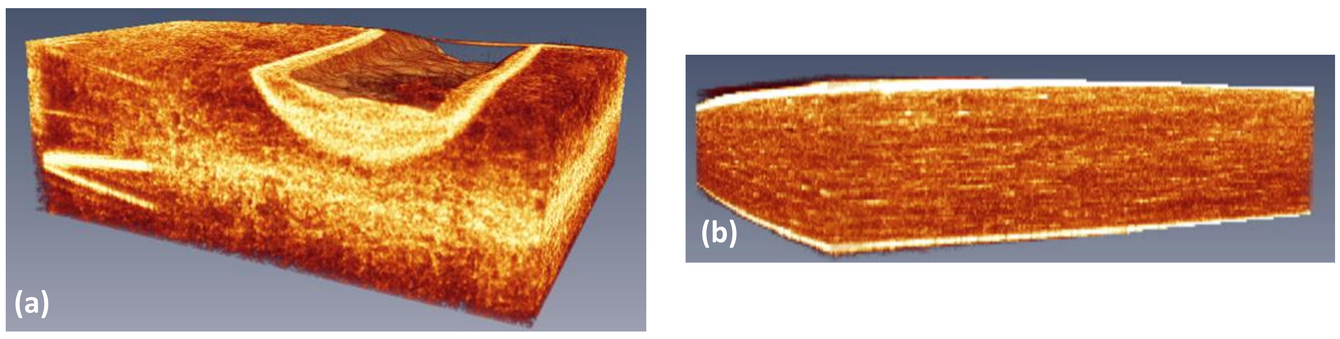


Figure 7. (a) OCT visualization of 3D corneal model with lesion of a size of 3 x 3 x 1 mm and (b) the collagen fibre bundles in the corneal model of a size of 3 x 3 x 0.7 mm.

- The cross correlation of OCT/OCE image is illustrated in Figure 8. The lesion area is noticed to be stiffer in stiffness with OCE, which is verified with an increase of cell density around the lesion boundary as shown in the confocal imaging.

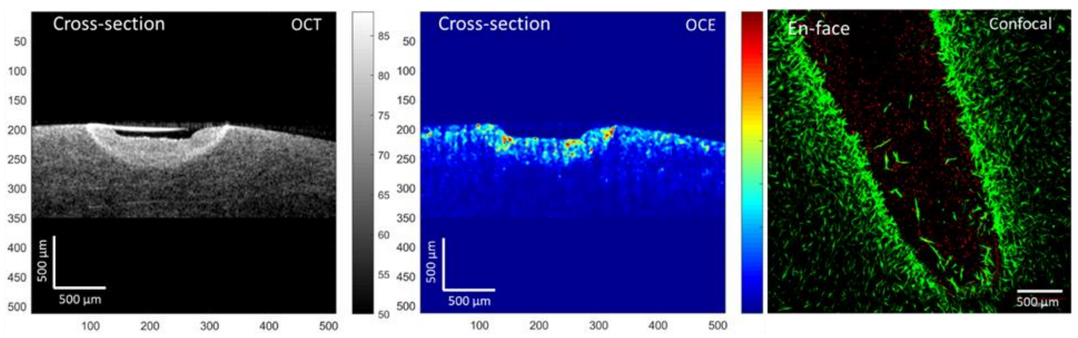


Figure 8. OCT, OCE and confocal imaging of the corneal lesion.

CONCLUSION

- A corneal 3D model was successfully established using tissue-engineering techniques. OCT/OCE was proved to be capable to characterize the lesion and demonstrate the stiffness variation between the lesion and benign area. After lesioning, the wound was shown with the higher stiffness owing to collagen alignment and interaction between the fibroblasts and epithelia.
- The future works will focus on quantifying the model stiffness. The ultimate goal is to use OCE to monitor the lesion change during would healing process and drug development.

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