Laser **A**ssisted **RO**botic **S**urgery of the anterior **E**ye **S**egment

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[1] Dissemination Level:
PU Public
PP Restricted to other programme participants (including the Commission Services)
RE Restricted to a group specified by the consortium (including the Commission Services)
CO Confidential, only for members of the consortium (including the Commission Services)

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Summary

1 Executive Summary

This document reports the approach followed for the analysis of the thermal effects induced in a porcine cornea by the diode laser used for welding. The results of Hematoxylin and Eosin staining of treated and not-treated corneal tissues are reported.

2 Introduction on cornea morphology

The cornea is the transparent part of the eye that covers the iris and pupil; whit the lens and the anterior chamber, it refracts the light. It's composed by five layers: the corneal epithelium, the Bowman's membrane, the stroma, the Descemet's membrane and the endothelium.

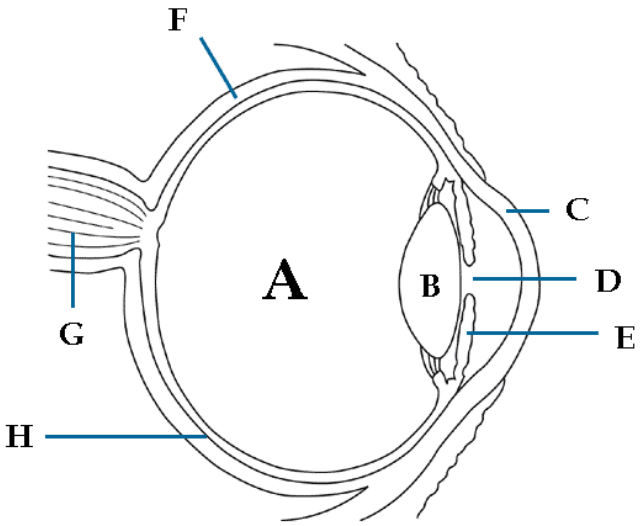

Figure 1: Sketch of an eyeball: A) vitreous humor, B) lens, C) cornea, D) pupil, E) iris, F) sclera, G) optic nerve, H) retina

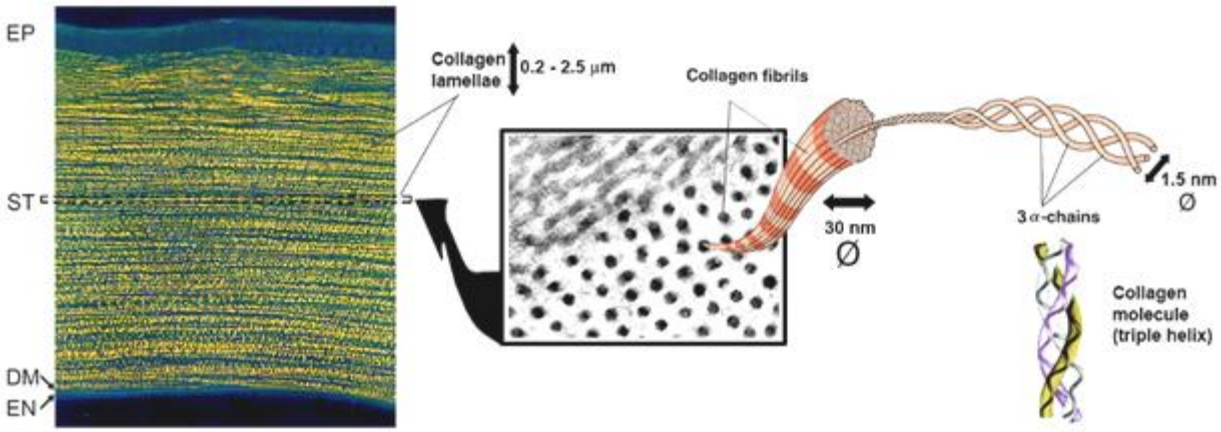

Figure 2: Corneal layers and collagen structure

The cornea is highly innervated and it is avascularized. This properties, together with the peculiar type I collagen distribution within the stroma, are essential to maintain the transparency and the refractive power. The refractive power of the cornea is about 43

dioptres and depends also on the cornea curvature. For this reason, it's of utmost importance to maintain the natural corneal curvature also during surgical interventions.

3 Analysis of the porcine corneas: Materials and Methods

In this experiment we used four pig's eyes. In three samples, the surgical incision was made with a punch suitable for corneal surgery; in another sample the incision was performed manually. All the interventions are described in D7.4.

After the treatments, the corneas were harvested from the bulbs, fixed in solution of 3.6% paraformaldehyde diluted in phospate buffer saline (PBS). The fixed samples were then sectioned with a surgical knife in order to extract only the portion of tissue treated with the welding laser.

The samples were included in a matrix suitable for cryosectioning (Tissue-Tek, Bio-Optica) and the slices were collected on a polarized slides (ThermoFisher). For this work we used a blade for hard tissue, in high-grade steel. A Leica CM1950 cryostat (Ag Protect) was used for cryosectioning.

The welding effects on corneal collagen were detected by histological staining of hematoxylin-eosin (ready to use solution) (Sigma-Aldrich).

The protocol is described here in the followings:

1) ethanol was applied on slides and allowed to evaporate at room temperature;
2) the slides were quikly washed in water;
3) hematoxylin was applied on the slides to cover all slices. It was left for 1 minute and 30 seconds;
4) the slides were washed in water to remove the hematoxylin in excess;
5) eosin was applied on the slides to cover all slices. It was left in place for 2 minutes;
6) the slides were washed in bidistilled water to remove the eosin in excess;
7) the slides were left 10 minutes at room temperature to dry out;
8) coverslips were mounted by applying a drop of distilled water;
9) the slides were observed under an optical microscope using a magnification of 4x and 10x and pictures were captured with a 5 megapixels camera.

Healthy corneas and laser treated corneas were observed.

4 Analysis of the porcine corneas: Results

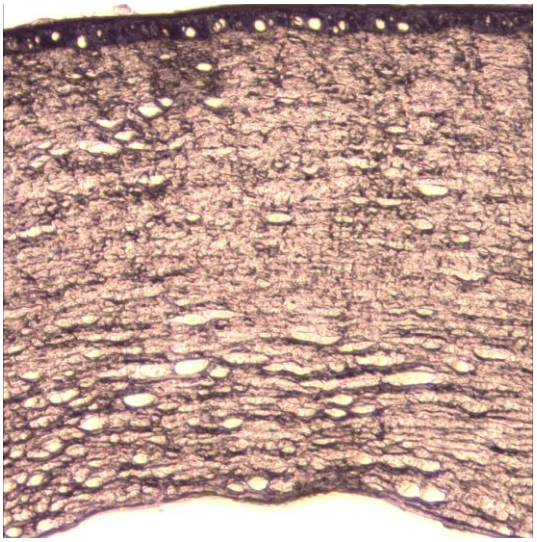

Figure 3: image of a healthy pig cornea

In these images the corneal epithelium is purple, and the corneal stroma is pink. In Figure 3 a portion of a healthy porcine corneal tissue is shown.

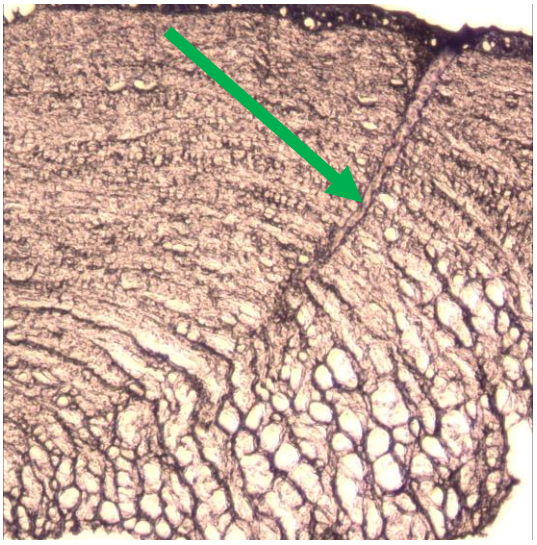

Figure 4: A laser welded cornea: it is clear the surgical incision filled with denatured collagen.

These picture shows an area of stromal collagen welded with the laser. No damage is observed in the surrounding tissue, which maintains the natural morphology and structure. The performed surgical cut is modeling a penetrating keratoplasty.

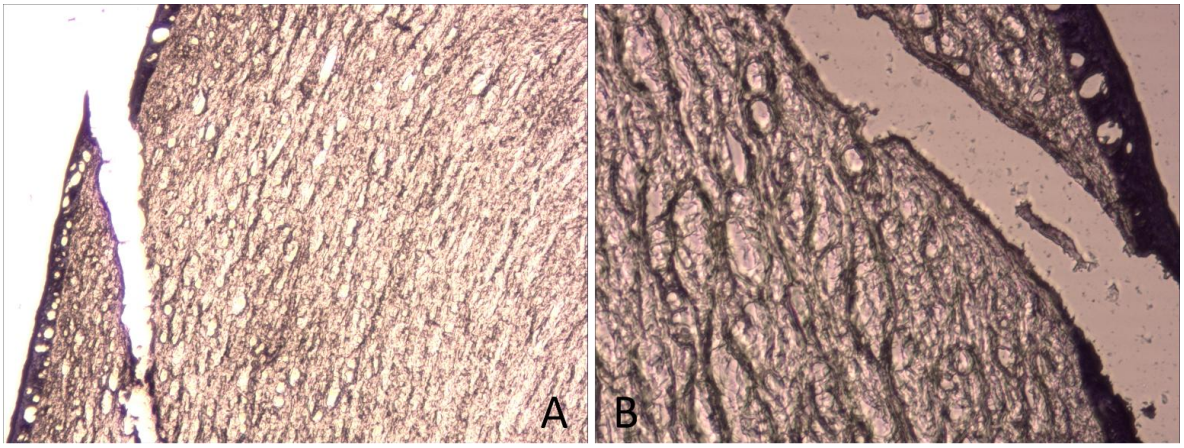

Figure 5: Two images of non-treated cornea. Image A: magnification 4x. Image B: magnification 10x.

In Figure 5 we can observe in A: surgical incision not-treated with ICG and wheren't has not been used the laser. This is not due to a strain because the margins are definite (in B).

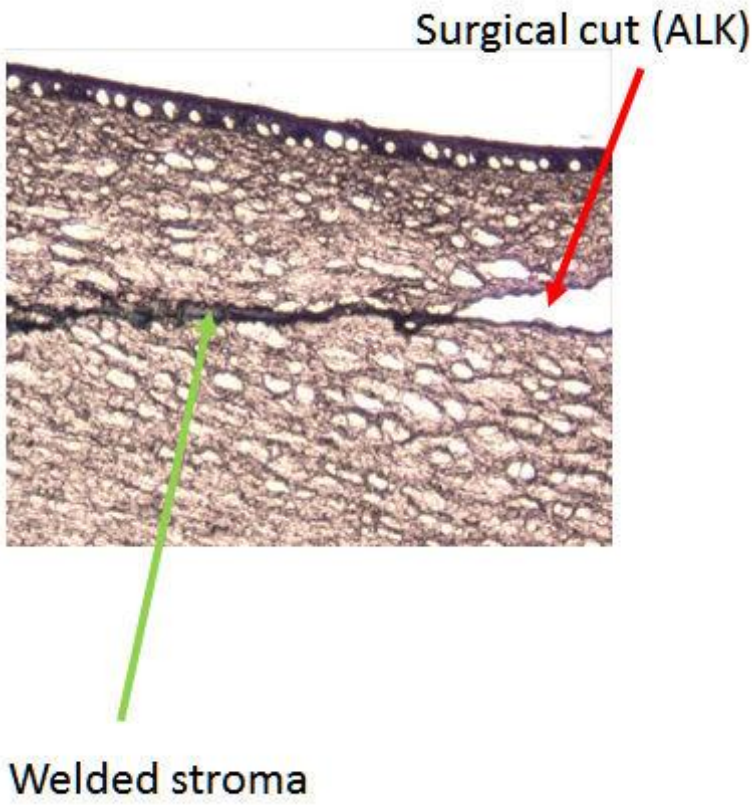

Figure 6: A welded stroma in a horizontal cut, simulating anterior lamellar keratoplasty.

Other cornea samples were used to model Anterior Lamellar Keratoplasty (ALK): histology evidenced that also in these irradiation conditions the wedling effect is induced in the deep collagen. No collateral side effects were found.