Pre-prepared Corneal Grafts for Facilitated Descemet Membrane Endothelial Keratoplasty (DMEK) — Controlled and Standardized Manufacturing in the Eye Bank May Lead to Reduced re-DMEK Rates

Nicola Hofmann, Ilka Wittmershaus, Regina Michaelis, Tanja Petrich, Lutz Blomberg, Martin Börgel

ABSTRACT

Purpose: Descemet membrane endothelial keratoplasty (DMEK) has become the gold standard for partial thickness corneal transplant surgeries. In the past most lamella were prepared by the surgeon directly before the operation in the operating room (OR). Since 2015, a preparation procedure in a DGFG[1] eye and tissue bank was established to supply transplant surgeons with pre-cut cornea grafts. However, the question arises whether this could have an influence on the clinical outcome of DMEK.

Method: Using our preparation procedure, pre-cut donor corneas are produced with a success rate of ≥ 90%. A comparison of the numbers of patients requiring regrafts according to DMEK/LaMEK was carried out to provide information about the success of the procedure when using pre-cut lamellae.

Results: Since December 2015, the DGFG holds a Paul-Ehrlich-Institute (PEI)[2] marketing authorization and has delivered more than 600 pre-cut donor corneas for DMEK procedures. marketed by DGFG as LaMEK. Compared to 2015 with grafts prepared exclusively in the operating room, in 2016/2017, with the prepared LaMEK grafts, a smaller number of regrafts were reported in four transplantation centers. In 2015, 22 out of 204 OR-prepared lamellae DMEKs had to be regrafted (MW 10.8% regraft rate). During the reference period 2016 to autumn 2017 in the same transplant centers, only six out of 147 LaMEK prepared corneas (MW 4.1%) were regrafted.

Conclusions: With the introduction of pre-cut donor corneas (LaMEK) for DMEK procedures, the technique for the surgeon has been significantly simplified, since a preparation risk is excluded and time and costs in the OR are reduced. In addition, use of LaMEK seems to reduce the risk of regraft for the patient.

For the treatment of corneal diseases caused by insufficiency of the corneal endothelium Descemet Membrane Endothelial Keratoplasty (DMEK) has evolved into the gold standard.[1] The most common indications for replacing the two inner layers of the cornea—the descemet membrane and the endothelium—are Fuchs endothelial dystrophy, forms of bullous keratopathy, and endothelial failure after penetrating keratoplasty (PK), DSAEK (Descemet Stripping Automated Endothelial Keratoplasty) or traumatically performed cataract surgery.

These are the most common corneal diseases in industrializations, and they tend to increase as a result of higher life expectancy. In this technique of keratoplasty, a descemetorhexis—removal of the descemet membrane and the diseased corneal endothelium—is performed in the recipient. These two corneal layers are then replaced by a donor graft consisting of the descemet membrane with healthy endothelium. The turbidity and decompensation of the cornea, caused by pathological changes in the endothelial layer, recede after the transplantation of the lamellar graft. Through DMEK, not only the functionality of the corneal endothelium, but also the anatomy of the cornea is correctly restored. The advantages of this surgical technique, compared to conventional PK, are a faster and better recovery of the visual acuity because only the affected endothelial cell layer on the descemet membrane is transplanted. Further advantages are a significant reduction in astigmatism as well as a reduction in the risk of immunological rejection reactions, since corneal tissue is avascular

Author Affiliation: German Society for Tissue Transplantation (DGFG) gGmbH, Hannover, Germany

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[1] The German Society for Tissue Transplantation (DGFG) is an independent non-profit organisation (gGmbH) with a nationwide network of eye and tissue banks, cooperating hospitals and transplanting institutions.

[2] The Paul-Ehrlich-Institute (PEI) is the senior federal authority in the field of medicinal products in Germany.

and generally has a low immunogenicity. In the separate transplantation of the two lower layers, the risk of immunological rejection reactions is further reduced since significantly less antigenic material is transferred from the donor to the recipient.[2]

Since very thin grafts are inserted into the anterior chamber of the eye through small incisions at the limbus, the risk of serious intra- and postoperative complications such as expulsive bleeding, infections and wound healing disorders is also significantly reduced. In addition, the surgical procedure is minimal and there are no suture-associated complications.

The approximately 15 µm thick transplants for DMEK are usually produced manually. To date, in Germany the vast majority of transplant grafts for DMEK are prepared by the ophthalmic surgeons in the operation theatre shortly before surgery. However, the manufacturing process of the grafts can be poorly standardized or validated. For example, corneas may be stored in organ culture as well as hypothermic media, requiring different preparation techniques to be used. Density, morphology and vitality of the endothelial cells usually cannot be controlled nor the microbiological purity after the preparation. The quality of the grafts produced in this way ultimately remains uncertain. In addition, the manufacturing process carries a relatively high risk of failure, which can lead to a problematic loss of donor tissue. By transferring the preparation of the corneal graft for DMEK to an eye bank, the manufacturing process is undoubtedly improved, making the grafts safer and of higher quality for the patient. Preparing cornea grafts in the eye bank thus reduces the risk of waste in the operating theatre, postoperative complications and the time and costs of the surgical procedure. The procedure also makes DMEK considerably easier and simpler for the surgeon. In 2014 in the US, 68% of DMEK-prepared corneas transplanted were prepared by eye bank.[3]

The aim of the DGFG was therefore to establish the preparation of prepared corneal grafts for DMEK in our eye and tissue bank in order to exclude the aforementioned deficits and risks. However, the production of the prepared grafts in an eye or bank is also associated with challenges. The focus here is on ensuring an optimal quality control of each prepared graft and the safe transport of the very thin and sensitive grafts to the transplant center.

Furthermore, it is of increasing interest whether the lamellae produced in this way have an influence on the clinical outcome of the DMEK procedure. Various studies are now tracking whether there are differences in the clinical course when comparing the use of the lamellae produced directly in the operating theatre with those prepared by the eye bank. In fact, several studies have been published in which it is reported that the use of prepared corneal grafts achieves comparable clinical results to grafts produced in the operating theatre. The graft failure of prepared lamellae is not rated higher in any of the published studies. Various authors emphasize that a standardized method of production in particular leads to high-quality grafts.[4-8] However, contrary to the results of these various studies, one paper[9] also describes that the use of 11 prepared DMEK grafts led to higher graft failure compared to 453 lamellae produced in the operating theatre. In order to clarify these contradictory results, data from several transplantation centers using prepared DMEK grafts from the DGFG were evaluated.

METHODS

To solve the above-mentioned manufacturing-related problems, we have developed the concept of an adhered prepared graft (Fig. 1). For the preparation of the prepared

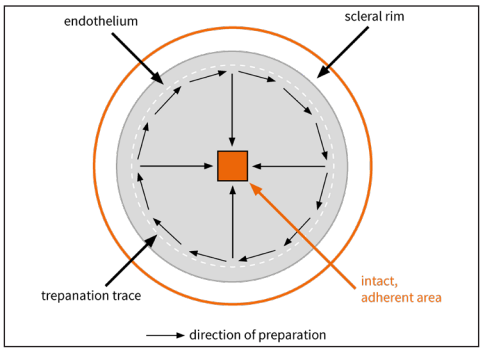

Fig 1. *Scheme of Preperation*

corneal graft for DMEK, the "human cornea, organ-cultured, DGFG" (already approved PEI.G.11566.01.1) is fixed on a punching block (see Fig. 2) with the endothelium side upwards and slightly punched from the endothelium

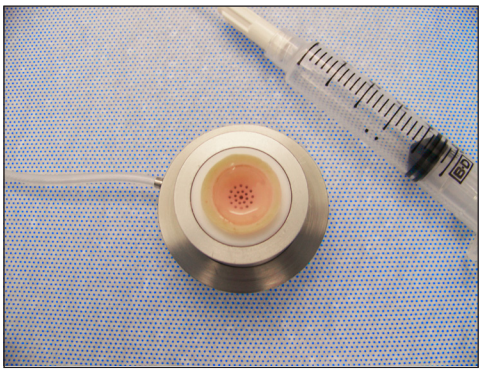

Fig 2. *Vacuum Punch Device with Fixed Corneo-scleral Rim*

side by means of a corneal punch. The standard diameter of the trepan used by DGFG is 8.5 mm, but other sizes may be requested. To visualize the trepanation trace, a trypan blue staining is applied after punching. Thereafter, the descemet membrane separated in the periphery is dissected starting from the trepanation trace so that a small area in the middle remains adhered, i.e., not separated from the corneal stroma (Fig. 3). After completion of the preparation, the descemet membrane with the endothelial cells is placed back on the stroma by dripping the de-swelling medium. The remaining intact area fixes the prepared graft on the inner surface of the cornea. The adhesive power between the detached areas of the descemet membrane and the corneal stroma are sufficiently strong to ensure thorough quality control and to securely transport the prepared grafts. Endothelial cell density, morphology and vitality are determined both before and immediately after preparation and 4 days after preparation for final release. In addition, a microbiological check of the prepared grafts is carried out before shipping (Fig. 4: shipping container).

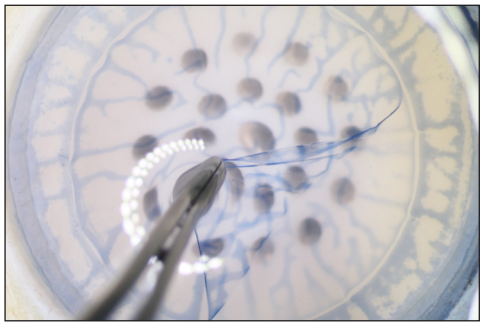

Fig 3. *A central area of the DM remains connected with the stroma*

The final detachment of the transplant occurs immediately before the start of the surgical procedure and is performed by the ophthalmic surgeon himself.

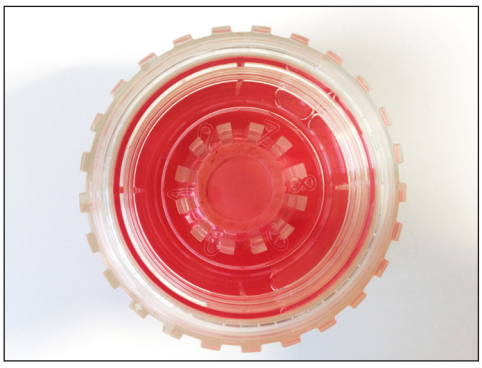

Fig 4. *Shipping Container*

Since 2016, more than 600 grafts produced in this manner have been delivered for transplantation. Against the background of these application experiences, it was then additionally possible to examine whether differences in the clinical outcome occur when using prepared grafts compared to grafts produced directly before the operation in the operating theatre.

RESULTS

For this study, organ-cultured corneas unsuitable for transplantation, but with an adequate endothelial cell density and vitality, were used to develop the preparation procedure as described herein. Examination of the endothelial cell density, morphology and vitality showed that the endothelial cell density decreased by only 0.02% immediately after preparation and by less than 5% within one day thereafter. Even 4 to 6 days after production, endothelial cell loss was acceptable at about 8% (Fig. 5).

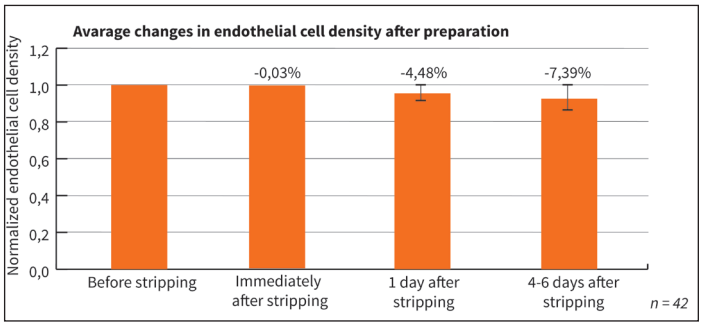

Fig 5. Avarage changes in endothelial cell density after preparation

These results ultimately made it possible to obtain a national marketing authorization for prepared transplant grafts in Germany. In December 2015, the Paul Ehrlich Institute granted the DGFG approval for the tissue preparation "LaMEK: pre-prepared corneal transplant for DMEK" (PEI.G.11785.01.1). Since then, more than 600 prepared LaMEK grafts have been delivered to various transplantation centers. The feedback from the transplant surgeons has been predominantly very positive (Tab. 1) with the respondents praising the easy handling and the good quality of the grafts.

In addition, in the investigation beyond these short-term results, data from several transplant centers have been compared with regard to the clinical outcome when using the prepared LaMEK.

The evaluation suggests that the rate of regraft after the use of prepared LaMEKs has been reduced compared to the previous year. The number of patients that were registered with DGFG for regrafts from 2015 to 2017 was considered.

When comparing the 2015 transplant outcomes with grafts prepared exclusively in the operating room, to the period of 2016 to autumn 2017 outcomes, using prepared LaMEK grafts, a clearly smaller number of re-enrollment patients were reported in four transplantation centers.

Four centers that previously transplanted cornea lamellae prepared exclusively in the operating theatre reported significantly fewer patients for regraft in 2016/2017 time period after utilizing prepared LaMEK tissue compared to the previous year 2015. In 2015, 22 out of 204 patients had to undergo regrafts in the four centers after receiving DMEK with lamellae prepared in the operating theatre (MW 10.8% regraft rate). During the reference period 2016 to autumn 2017 in the same centers, only six out of 147 patients required regrafts after receiving DMEK using LaMEK tissue (MW 4.1%) (Tab. 2). In two of these centers, no regrafts were necessary after patients received prepared LaMEK tissue.

DISCUSSION

Descemet membrane endothelial keratoplasty (DMEK) has increasingly become the method of choice for the treatment of corneal diseases caused by functional loss of the endothelium. After the technique was first described by Melles in 2006,[10] numerous groups have dealt with this method and published various different procedures.

However, due to the greater challenge that the transplant production poses to the cornea surgeon, the technique was initially used only hesitantly. With the provision of prepared lamellar corneal grafts by eye banks, however, this limitation becomes increasingly less severe.

The DGFG has successfully developed a method to produce prepared grafts for DMEK, known as LaMEK, in the tissue bank with a graft success rate of ≥ 95% and a mean endothelial cell loss of less than 10%. The preparation of the graft tissue in the eye bank allows a production under clean room conditions and a standardization of the manufacturing

Table 1. *Feedback from transplant surgeon via questionnaire (n=182)*

[%]	transport	position	necroses	final detachment	roll forming	transfer	quality
not specified	1,1	0,0	15,9	0,0	4,4	2,2	0,0
very good	85,2	94,5	56,6	35,2	43,4	31,3	44,0
good	13,7	5,5	21,4	56,0	36,8	60,4	48,4
deficient	0,0	0,0	4,4	7,7	13,5	5,5	6,0
poor	0,0	0,0	1,6	1,1	2,2	0,5	1,6

Table 2. *Comparison of re-grafts in the period 2015 compared to 2016 till autumn 2017 from four transplantation centers. The lamellae used for transplantation in 2015 were prepared the centers directly before surgery, whereas in 2016 and 2017 prepared LaMEK from the tissue bank were used in these centers.*

Comparison of registrations for regrafts of patients from 4 transplant centers (TX)				
2015 compared to 2016 - 09/2017				
Center	Period DMEK/LaMEK	OP number	Regraft after graft failure	percentage [%]
TX1	2015 DMEK	129	**8**	**6,2**
TX1	2016 - 09/2017 LaMEK	33	**0**	**0**
TX2	2015 DMEK	29	**6**	**20,6**
TX2	2016 - 09/2017 LaMEK	55	**4**	**7,3**
TX3	2015 DMEK	31	**5**	**16,1**
TX3	2016 - 09/2017 LaMEK	34	**0**	**0**
TX4	2015 DMEK	15	**3**	**20**
TX4	2016 - 09/2017 LaMEK	25	**2**	**8**

process. As a result, through microbiological controls after the production as well as the quality control of each lamellar graft before distribution, only LaMEK grafts with excellent quality are subsequently delivered for transplantation. The postoperative complications potentially associated with a less than high quality graft can thus be minimized and the time and costs of the surgical procedure significantly reduced. Final detachment of the graft is straightforward and can be performed directly before surgery.

Since December 2015, prepared corneal grafts for DMEK have been delivered by the DGFG with the approval of the Paul Ehrlich Institute and more than 600 so-called LaMEK have been allocated and transplanted. Our data indicate that compared to the previous year, the use of prepared grafts has considerably reduced the rate of regraft. An experimental study conducted in parallel at the Eye Clinic Sulzbach Saar seems to confirm these data.[11]

As this result was obtained indirectly by recording the re-registration of patients for a new operation, it should be mentioned that not all patients requiring further surgery may have been treated with a transplant from the DGFG. In addition, the learning curve of the surgeons certainly plays a supplemental role.[12-13]

Nevertheless the data underline the realization that the preparation of the lamellae has clear advantages for the surgeon and patient.

At the same time, the reduced regraft rate could indicate a lower tendency to graft failure. It seems plausible that the period of time between the pre-preparation and the operation represents a rest phase for the endothelial cells in which it is possible to recover from the stress of the manipulations. In the absence of further stress during the pause, the induced regulatory processes of the cell could predominate in favor of the repair mechanisms, so that cells that have already been damaged by the preparation can also recover, which would otherwise undergo the apoptosis program once activated during subsequent surgery. A future study will further clarify the physiological relationships that could underlie the reduced regraft rate.

On the other hand several factors have already been published in the literature that could lead to an increased risk of transplant failure. Although the use of pre-prepared corneal lamellae indicates a lower rate of regraft, graft failure cannot be ruled out in the future either. The following other reasons can lead to graft failure in the context of a DMEK:

- Learning curve of the transplant surgeon and necessary standardization of the surgical process, e.g. by avoiding a plastic implant cartridge: As a result of standardization of the technique, the rate of postoperative complications decreased from 23.2% to 10% (P<0.001) and the rate of necessary second operations from 6.8% to 3.6% (P=0.10).[12-13]
- Intraoperative difficulties (graft unfolding, upside-down localization, overlap): Loss of the endothelial cell layer to varying degrees and a positive correlation with intraoperative difficulties are the outstanding features of primary and early DMEK graft failure. The rate of graft detachment and endothelial cell loss increases significantly with more difficult graft development in DMEK surgery.[14]

  Results show the importance of a central, well-positioned graft and the relationship to the severity of the disease. A well-positioned central graft can reduce the incidence of graft detachment. An overlap of the donor lamella and the Descemet membrane of the recipient seems to be responsible for the detachment. One way to improve adhesion could be a greater descematorhexis that avoids overlap. Greater descemetorhexis in DMEK correlates with better graft adhesion and lower rebubbling rates. Therefore, patients with a larger descemetorhexis need less intensive follow-up. [12, 15, 16, 17]
- Intrinsic recipient factors: Conclusion from graft failure due to Re-DMEK complications can be better prevented than primary DMEK, as graft detachment and graft failure tend to recur. This suggests that intrinsic properties of the host eye play a role in graft adherence and graft failure.[18]
- Transplant failure may be gender-specific: mismatch in a transplant of a male donor and a female recipient can lead to an increased risk of transplant rejection, especially in Fuchs' endothelial dystrophy: H-Y-antigen-mismatched patients had a higher risk of rejection or graft failure.[19]
- Cooperation and compliance of the patient after the operation

CONCLUSION AND OUTLOOK

In summary, it can therefore be concluded that the use of prepared corneal grafts from the eye bank offers outstanding advantages, taking into account the findings already published. Since the preparations are quality-tested, a loss of grafts in the operating theatre is prevented and time and costs in the operating theatre are reduced. In the meantime, results of clinical studies over a period of up to 8 post-operative years after DMEK surgery[20] prove that DMEK is a safe and successful surgical method in the long term.

The use of the prepared corneal transplants for DMEK makes the application in the operating room easier for the ophthalmic surgeon and thus also makes the procedure more secure for the patient. In the future, the handling of the sensitive transplant tissue in the operating room should be made even easier. For this purpose, investigations are currently being conducted on the delivery of the pre-loaded corneal lamellae in implantation cartridges prepared under clean room conditions in the eye bank. The aim of the DGFG is to obtain authorization from the PEI for this shipping procedure as well.

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