P18-A148 ASSYMETRICAL INJECTOR FOR ENDOTHELIUM-IN DMEK WITHOUT THE NEED OF PULL-THROUGH TECHNIQUE

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Purpose One of latest surgical development of preloaded Descemet membrane endothelial keratoplasty (DMEK) is the delivery of the graft with the endothelium inwards, which allows for a very fast operation, but requires a pull-through surgical technique. Although the tri-folded, endoth-in DMEK technique has significant advantages, the absence of proper surgical instruments that could allow their use without the ‘pull-through’ technique still restricts the wide use of such an operation. None of the available commercial DMEK injectors could be used for tri-folded DMEK (endothelium-inward) orientation, as it requires the graft to be intently secured within the injector. This report presents a retrospective eye bank validation study of an asymmetrical injector designed to orientally implant a tri-folded DMEK graft without needing a pull-through technique.

Methods The injector is made from transparent plastic, allowing microscopic tissue validation directly before injection. The device is asymmetrical, so the orientation of the graft can be controlled and validated according to the best eye bank practice, which is critical for successful tri-folded DMEK graft clinical application. Four different designs of the internal compartment of the injectors were evaluated with DMEK tissues. Mates from two pairs were tested on each device type, totaling 16 grafts, all loaded with folded, endoth-in grafts. The tissue was prepared, loaded into the injector, and ejected to simulate the tissue manipulation in DMEK operation.

Results After graft loading the delivery of the endothelium-in grafts was performed by injection, without the need for a pull-through technique. One graft (6.25%) has double-scrolled (changed its folding) within the injector with a larger (1.5 mm) internal compartment. The loss of valuable cells was limited to 3-23% (13.98% average). No significant differences in cell loss were observed between injectors with different tissue volumes. Higher viability loss (17.3% +/- 5.7) was observed for the grafts with >20 days death to prep-days in comparison with grafts stored with less than two weeks (10.9% +/- 2.1).

Conclusion The TissueGUARD injector is the only injector that currently allows oriented, tri-folded DMEK injection without the need for a pull-through technique. The average cell loss after loading and ejection was 13.98%, which is comparable/better than the current best practice with the pre-cut-preloaded technique of naturally folded DMEK.

P19-A118 BOWMAN LAYER ONLAY: PREPARATION AND TRANSPANTATION (BLOT)

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Purpose With the introduction of Bowman layer onlay transplantation (BLOT), the need for BL transplants increases. In this study, the clinical outcomes of BLOT are described and the results of three different BL graft preparation methods are evaluated: manually (m-BL), femtosecond laser-assisted (fs-BL), and femtosecond laser-assisted followed by excimer laser (fs/ex-BL).

Method Twenty-one eyes with advanced progressive keratoconus underwent BLOT with m-BL. Best spectacle- and/or best contact lens-corrected visual acuity (BSCVA/BCIVA), corneal tomography, and complications were recorded. Follow-up ranged from 6-36 months with a mean follow-up time of 21 ± 12 months.

To evaluate BL preparation methods, Descemet membrane-denuded donor corneas (n=41) were used (n=2 for m-BL, n=18 for fs-BL and n=21 for fs/ex-BL). For fs-BL, corneas were placed on an artificial anterior chamber and different depth cuts were performed with decreasing decrements starting from 30 μm (diameter 9.0 mm). For fs/ex-BL, a superficial flap of 80 μm was created by the femtosecond laser (FEMTO-LDV Z8, Ziemer). Followed by residual stroma ablation by excimer laser (Schwind Amaris 750S) with increasing increments. Grafts were analyzed visually, and graft thickness regularity was evaluated by histological analysis and Transmission Electron Microscopy (TEM).

Results All twenty-one surgeries could be performed without intraoperative complications. Average maximum keratometry changed from 75.8 ± 12D preoperatively to 72.2 ± 9D at the last available follow-up (n=21, P<0.05), and BSCVA/BCIVA improved. Five patients required a regraft; four of those because of a graft detachment within one week.

Evaluation of BL-preparation methods: Fs-BL preparation was successful until 14μm cuts (success rate: 12 out of 14, 86%). Fs/ex-BL graft preparation was most successful after an 80μm cut by femtosecond laser with subsequent 60μm ablation by excimer laser (success rate: 15 out of 21, 71%). After the femtosecond laser cut, traces of the femtosecond laser treatment were visible on the flap. While m-BL showed long protruding stromal fibers, they were shorter in fs-BL and absent in fs/ex-BL.

Conclusion BL-onlay grafting may be a feasible surgical technique, providing on average -3D of corneal flattening in eyes with advanced progressive keratoconus, while improving patient’s visual acuity.

Fs-BL and fs/ex-BL preparation may be faster alternatives to manual BL graft preparation.

P20-A129 AN INNOVATIVE WOUND HEALING METHOD REVEALS A DONOR AND POST-MORTEM TIME-DEPENDENT REGENERATION OF CORNEAL EPITHELIUM

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Purpose The aim of this study was to establish and optimize a new and reproducible epithelial wound healing model on human corneas. This assay was used to study the kinetics of epithelial regeneration following a chemical injury.

Methods Thirty (n=30) human corneas unsuitable for transplant were used for the experiments. Corneas were cultured in Storagix medium (FBOV) at 31°C. Epithelial integrity...
before the beginning of the experiments (pre-wound) was assessed using the vital dyes trypan blue (TB, TB-S 0.25%, ALCHI.MIA. 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 (HRAVG 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, septicaemia, 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 (Pearson correlation coefficient = 0.630; p>0.05). A negative linear correlation was observed between PMT 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 scientists investigating the regenerative properties of the corneal epithelium, as well as for eye bank personnel aiming to preserve the regenerative potential of corneal epithelium.

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.