before the beginning of the experiments (pre-wound) was assessed using the vital dyes trypan blue (TB, TB-S 0.25%, AL.CHI.MI.A. srl) and sodium fluorescein (Fluo). 1-heptanol soaked paper disks (6 mm) were applied in the centre of the corneas for 1’ to trigger a chemical damage at the epithelial layer. Afterwards, sodium fluorescein and TB stainings were repeated to quantify the damaged area and to monitor healing progression. The damaged area (mm2) was calculated for each time point with Fiji software. Wound healing rate (HR, mm2/die) was calculated for both Fluo (HRF) and TB (HRTB) measurements using the previously described formula:

Arithmetical averages (HRFAVG and HRTBAVG) of HRs were calculated and correlated by Pearson correlation coefficient with the following donor’s parameters: age, sex, post-mortem time (PMT, time between death and tissue procurement), stromal defects, sepsicaemia, body temperature, diabetes.

Results The execution of the heptanol wounding is highly reproducible, as highlighted by Fluo and TB staining. The average time for full recovery from wounding was 3.8 ± 0.41 days for Fluo and 3.5 ± 0.63 days for TB. Fluo and TB stainings are interchangeable as they significantly correlate (Pearson correlation coefficient = 0.630; p>0.05). A negative linear correlation was observed between HR and PMT (HRFAVG: corrected R2: 0.243, p = 0.003; HRTBAVG: corrected R2: 0.132, p = 0.028), but not with the other donors’ parameters.

Conclusion Our wound/healing model might be of great interest for studies of epithelial regeneration kinetics and validation of drugs for the treatment of ocular defects. The inverse correlation between PMT and HR provides valuable insights for understanding of what components of SED contribute to their activity is limited. SEDs are produced from a patient’s own blood or from an allogeneic donor source. The serum component is separated from the whole blood which is then diluted 50/50 with sterile saline, and contains bioactive molecules that are believed to help heal and maintain the ocular surface. The objective of this study is to quantify the amount of bioactive molecules in donor serum, and to understand how processing variables effects these factors.

Methods Samples of SEDs from 28 male allogenic donors were taken from ultra-low temperature storage and thawed. They were then centrifuged at 13,000 rpm at 4oC to remove potential contaminants such as residual red blood cells. Duplicate test samples were analysed for epidermal growth factor (EGF) and fibroblast growth factor (FGF) using ELISA kits. Analysis was carried out using Excel.

Results The age range of the donors was 17 to 79 years (mean 47.9).

Mean time from venepuncture to refrigerated storage was 6 hours 12 minutes with time ranging from 2 hours 40 minutes to 9 hours 35 minutes.

The concentration of EGF found in the diluted serum ranged from 0.048 to 1.90 ng/ml (mean 0.87 ng/ml), and FGF concentration ranged from 4.88 to 39.50 pg/ml (mean 12.37 pg/ml).

Analysis showed that there was no correlation between either age of the donor, or sample transfer time and growth factor concentration.

Conclusion Our study demonstrated that with both types of growth factors measured in the SED, a wide range of concentrations were found in the donor samples. Compared to published data EGF was at higher range while FGF was lower. Further analysis of other factors present in the donor serum is being undertaken to determine if any pattern can be found.