# Possibilities of Using Corneal Stromal Lenticules Obtained During Relex SMILE Refractive Surgery for Transplantation Purposes. A Review

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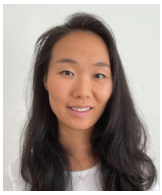

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## SUMMARY

A corneal stromal lenticule (CSL) is a part of the corneal stroma that forms as a waste product during the refractive surgery ReLEx SMILE (Small Incision Lenticule Extraction) and is no longer used.

With the increasing number of ReLEx SMILE procedures and the number of potentially available CSLs, two main aspects of their usage are currently being investigated. The first aspect includes the biological properties of CSLs and their proper preservation with respect to long-term storage. The second aspect is related to the potential clinical use of CSLs. As a high-quality biomaterial, CSLs have substantial potential to be used for alternative solutions in the treatment of specific eye diseases.

In a number of studies it has been shown that RSL transplantation could be a safe and effective method that does not cause any serious complications, for example in terms of immune reaction.

The aim of this article is to present an overview of the possibilities for using CSLs for transplantation purposes, and at the same time to discuss our methodology for processing and preserving CSLs with the protocol used at the Eye Tissue Bank of the Královské Vinohrady University Hospital.

**Key words:** corneal stromal lenticule (CSL), ReLEx SMILE, cryopreservation

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## INTRODUCTION

A corneal stromal lenticule (CSL) forms as a waste product during ReLEx SMILE refractive surgery, a method in which a stromal lenticule with a diameter of approx. 8 mm and thickness of 50–150 µm, approx. 14 µm per one removed diopter (D) is created with the aid of a femtosecond (FS) laser in the central part of the cornea of refractive surgery patients. This lenticule is subsequently mechanically separated and removed, thereby causing a change of curvature of the anterior surface of the cornea and a change of its basic refraction. The ReLEx SMILE method is used primarily for the correction of medium and higher myopia. The CSL as waste tissue is subsequently liquidated as biological waste in the regular manner.

A CSL is composed predominantly of corneal collagen and a smaller amount of keratocytes. With reference to the fact that this concerns quality, healthy tissue, possibilities for its further use have been described in the professional literature. One potential application we can present is its use for allotransplantation, for example in order to cover corneal defects, in the case of small perforating traumas, in patients with keratoconus, for correction of far-sightedness or presbyopia. To date mainly corneas from deceased donors have been used as standard in these cases, prepared according to the standardized procedures in tissue bank conditions. However, limiting factors are above all the limited availability of such cadaverous tissues and also the high price of donor corneas, from which only a small part is subsequently used in the above-stated indications.

It would therefore appear highly advantageous to use CSLs in such cases. Nevertheless, the use of fresh tissue is complicated, primarily with regard to logistics. Refractive procedures of the ReLEx SMILE type are performed only in a small number of predominantly private refractive centers, while by contrast patients for whom the use of such a lenticule might come into consideration often visit highly specialized corneal centers, mainly university clinics. A further complication is the need to perform all the necessary and prescribed examinations 6 months from the taking of the tissue sample from the live donor in order to eliminate the risk of transmission of infectious diseases.

From the above it is evident that the use of tissue in a fresh state is an extremely complicated issue. It is therefore desirable to use a method that enables the long-term storage of CSLs. The processing and storage of CSLs using the technique of cryopreservation appears to be the optimal solution. This solution would be highly relevant due to the unavailability of donors of corneal tissue, especially in developing countries. The method of corneal cryopreservation has not been adopted in regular practice due to the risk of damage to the corneal endothelium. However, in the case of CSLs and their use in the above-stated indications, no role is played by the influence of cryopreservation on the cornea. Furthermore, studies confirm that cryopreservation does not cause damage to collagen fibers, which is of key importance for preserving transparency of the cornea after CSL implantation [1,2].

**Obtaining, processing and storing CSLs**

In the case that a donor consents to provide a CSL for medical purposes or research, a patient consent form is signed before the planned ReLEx SMILE operation, and at the same time a blood sample is taken for serological examination for bloodborne infections (hepatitis B, C, HIV, syphilis). The CSL is obtained perioperatively during the ReLEx SMILE refractive operation, in which it is sterilely removed at the end of the procedure using forceps. Immediately after the operation the tissue is inserted into a sterile cryotube vial containing a preservative solution (Eusol) at a temperature of 4°C and placed in a protective transit container. It is then transported at this controlled temperature to the Eye Tissue Bank of the Královské Vinohrady University Hospital (FNKV), where it is marked with an identification label in accordance with the approved specifications and stored in BSS solution in a freezer box (-80°C). All donors must show a negative result of the serological examination at the time of tissue sampling and after an elapse of 6 months after the sampling in order for the tissue to be released for use.

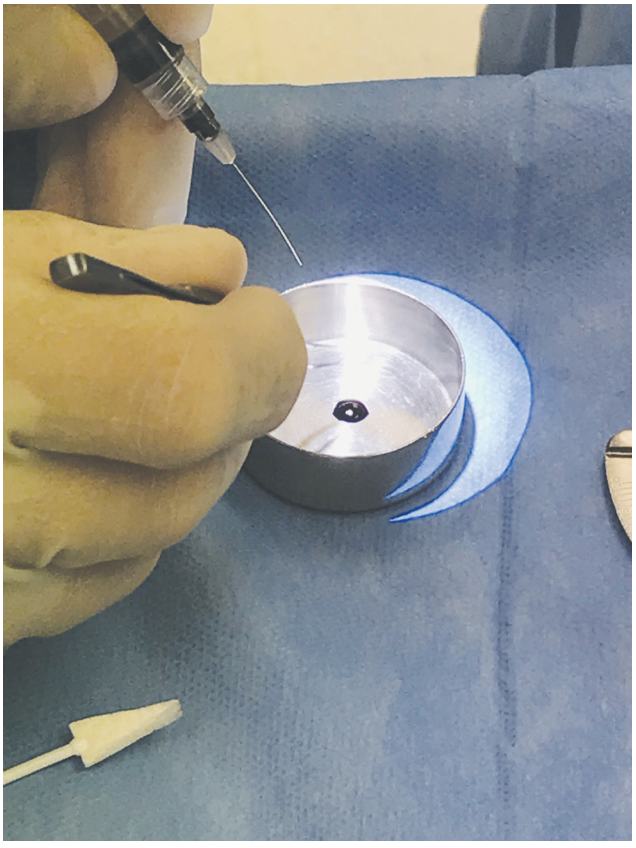

**Figure 1.** CSL staining with trypan blue dye
*CSL – Corneal Stromal Lenticule*

**Preparation of tissue for use**

If the tissue is approved and released for use, the vial containing the cryopreserved CSL is taken from the freezer box and left at room temperature for 30 minutes. It is subsequently placed in saline solution. For better visualization, a drop of trypan blue 0.05% (RS-blue, Alchimia, Padova, Italy) is applied in the operating theater immediately before the use of the tissue. Fig. 1 and 2.

**Indication for CSL transplantation**

Today there are a relatively large number of publications that describe CSL as an alternative option for the treatment of various ocular pathologies or as a solution in the case of failure of the standard method of treatment.

**1. Treatment of ametropia**

Hypermetropia

CSL treatment of hypermetropia consists in the implantation of a CSL into the cornea of the eye whose refractive properties are to be corrected, in order to attain a change of corneal curvature. Pradhan et al. were the first to describe a case of fresh allogenic transplantation in a human in 2013 [3], in which the recipient had high secondary hypermetropia as a consequence

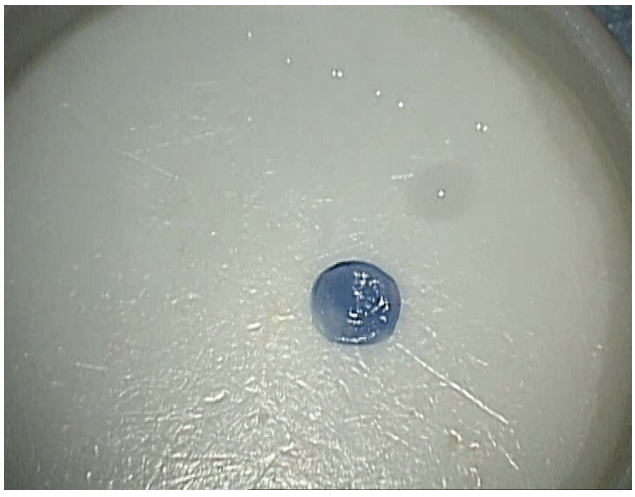

**Figure 2.** Stained and BSS-washed CSL in greater detail
*BSS – Balanced Salt Solution*
*CSL – Corneal Stromal Lenticule*

of aphakia. Although the patient still had high hypermetropia postoperatively even despite a relatively large reduction of refraction, no adverse side effects were recorded in this case in terms of change of corneal transparency or immune response.

The first clinical series of cryopreserved CSL implantations was performed by Ganesh et al. [2]. CSLs were implanted into the corneal stroma of a hyperopic eye. Studies have since been published describing experiences of CSL transplantation in the treatment of hypermetropia [4–11]. The studies also pointed to the relatively poor predictability of the refractive result, and as a result this procedure has now been abandoned.

**Presbyopia**

Presbyopia is the most common ocular defect in people aged over 40 years, and the use of a CSL from ReLEx SMILE could potentially be a new method of treatment. In 2017 Jacob et al. [12] were among the first to demonstrate the safety and efficacy of the PEARL method (PrEsbyopic Allogenic Refractive Lenticule) in the correction of presbyopia. They used an allogenic corneal inlay (implant placed in the corneal stroma for correction of presbyopia) with a diameter of 1 mm, which was implanted under a pocket created by femtosecond laser in the non-dominant eye of presbyopic patients.

**2. Treatment of corneal and tissue defects**

Corneal ulcers and perforations

Another possibility of the use of CSL is the treatment of corneal ulcers or small perforations [13,14]. The main therapeutic methods at present include the application of therapeutic contact lenses, tissue adhesive, conjunctival suturing, amniotic membrane transplantation and corneal transplantation [15–18]. The treatment of corneal ulcers is often complicated. Damaged and missing corneal tissue is only minimally and very slowly restored. Cases of progressive keratolysis lead to corneal perforation, which requires an urgent solution by means of covering the defect in such a manner as to restore the integrity of the eyeball and reduce the risk of infection of the intraocular tissues.

An advantage of CSLs is that with their "thickness" they can fill this corneal defect. There are studies with the use of individual CSLs, or in combination with a tissue adhesive [19] or the use of several layers of CSLs [20]. A combination of CSL transplantation with an amniotic membrane containing a large quantity of growth factors and cytokines appears to be highly advantageous [21,22].

The first experience is also with the use of CSLs in the treatment of limbal dermoid or recurrent pterygium, in which the principle is the replacement of missing tissue and the restoration of the integrity of the cornea [23–25].

**3. Treatment of ectatic diseases**

Keratoconus

Keratoconus is a progressive degenerative disease of the cornea, in which its progressive buckling and thinning occurs. The only option for the treatment of this pathology is the method of corneal crosslinking (CXL). However, in certain patients with keratoconus, corneal thickness is insufficient for CXL to be performed. Sachdev et al. [26,27] described a new method of thickening the corneal stroma with the aid of CSL with the possibility of subsequent performance of standard CXL. It was demonstrated that this technique was safe and effective. In further studies Ganesh and Cagini [28,29] confirmed this fact, and demonstrated that a combination of CSL implantation and CXL may be a suitable method of choice for patients with insufficient corneal thickness, enabling the deferral or in some cases avoidance of keratoplasty.

**Iatrogenic (postoperative) keratectasia**

Li et al. [30,31] published studies with the use of CSL for the treatment of postoperative keratectasia following LASIK refractive surgery. The referred patients had satisfactory results, and no significant complications occurred.

## DISCUSSION

At present there are no recommended guidelines, i.e. standardized surgical procedures, including the working procedure of preservation, in the field of CSL transplantation. A variety of publications exist, but they often differ in their method. For example, we could present the differences in the use of autologous or allogenic stromal tissue, cryopreserved of fresh CSL. The surgical method differs according to whether the CSL was sutured or implanted into a pocket created manually or a pocket created by FS laser, or implantation of CSL beneath the flap.

Even despite the substantial variability of the method, CSL transplantation itself has proven itself to be a feasible procedure with a minimum of perioperative and postoperative complications. Frequent postoperative complications include transitional corneal edema and folds of the Descemet's membrane, edema of CSL tissue which could be attributed to cryopreservation, and opacities in the interface (between the host and donor tissue).

An important fact is that to date no case of failure or rejection of CSL tissue has been described in any of the published literature. This is in accordance with the assumption that CSLs are implanted into immunologically privileged tissue of the cornea, and at the same time they are composed predominantly of collagenous tissue, thereby minimizing the risk of rejection [32]. In order to eliminate the potential risk of rejection, a question remains concerning the benefit of decellularization of an allogenic CSL in order to remove the residual cellular material which could lead to rejection of the tissue [33].

## CONCLUSION

In conclusion we may state that transplantation of a CSL obtained by the method of ReLEx SMILE refractive

surgery is a new alternative method of treating certain ocular pathologies, and at the same time that it concerns effective utilization of a waste product. The most important criterion of this method is naturally safety in preserving minimal damage to the tissues and the possibility of long-term storage enabling further use.

Although several studies have demonstrated its safety and efficacy, the clinical use of CSLs is still in its initial and experimental stage, and it is therefore necessary to conduct studies on a larger scale, with a longer observation period in order to determine the long-term results and confirm its efficacy in comparison with the current treatment.

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