Amniotic membrane welded to contact lens by 1470–nm diode laser: a novel method for sutureless amniotic membrane transplantation

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Abstract

• AIM: To avoid the side effects of the suture usage by welding amniotic membrane (AM) to contact lens (CL) with laser.

• METHODS: AM was taken from pregnant women and cleaned from blood clots with sterile phosphate–buffered physiological saline solution which included antibiotics. Stromal side of the AM was spread inside of the CL and it was welded to CL by 1470 nm diode laser. And 600 μm diameter fiber tip of the laser was contacted with the epithelial side of the AM from 4 separate points. After welding excess amniotic membrane around the CL was cut with a scalpel.

• RESULTS: Stromal side of the AM was spread inside of the CL and then with laser fiber, different power levels and exposure times were applied on the epithelium of AM and 340 mW for seven seconds was found optimal. CL and AM attached with the spot welding effect in 4 points by touching fiber tip. CL–AM welded complex did not separated from each other while holding AM that extend beyond the CL with the help of two forceps.

• CONCLUSION: As a conclusion, it was aimed in this study to achieve the success of the conventional amniotic membrane transplantation (AMT) with the easiness of applying a CL and to avoid risks and side effects of corneal or conjunctival suturing. The results showed that the application of the CL–AM complex will be as easy as the application of a CL and lasts shortly.

• KEYWORDS: amniotic membrane; contact lens; laser; welding

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INTRODUCTION

Human amniotic membrane (AM) is derived from the fetal membranes. AM is the most interior layer of the placenta. Histologically, AM consists of three layers, namely, single layer cubic epithelium, basement membrane and stroma. Macroscopically one side of the membrane is smooth, shiny, transparent and non-adhesive epithelial face and the other is matte and adhesive stromal face. Epithelial and stromal side can be determined with the help of triangle sponge. When sponge touched to the membrane it is the stromal side that attached. The thickest basement membrane of the human body is AM. Clinically this allows the storage of frozen membrane at -80°C so epithelium cells can be protected for a long time and stay alive. Histochemical examinations on the basal membrane of AM show that it is similar to the conjunctiva[1].

After the first usage of fetus membranes in skin transplantation by Davis[2] in 1910 and with the publication of studies supporting the idea that human amnion membrane facilitates epithelialization, prolongs the life of epithelial cell and decreases the formation of inflammation and scar, AM transplantation (AMT) has been widely used especially in the fields of plastic surgery, gynaecology and otolaryngology. Usage in ophthalmology for the first time in 1940 performed by De Rotth[3] and used for conjunctiva surface reconstruction, fresh and with chorionic layer but has not been successful. In 1946 and 1947 Sorsby and Symons[4] administered AMT in caustic burns of the eye and has been successful. Studies on AMT accelerated after 1990s and in 1995 Kim and Tseng[5] carried out studies on rabbits involving peeling of corneal surface epithelium completely and the re-creation of the ocular surface with AMT. In 1997, Lee and Tseng[6], developed methods of warehousing and storage AM.

In humans, AMT increases the life cycle of epithelial stem cells and in eyes with partial stem cell deficiency and clonogenity and re-creates the corneal surface[7]. Therefore limbal epithelial progenitor cells in human ex vivo extended with AM are used for repairing the corneal surfaces with full or partial limbal stem cell deficiency [8]. AM, covering over the wound creates a sterile environment and contributes to reducing the risk of post-operative infection. In a study of
Chakraborty and Bhattacharyya[9], AM is shown to inhibit the growth of microorganisms in vitro environment. Schroeder et al[10] showed that neuronal cells in in vitro environment grows on the stroma of AM and basement surface so AM is effective in the growth of neurons. In ophthalmology, AM is used for its contribution to the improvement, and also to reduce pain and discomfort. Suppressing inflammation and neurotrophic effects of the AM and its biological bandage effect contributes to the provision of epithelialization. Due to the secretion of growth factors stimulates reepithelialization. It has a good feature that facilitates the migration of epithelial cells from environment [11]. Amniotic basement membrane strengthens the adhesion of epithelial cells [12]. It facilitates proliferation and differentiation of epithelial cells, supports the continuation of the original epithelial phenotype and facilitates the differentiation of goblet cells [13]. However, the amount of these factors decrease with long term storage [14]. The poor clinical outcomes might have been due to inefficient tissue processing leading to loss of the biologic properties of AM; the present success is in part due to cryopreservation of AM resulting in maintenance of its physiologic properties and loss of epithelial cells which results in lack of immunogenicity. The preservation method by Lee and Tseng [6] is still the best way for maximal maintenance of its biologic properties. It has positive effects such as faster differentiation of healing epithelium under contact lens (CL) with the protective effect of therapeutic CL application after AMT, reduction in corneal stromal oedema due to the structure of the hydrophilic lens, the accumulation of keratocytes at the wound site, inhibition of leukocyte migration to cornea. Sufficient oxygen permeability of therapeutic CLs help to improve the surface of the cornea. It can also be used as a mechanical barrier to protect the surface of the eye from external influences[15]. In this study we aimed to avoid the side effects of the suture usage by welding AM to CL with laser and thus simplifying the procedure of AMT to a CL procedure that do not require a surgery.

SUBJECTS AND METHODS
AM was taken from seronegative pregnant women for HIV, Hepatitis B, C and syphilis during elective caesarean section under laminar flow and sterile conditions. AM was cleaned from blood clots with sterile phosphate-buffered physiological saline solution which included penicillin, 50 mg/mL, streptomycin 50 mg/mL; neomycin 100 mg/mL amphotericin B 2.5 mg/mL. Amnion was separated from the rest of the chorion by blunt dissection[6]. AM was aseptically stored in the same saline solution for 1h and then used in the laboratory. AM can be used in three techniques. Placed into the area to cover defects "inlay" (graft) technique, placed on the cornea and limbuscover to cover "overlay" (patch) technique and to close the thin region “filling” (gap) technique. In this study, sutureless overlay (patch) technique was used. In this procedure, the upper face was stromal side and epithelial side was in contact with the ocular surface. In this sutureless overlay technique with CL, it is obvious that the patient vision will be clear immediately after epithelialization under the AM and after the CL removal. The longer the duration of the AM on ocular surface, epithelialization will be more successful. Primarily, AM was spread on ocular surface to understand which side of the AM is epithelium and which side is stroma. Epithelial face was bright and stromal face was more matte when touched with the tip of forceps to the epithelium, gel-like vitreous elongation was formed. After deciding the epithelial surface with this method, stromal side of the membrane was spread inside of the CL with the specifications of 14 mm diameter, base curve 8.6, -0.25D refraction, 36% water content silicone hidrogel [PureVision (balafilcon) Bausch&Lomb, Rochester, NY, USA] and it was welded to CL by the 1470 nm diode laser (DILAS Mini Diode Laser System, Meinz, Germany) which was especially customized production for our biophotonics laboratory. 600 μm diameter fiber tip of the laser was contacted with the epithelial side of the AM from 4 separate points (Figure 1). After welding of the membrane to the CL excess AM around the CL was cut with a scalpel.

RESULTS
Stromal side of the AM was spread inside of the CL which has the specifications of 14 mm diameter, base curve 8.6, -0.25D refraction, 36% water content silicone hidrogel [PureVision (balafilcon) Bausch&Lomb, Rochester, NY, USA]. Then with the 600 μm diameter fiber, laser applied with the power varying from 200 mW to 800 mW on the layer of the epithelium of the AM by contacting with fiber tip. Different power levels and exposure times were tested and 340 mW for seven seconds was found optimal. CL and AM attached with the welding effect in 4 point spot welding by touching fiber tip without excessive contraction or burn effect of AM. After the application of the laser with the appropriate dose and duration, it was observed that the AM and CL were not separated from each other while holding AM portions that extend beyond the CL with the help of two forceps (Figure 2). When the AM was hold in the air with the help of two forceps, 4 point of spot welding points which was done with laser fiber, visualized clearly on the transparent surface of the CL. (Figure 3). When laser applied with higher energy (450 mW for 5s, 600 mW for 5s, 800 mW for 3s), burning of the AM, contraction of the membrane tissue or melting of CL structure was determined. If the laser applied with lower energy (150 mW for 15s, 250 mW for 10s) than specified values, which are 340 mW for 7s, when tissue was hold from sides of the excessive membrane, it was viewed that membrane did not weld to CL and separated from the CL.
After the optimum dose application when it was observed that CL and AM was welded, the excessive tissue which was extended beyond the CL was cut with a scalpel (Figure 4).

DISCUSSION

Mitotic division of the basal cell layer of the corneal epithelium is normally renewed in 7-10d on a regular basis. Epithelial cells on the surface shed to precorneal tear film. The basal layer cells are constantly replaced by multiplying the cells. Corneal wound healing takes place in three stages these are: migration of healthy epithelial cells to this area, regeneration and stromal wound healing to close the defect. [16,17].

If corneal epithelial injury is minor, after injury damage is tries to be closed by cell migration as soon as possible. Epithelial migration begins within minutes, there may be delays up to 4-5h of a wider damage. This delay time prior to the rapid cell division required for anatomical, physiological and biochemical preparation. Clinically, corneal epithelial migration is sufficient to close many lesion [16,17].

AM accelerates epithelialization, maintains normal epithelial phenotype and inflammation, cicatrization, reduces neovascularization. These effects depends on suppression of inflammation with the effect of AM antiprotease, suppression of lipopolysaccharide that increase IL-1α and IL-1β, reducing TGF-β signaling system and scar system by reducing the conversion of myofibroblasts and fibroblast, the creation of epithelial progenitor cells and colony survival by being basal membrane and increasing growth factors carring with [13-18].

AM acting as a bandage CL protects the epithelium from the eyelid movements. It is claimed that there is growth factors in AM needed for treatment of deep ulcerations and desmatocells in patients at risk of perforation of the cornea, inflammation will suppressed with the use of AM, also increases the success of future penetrating or lamellar keratoplasty through containing basal membrane and collagen but not allogenic cells [19].

AMT in the cornea therapeutic CL can be used in almost all applications. Gris et al [20] study showed that the AM implant remained in place for a mean of 12.5d (range, 3-34). In 11 of the 20 patients, the AM implant became detached within the first 8d. When the corneal implant was postoperatively covered with a soft CL, duration of attachment was increased. Therapeutic CLs are used to increase the regeneration of corneal epithelial healing and lenses. Therapeutic CLs cover elements that help in reducing pain, hydration and protection of the cornea to reduce oedema, corneal epithelial healing maintaining the mechanical...
disorders of the cornea \[24\]. With sufficient oxygen permeability of therapeutic CLs help to improve the surface of the cornea. Also be used as a mechanical barrier to protect the surface of the eye from external influences. Therapeutic CLs can be use after AMT for decreasing inflammation until completion of reepithelialization\[25\].

Today, that has been practiced cover (overlay) technique; biological AM acts as a CL and at the end separates from the receiving tissue. In the cover technique AM applied to cover limbus \[23,24\]. In this technique stromal face should be above and epithelial side should be in contact with the ocular surface. In the cover technique result of separation of AM from ocular surface after epithelisation under AM, patients immediately reach to clear vision. Separation of CL-AM complex from ocular surface can be easily done by removing the CL. The longer the duration of AM in the ocular surface, epithelialization are more successful. However, in this method in some patients, AM may leave before corneal epithelialization is completed \[20\]. Stay on the surface can be controlled much more comfortable with our welded CL-AM complex. While CL is on the surface of the cornea, AM cannot be separated easily before epithelisation is completed. AM is attached to the peripheral conjunctiva and episclera with 8-0 polyglactin sutures. CL-AM welded complex do not require any sutures and eliminates the many problems resulting from suture such as suture abscess, subconjunctival haemorrhage, infection due to sutures, irritation of the eye lids. Another disadvantage of suturing AM in children is the necessity of general anaesthesia and especially to children general anaesthesia during AMT is a risk by itself.

Adhering AM to cornea or conjunctiva with tissue adhesive is an another method that can be applied except suturing AM to conjunctiva and episclera. In the present study, instead of welding AM to CL with laser, AM could be pasted to CL with these adhesives but very serious side effects of these adhesives can be seen. Cyanoacrylate-based glues have traditionally been the most widely used glues for ophthalmic surgery\[20\]. The major draw back of cyanoacrylate glue is that they form a solid, impermeable mass \textit{in situ}. This persists as a foreign body causing inflammatory reactions like giant papillary conjunctivitis and corneal neovascularization \[25\]. They are also impermeable to fluids and metabolites. Though these disadvantages preclude its intraocular use, they are not very significant if the glue is applied superficially\[20\].

Fibrin glue is another substance to adhere tissues. Hick \textit{et al}\[20\] and Kheirkhah \textit{et al}\[10\] reported that fibrin glue use in AMT and it was found to be safe and effective in fixing the AM to the ocular surface. Sekiyama \textit{et al}\[11\] evaluated the efficacy and safety of transplantation of fibrin glue coated freeze dried AM for ocular surface reconstruction. They found out that the freeze dried AM retained most of its biological characteristics indicating that it was safe and efficacious for ocular surface reconstruction. Szuroman \textit{et al}\[22\] showed that general feasibility of reproducible and reliable sutureless AM fixation onto the corneal surface by fibrin glue in rabbits, and also they demonstrated several advantageous characteristics as increased biocompatibility, better epithelialization pattern and the lack of membrane shrinkage. But the major drawback to fibrin glue usage is the risk of transmitted disease from pooled and single-donor blood donors \[15,33\]. This problem can be minimized by obtaining the blood from screened healthy donors but the safest preparation is by using the patient's own blood to prepare fibrin glue\[13,34\]. It is expensive and autologous donation requires at least 24h for processing. The resultant product often has variable concentrations thereby resulting in an unpredictable performance. Moreover, tensile strength of fibrin glue has not been adequately determined and precludes quantification, being dependant on various extraneous factors also. Hence laser welding does not contain any chemical or biological substance, the laser welding procedure we used in our study is not supposed to contain any side effects like risk of transmitted disease or inflammatory reactions which can be seen in tissue glues.

The fact that the welding procedure has been applied to a standard CL is considered to be the weak point of our study. The CL to be weld should cover the perilimbal area as well, so the procedure has to be applied also with a wider lens. The scleral CLs seem appropriate.

Scleral CLs are large-diameter gas permeable (GP) lenses with diameters varying from 13 mm to more than 20 mm. Unlike standard GP lenses, scleral CLs completely cover the cornea and extend onto the sclera. Corneal-scleral lenses may provide better initial comfort, centration, and stability compared to a corneal GP lens for many patients. Nearly anyone who can wear a corneal GP could be wearing a corneo-scleral GP instead. Following a fitting guide, these lenses are easy to fit and inexpensive. The material of the scleral CLs is similar to that of Bausch&Lomb CL, which we used in this project. Because of that the AM is thought to be weld to the scleral lenses as well. Many corneo-scleral CLs are available including Semi-scleral 13.5 (ABBA Optical); SO3Clear (Dakota Sciences); Perimeter (Essilor Contact Lenses); and DigiForm (TruForm Optics).

Because of the difficulty of obtaining these optional lenses in our country we designed our work according to a normal diameter CL. In addition to that a CL with 14 mm diameter can provide sufficient corneal-amnion contact for pathologies on corneal surface.

As a conclusion, it was aimed in this study to achieve the success of the conventional AMT with the easiness of applying a CL and to avoid risks and side effects of corneal or conjunctival suturing. The results showed that the application of the CL-AM complex will be as easy as the
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application of a CL and lasts shortly. This study is a beginner study for this application. An in vivo animal study should be designed as the continuation to compare the results of CL-AM welded complex with conventional AMT. The long term effects of the CL-AM complex to the corneal surface need to be investigated with future studies.

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REFERENCES