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(54) **Title**: METHOD FOR PERFORMING A REVERSIBLE REFRACTIVE PROCEDURE

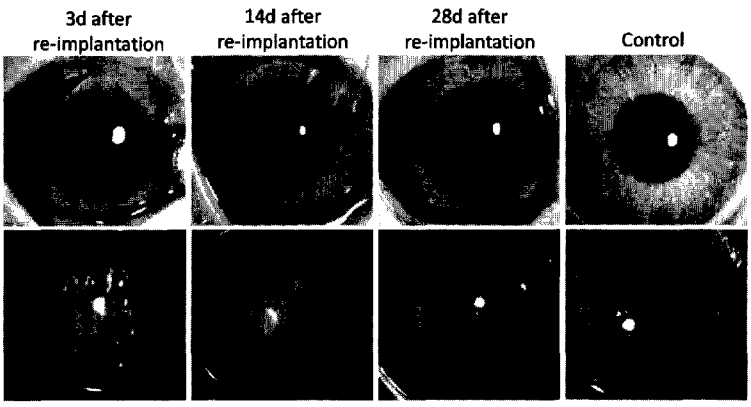

Figure 6a

(57) **Abstract**: The present invention is directed to a method for performing a reversible refractive procedure by reimplanting a refractive lenticule that has been obtained by a refractive procedure on the eye, the method comprising the steps of preparing the refractive lenticule for reimplantation, creating a corneal flap in an eye of an acceptor, transferring the refractive lenticule under the corneal flap, and repositioning and covering the acceptor's corneal flap; thereby reimplanting the refractive lenticule. The present invention is also directed to a refractive lenticule obtained by a refractive procedure on the eye of a donor for use in a method for subsequent reimplantation in a reversible refractive procedure on an acceptor, wherein the refractive lenticule is stored at a temperature below 0°C.

WO 2012/035403 A1

Method for Performing a Reversible Refractive Procedure

Cross-Reference To Related Application

[0001] This application makes reference to and claims the benefit of priority of an application for "Storage of Lenticules Following Femtosecond Laser Refractive Lenticule Extraction Procedures from Cornea for Subsequent Reimplantation" filed on September 13, 2010 with the United States Patent and Trademark Office, and there duly assigned serial number 61/382,037. The content of said application filed on September 13, 2010 is incorporated herein by reference for all purposes, including an incorporation of any element or part of the description, claims or drawings not contained herein and referred to in Rule 20.5(a) of the PCT, pursuant to Rule 4.18 of the PCT.

Technical Field

[0002] Various embodiments relate to the field of refractive procedures or surgeries, in particular, reversible refractive procedures on refractive lenticules.

Background

[0003] Surgical correction for refractive errors may be performed by a number of procedures. Laser correction involves procedures such as EpiLASIK, LASIK or conductive keratoplasty, while lens based corrections involve the use of phakic intra-ocular lenses. Despite the emergence of other procedures, due to its fast visual rehabilitation and painless postoperative course, LASIK is still the procedure of choice for both patients and refractive surgeons.

[0004] LASIK consists of two steps: the first step involves the formation of a corneal flap and the second step is when the excimer ablation is performed in the corneal stromal bed. Femtosecond lasers (FL) have been widely used in LASIK surgery to fashion the corneal

flap. The laser will cut a lamellar flap followed by a vertical corneal incision. A vertical cut may be produced since the femtosecond laser can deliver laser pulses of 1 micron diameter at a preselected depth in the cornea, which then expands to 20/30 microns with the formation of a cavitation bubble. These pulses create micro-photodisruption of the corneal tissue by the formation of an expanding bubble of carbon dioxide and water, which in turn cleaves the tissue and creates a plane of separation. The flap is then removed to expose the stroma and the refractive treatment is done by the excimer laser over the denuded stroma. The advantages of femtosecond laser over microkeratome LASIK flaps have been reduced postoperative dry eye symptoms, less chance of dislocation from trauma and reduced incidence of button holes or free caps.

[0005] Even though Femto-LASIK has demonstrated good efficacy, it can still be associated with side effects found in standard LASIK, for example, glare, haloes, dry eye and still requires the use of two separate laser machines, one for the flap the other for the excimer ablation. This increases the length of time of the overall procedure and the costs.

[0006] Recently, a "one laser" refractive surgery known as Refractive Lenticular Extraction (ReLEx) has been proposed. It consists of performing the entire refractive procedure without an excimer laser. ReLEx surgery involves 2 different surgical approaches, Femtosecond Lenticule Extraction (FLEx), and Small Incision Lenticule Extraction (SMILE).

[0007] FLEx mimics conventional LASIK surgery by performing a LASIK type hinged flap to extract the lenticule. The femtosecond laser creates four stromal incisions to create a corneal lenticule and a corneal flap. The size and shape of the lenticule is calculated based on the patient refractive error. The lenticule is separated from the flap above and the rest of the cornea below using a small spatula. Following this, the lenticule is grasped with a forceps and extracted from the eye. Finally, the flap is repositioned and the interface washed. At this time the refractive error is already corrected without the use of a second laser.

[0008] The SMILE procedure is a flap-less form of small incision surgery, in which the lenticule is extracted through a small superior pocket incision, without the formation or lifting of a full flap, which has the advantages of minimal incision or disturbance to the corneal surface, greater wound strength as a full flap is not raised, the possibility of less

flap-related optical aberrations, with enhanced visual quality and less loss of contrast sensitivity, and less neurotrophic keratopathy or dry eye, as less corneal nerves may be disrupted at the corneal periphery when a full flap is used. Both procedures come under the umbrella term of Refractive Lenticule Extraction (ReLEx).

[0009] The advantages of FLEx and SMILE treatments are obviation of the need for a separate excimer laser, shortened length of the refractive procedure, reduced patient inconvenience from moving from one laser to another, and more accurate ablation especially in high myopia as it appears that no algorithm adjustment is needed in ReLEx unlike that of excimer based LASIK surgery. The advantages of a pocket incision in SMILE as apposed to a full LASIK type flap have also been described above.

[0010] There are few publications on FLEx or SMILE since as yet it is not commercially released and the procedure is only carried out by a few centres. Currently, following the creation of the refractive lenticule, the lenticule is removed from the eye and then disposed of.

[0011] Presently, there are no laser based reversible refractive procedures. The only reversible refractive procedures currently available are artificial lens based systems that require intraocular surgery and considerable risks involved in reversal.

[0012] Thus it is an object of the present invention to provide a method for storing the refractive lenticule upon its creation and subsequently performing a reversible refractive procedure thereon.

Summary of the Invention

[0013] In a first aspect, the present invention relates to a method for performing a reversible refractive procedure by reimplanting a refractive lenticule that has been obtained by a refractive procedure on the eye, the method comprising the steps of preparing the refractive lenticule for reimplantation, creating a corneal flap in an eye of an acceptor, transferring the refractive lenticule under the corneal flap, and repositioning and covering the acceptor's corneal flap; thereby reimplanting the refractive lenticule.

[0014] According to a second aspect, the present invention relates to a refractive lenticule obtained by a refractive procedure on the eye of a donor for use in a method for

subsequent reimplantation in a reversible refractive procedure on an acceptor, wherein the refractive lenticule is stored at a temperature below 0°C.

Brief Description of the Drawings

[0015] In the drawings, like reference characters generally refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the invention. In the following description, various embodiments of the invention are described with reference to the following drawings, in which:

[0016] Figure 1 shows a flow diagram illustrating the processes performed for short term study of FLEx vs LASIK, in accordance to various embodiments;

[0017] Figure 2 shows *in vivo* confocal microscopy of keratocyte activation, according to various embodiments;

[0018] Figure 3a shows immunohistochemistry images of fibronectin expression of the central cornea, according to various embodiments;

[0019] Figure 3b shows immunohistochemistry images of fibronectin expression of the periphery of flap, according to various embodiments;

[0020] Figure 4a shows immunohistochemistry images of inflammation of the central cornea, according to various embodiments;

[0021] Figure 4b shows immunohistochemistry images of inflammation of the periphery of flap, according to various embodiments;

[0022] Figure 5 shows light microscopy of an extracted lenticule at (a) 100x magnification; (b) 400x magnification of the anterior edge; (c) 400x magnification of the posterior edge; and (d) 5600x magnification of a keratocyte, according to various embodiments;

[0023] Figure 6a shows slit lamp (SL) images of the lenticule following re-implantation, in accordance to various embodiments;

[0024] Figure 6b shows confocal microscopic images of the lenticule following re-implantation in accordance to various embodiments;

[0025] Figure 6c shows a plot of corneal thickness, in accordance to various

embodiments;

[0026] Figure 7 shows *in vivo* confocal microscopy of the lenticule, according to various embodiments;

[0027] Figure 8 shows topography results 28 days after re-implantation of the lenticule, in accordance to various embodiments;

[0028] Figure 9 shows immunohistochemistry images of inflammation of the eye 28 days after re-implantation of the lenticule compared against a control (in the rightmost column), according to various embodiments;

[0029] Figure 10 shows confocal microscopic images of inflammation similar to Figure 9 using various labels and stains, in accordance to various embodiments;

[0030] Figure 11a shows a light microscopic image of extracted stromal lenticules, according to various embodiments;

[0031] Figure 11b shows a magnified light microscopic image of the extracted stromal lenticules, according to various embodiments;

[0032] Figure 11c shows a light microscopic image of a cell culture of the extracted stromal lenticules, according to various embodiments;

[0033] Figure 11d shows a light microscopic image of the cell culture of Figure 11c after adding the stock freezing solution, according to various embodiments;

[0034] Figure 11e shows a light microscopic image of the cell culture following a culture of a fresh lenticule, according to various embodiments;

[0035] Figure 11f shows a light microscopic image of the cell culture after 1 month of cryopreservation, according to various embodiments; and

[0036] Figure 11g shows gel electrophoresis for real-time PCR, in accordance to various embodiments.

Detailed Description

[0037] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the invention may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the invention. Other embodiments may be utilized and

structural, and logical changes may be made without departing from the scope of the invention. The various embodiments are not necessarily mutually exclusive, as some embodiments can be combined with one or more other embodiments to form new embodiments.

[0038] In one aspect, the present invention relates to a method for performing a reversible refractive procedure by reimplanting a refractive lenticule that has been obtained by a refractive procedure on the eye. The method comprising the steps of preparing the refractive lenticule for reimplantation, creating a corneal flap in an eye of an acceptor, transferring the refractive lenticule under the corneal flap, and repositioning and covering the acceptor's corneal flap; thereby reimplanting the refractive lenticule.

[0039] In the context of various embodiments, the term "refractive procedure" may generally refer to any eye surgery used to improve the refractive state of the eye. This may include methods of surgical remodeling of the cornea or cataract surgery. For example, use of excimer lasers to reshape curvature of the cornea is a refractive procedure. The term "reversible refractive procedure" may refer to a procedure to invert or undo the refractive procedure.

[0040] The term "refractive lenticule", as used herein, relates to a piece of stromal corneal tissue that is removed to correct refractive error of the eye. For example, the refractive lenticule may be peeled off the corneal bed. The refractive lenticule may have any shape and can, for example, be lense-shaped.

[0041] In various embodiments, the refractive lenticule has been stored at a temperature below 0 °C. For example, the refractive lenticule may have been stored at about -80 °C.

[0042] For storage, the refractive lenticule may be freezed and the frozen refractive lenticule may be stored in a freezer or a cryopreserving environment. During storage, the refractive lenticule may be held by a support to maintain a curved surface comparable to a corneal surface. For example, the support may be a rigid gas permeable contact lens or a mounting structure comprising a curved surface. As used herein, the term "mounting structure" may refer to any structure or mechanism to hold the refractive lenticule in place. The mounting structure may include a right and left mount to hold the refractive lenticule at each end such that the refractive lenticule is spreaded like a flat sheet. The mounting structure may also allow for easy removal of the refractive lenticule without

damage.

[0043] In various embodiments, the step of preparing the refractive lenticule for reimplantation may comprise thawing the refractive lenticule in a water bath of about 37 °C. Upon thawing, the refractive lenticule may be washed in a buffer solution. For example, the buffer solution may be selected from the group consisting of morpholinepropanesulfonic acid, a Phosphate Buffered Solution (PBS), a trehalose buffer, and a sucrose buffer.

[0044] In various embodiments, the step of preparing the refractive lenticule for reimplantation may further comprise enzymatically digesting the thawed refractive lenticule for culture of corneal stromal fibroblast. The step of preparing the refractive lenticule for reimplantation may also further comprise adjusting refractive errors of the refractive lenticule to restore near vision, or to correct presbyopia, or to treat complications of keratectasia.

[0045] According to various embodiments, the step of repositioning and covering the acceptor's corneal flap may comprise placing a bandage contact lens over the repositioned corneal flap.

[0046] In various embodiments, the refractive lenticule for reimplantation may have been obtained by a method comprising the steps of creating an incision in an eye of a donor, and extracting the refractive lenticule through the incision. The incision may be made using a one-laser refractive surgery. The one-laser refractive surgery may be Refractive Laser Extraction (ReLEx).

[0047] In one embodiment, the ReLEx may comprise Small Incision Lenticule Extraction (SMILE).

[0048] In other embodiments, the incision may comprise one or two or three or four stromal incisions. For example, the four stromal incisions may further create a corneal flap in the donor's eye. The ReLEx in this embodiment may be Femtosecond Lenticule Extraction (FLEx).

[0049] In various embodiments, the donor and the acceptor may be the same subject. In other embodiments, the donor and the acceptor may be different subjects.

[0050] In another aspect, the present invention relates to a refractive lenticule obtained by a refractive procedure on the eye of a donor for use in a method for subsequent

reimplantation in a reversible refractive procedure on an acceptor, wherein the refractive lenticule is stored at a temperature below 0°C.

[0051] In various embodiments for storage, the refractive lenticule may be snap-freezed in liquid nitrogen for about 10 minutes. The snap-freezing process may be carried out for about 5 minutes to about 15 minutes, or about 5 minutes to about 8 minutes, or about 12 minutes to about 15 minutes. The refractive lenticule may be stored at about -80 °C.

[0052] In various embodiments, the refractive lenticule may be cryopreserved. For the cryopreservation, the refractive lenticule may be suspended in a serum-containing medium in a vessel, a freezing solution of about 10% serum and about 20% organic solvent is added in a dropwise manner into the vessel to form a mixture of about 10% serum and about 10% organic solvent, the vessel containing the mixture is placed in a -80 °C freezer for at least 4 hours; and the frozen mixture is transferred into liquid nitrogen for storage.

[0053] The organic solvent may be selected from the group consisting of organic solvent is selected from the group consisting of hexane, heptane, cyclohexane, benzene, pyridine, dichloromethane, chloroform, carbon tetrachloride, carbon disulfide, tetrahydrofuran, dioxane, diethyl ether, diisopropylether, ethylene glycol monobutyl ether, methyl ethyl ketone, methyl isobutyl ketone, acetone, cyclohexanone, ethyl acetate, isobutyl isobutyrate, ethylene glycol diacetate, dimethylformamide, acetonitrile, N,N-dimethyl acetamide, nitromethane, acetonitrile, N-methylpyrrolidone, dimethylsulfoxide water, methanol, ethanol, butyl alcohol and formic acid. The serum may be a fetal bovine serum.

[0054] In various embodiments, the refractive lenticule may be obtained by creating an incision in an eye of the donor; and extracting the refractive lenticule through the incision. The incision may be created using a one-laser refractive surgery. For example, the one-laser refractive surgery is Refractive Laser Extraction (ReLEx). The ReLEx may comprise Small Incision Lenticule Extraction (SMILE) or Femtosecond Lenticule Extraction (FLEx).

[0055] In one embodiment, the donor and the acceptor may be the same subject. In another embodiment, the donor and the acceptor may be different subjects.

[0056] Prior to the subsequent reimplantation, the stored refractive lenticule may be

processed to adjust its refractive errors to restore near vision, or to correct presbyopia, or to treat complications of keratectasia.

[0057] In the context of various embodiments, the term "about" or "approximately" as applied to a value may encompass the exact value and a variance of +/- 5% of the value.

[0058] The phrase "at least substantially" may include "exactly" and a variance of +/- 5% thereof. As an example and not limitations, "A is at least substantially same as B" may encompass embodiments where A is exactly the same as B, or where A may be within a variance of +/- 5%, for example of a value, of B, or vice versa.

[0059] In order that the invention may be readily understood and put into practical effect, particular embodiments will now be described by way of examples and not limitations, and with reference to the figures.

Examples

[0060] Various embodiments provides the storage of refractive lenticules following the FLEx or SMILE ReLEx or other similar stromal lenticule extraction procedures to make these completely reversible or modifiable refractive procedures.

[0061] The main advantage of FLEx/SMILE is that in theory it may be able to correct higher refractive powers with removal of less tissue and also it is a potentially reversible procedure. A FLEx model in an animal is firstly established. A rabbit model is chosen since this has been previously chosen to study refractive surgical procedure, for example, LASIK and PRK so as to provide basis for comparison.

[0062] In an experimental model, the short term effect of the FLEx procedure compared to LASIK is assessed. Subsequently a model of re-implantation in an animal model is established and the viability of the lenticule following the cryopreservation method is assessed.

Method

[0063] Example A: Comparison to LASIK – Establishing ReLEx model

[0064] Thirty six (36) New Zealand White rabbits (about 3-4 kg body weight) have been procured from animal holding unit of National University of Singapore, Singapore. Rabbits have been housed individually at room temperature on about 12 hours:12 hours of light:darkness, with rabbit pellets and water available ad libitum.

[0065] Eighteen rabbits (18) are used to study the short term effect of the surgery (i.e., to be sacrificed 1 day after surgery):

[0066] • FLEx (mild correction: -3.00D) on right eye and control left eye (naïve)→ 3 rabbits

[0067] • FLEx (moderate correction: -6.00D) on right eye and control left eye (naïve) → 3 rabbits

[0068] • FLEx (severe correction: -9.00D) on right eye and control left eye (naïve) → 3 rabbits

[0069] • LASIK (mild correction: -3.00D) on right eye and control left eye (naïve) → 3 rabbits

[0070] • LASIK (moderate correction: -6.00D) on right eye and control left eye (naïve) → 3 rabbits

[0071] • LASIK (severe correction: -9.00D) on right eye and control left eye (naïve) → 3 rabbits

[0072] Rabbits are anesthesized with a combination of xylazine hydrochloride (20 mg/kg) and kentamine hydrochloride (40 mg/kg) intramuscularly. In addition, preservative-free oxybuprocain hydrochloride eyedrops (Benoxinat SE 0.4%) are applied to each eye just before the surgery. After the surgery, the eyelids are closed with a temporary tarsorrhaphy placed with a 6-0 suture.

[0073] The rabbits are then sacrificed under anesthesia by intracardiac injection of 5 ml embutramine 0.2 g/ml, mebezonium iodide 0.05 g/ml, and tetracaine hydrochloride 0.005 g/ml. All rabbits are treated in accordance with the ARVO Statement for the Use

of Animals in Ophthalmic and Vision Research. The experimental protocol is approved by the Institutional Animal Care and Use Committee of SingHealth.

[0074] Example B: Storage and re-implantation of stromal lenticule

[0075] Eighteen different rabbits (18) are used to study the long term effect of the surgery (i.e., to be sacrificed 28 day after surgery).

[0076] The extracted stromal lenticules are carefully transferred on the Rigid Gas Permeable (RGP) contact lens. A marking at 6 o'clock position is made on the lens to indicate the anatomical position of the lenticule on the cornea before extraction. A drop of 10% DMSO (Sigma, St. Louis, MI) is applied on the lenticule before a 10-minute snap freezing in the liquid nitrogen (N_2). The lenticule is stored in -80 °C until further use.

[0077] The re-implantation of the lenticule is performed 28 days after the initial ReLEx procedure. A Siebel spatula is inserted under the flap near the hinge and the flap is then lifted. The lenticule is transferred onto the exposed stromal bed by a sliding motion from the RGP contact lens. After the flap is repositioned, a bandage contact lens (Bausch & Lomb, Rochester, NY) is placed over the flap and the eyelid is closed with a temporary tarsorraphy for 3 days. Post-operative follow up is done on day 3, 14, and 28 after the re-implantation. Gentamycin and Dexamethasone of 1 ml each are injected subconjunctivally following the re-implantation procedure and each post-operative follow up.

[0078] Figure 1 shows a flow diagram illustrating the processes performed for short term study (sacrifice 1 day after surgery in Example A) and long term study (sacrifice 28 days after surgery in Example B) of FLEx vs LASIK.

In the context of various embodiments, the term "OCT" for example in Figure 1 refers to optical coherence tomography. The term "immunofluorescent staining" may generally refer to fluorescent dyeing for detection and imaging of biological samples using light microscopy with a fluorescence microscope. The term "TUNEL" may generally refer to terminal deoxynucleotidyl transferase dUTP nick end labeling for detecting DNA fragmentation by labeling the terminal end of nucleic acids. The term "in vivo confocal" refers to *in vivo* confocal microscopy (IVCM), wherein confocal

microscopy may generally refer to a microscopy for obtaining increased resolution by limiting the illumination and observation systems to a single point. The term "refractive states" may generally refers to a determining procedure for the purpose of obtaining glasses and includes specification of lens type (monofocal, bifocal, other), lens power, axis, prism, absorptive factor, impact resistance, and other factors.

[0079] Example C: Lenticule Viability – Before and after Cryopreservation

[0080] Extracted stromal lenticules from clinical patients and also from human cadaver eyes have undergone TUNEL assay, transmission electron microscopy (TEM), cell culture and real-time PCR. Extracted human stromal lenticule is washed twice (about 10 minutes each) in a phosphate buffered saline (PBS) buffered antibiotic/antimycotic solution. The lenticule is then transferred into a cryovial and resuspended in 500 μl medium containing 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA). A stock freezing solution containing 10% FBS and 20% dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA.) are added slowly in a dropwise manner to an equal volume ratio, to make a final volume of 1 ml freezing solution containing 10% FBS and 10% DMSO.

[0081] Freezing of the cryovial containing the stromal lenticule is carried out in a cryo-container ("Mr. Frosty", Nalgene, Denmark), within a -80 °C freezer overnight, and transferred into liquid nitrogen the following day for long-term storage.

[0082] Thawing of frozen stromal lenticule is carried out after one month of cryopreservation where the vial containing the frozen stromal lenticule is rapidly thawed in water bath at 37 °C. Immediately after thawing, the stromal lenticule is rinsed twice in a PBS solution to remove the cryoprotectant agents. Thawed lenticules are either enzymatically digested for the culture of corneal stromal fibroblast, or immersed in a tissue-freezing compound and frozen for cryosection to be analyzed with TEM and TUNEL assay.

Discussion

[0083] Table 1 shows the data extracted from topography results for various refractive procedures as carried out in the short term study of Example A.

[0084] Table 1

Control	44.81 ± 0.75	46.77 ± 0.32
LASIK (-3.00D correction)	37.00 ± 1.44	39.15 ± 1.62
FLEx (-3.00D correction)	37.16 ± 1.02	40.52 ± 0.08
LASIK (-6.00D correction)	35.68	33.25
FLEx (-6.00D correction)	38.34 ± 1.84	36.59 ± 1.35
FLEx (-9.00D correction)	35.45	34.09

[0085] In Table 1, Kh and Kv represent horizontal Keratometry readings and vertical Keratometry readings, respectively, to determine the exact curvature (steepness) of an eye with respective standard deviations. That is, the larger the Keratometry reading, the steeper or the more curved is the eye. The control procedure is an unoperated eye. For the same myopic correction, less corneal flattening using the FLEx procedure as compared to using the LASIK procedure is observed. For example, with -3.00D correction, using the FLEx procedure Kh is about 37.16 diopter, while using the LASIK procedure Kh is 37.00 diopter. The Kh using the FLEx procedure is larger than that using the LASIK procedure; thus the eye using the FLEx procedure is steeper or more curved, therefore less flattening.

[0086] In Figure 2, *in vivo* confocal microscopy shows that there is no increase in keratocyte activation following FLEx as compared to LASIK for the same myopic

correction. For example, keratocyte activation at the direct anterior of the keratotomy site is represented by the solid small arrows. However there is evidence of more inflammation in the LASIK group at higher corrections (large arrows).

[0087] Figures 3a and 3b show immunohistochemistry images of increasing fibronectin expression with increasing power of myopic correction with both ReLEx and LASIK. As used herein, the term "ReLEx" may be interchangeably referred to as "FLEx". In this regard, the myopic correction of –6.00D is of a larger or increased power as compared to the myopic correction of –3.00D. For example in Figure 3, increasing fibronectin expression in term of higher intensity shades are observed as indicated by the arrows.

[0088] Figures 4a and 4b show immunohistochemistry images of inflammation. In Figure 4b, it is observed that there are similar levels of inflammation at low correction (i.e., at -3.00D) between LASIK and ReLEx (as indicated by the area enclosed by the double-dashed rectangle). However, at higher corrections (i.e., at -6.00D) there is more inflammation in the LASIK group (as indicated by the arrows in Figure 4a and the area enclosed by the single-dashed ellipse in Figure 4b).

[0089] Light microscopy of an extracted lenticule shows evidence of a biconvex shaped lenticule (as seen in Figure 5a, magnified 100x), and high power TEM shows in Figure 5d that the keratocytes in the lenticule are viable indicating no evidence of degeneration or cell death. Figure 5b shows a 400x magnified image of the anterior edge and Figure 5c shows a 400x magnified image of the posterior edge. Figure 5d shows a 5600x magnified image of a keratocyte.

[0090] Slit lamp (SL) images in Figure 6a and the confocal microscope images of Figures 6b show clearing of lenticule following re-implantation in accordance to various embodiments, for example, in Example B. Figure 6c shows an increase in corneal thickness back to preoperative stat on OCT.

[0091] *In vivo* confocal microscopy of the lenticule in Figure 7 shows an increased hyper intense signal (as indicated by arrows) in the early postoperative period that reduces (in intensity) by 14 days and 28 days after re-implantation.

[0092] The topography results of Figure 8 shows that 28 days after re-implantation of the lenticule, the keratometry readings are within -0.6 ± 0.8D of the preoperative keratometry.

[0093] Figure 9 shows immunohistochemistry images of inflammation of the eye 28 days after re-implantation of the lenticule compared against a control (in the rightmost column). The leftmost column of Figure 9 shows 100x magnified immunohistochemistry images of the eye 28 days after re-implantation of the lenticule while the center column shows respective 200x magnified images of the enclosed rectangular areas correspondingly marked out in each of 100x magnified images in the leftmost column. It is shown from the images of the eye 28 days after re-implantation of the lenticule, more clearly in the enlarged images of the center column that new extracellular matrix formation and minimal inflammation are present, as indicated by the light shades within the area enclosed by the dashed ellipses. However, extracellular matrix formation and inflammation are minimal as the leftmost column is comparatively similar to the rightmost column of the control in terms of fluorescence intensity and distribution. Thus Figure 9 shows that there is still evidence of minimal extracellular matrix formation (e.g., Fibronectin, CD18, Tenascin-C) and tissue inflammation (e.g., α-SMA, Thy-1) deposition at 28 days of post-operation.

[0094] Figure 10 shows confocal microscopic images of inflammation similar to Figure 9 using various labels (TUNEL) and stains (Ki-67 and Phalloidin).

[0095] There is minimal evidence of proliferating cells and myofibroblasts at 28 days of post-operation, indicating minimal haze formation.

[0096] Figure 11a shows a light microscopic image of extracted stromal lenticules as described in Example C. Figure 11b shows a magnified light microscopic image of the extracted stromal lenticules before cryopreservation following collagenase digestion as described in Example C. Figure 11c shows a cell culture of the extracted stromal lenticules. After adding the stock freezing solution containing 10% FBS and 20% DMSO to the cell culture, longish cells (fibroblasts) are observed in Figure 11d. Figure 11e shows the cell culture following a culture of a fresh lenticule, and Figure 11f shows cell culture after 1 month of cryopreservation. Figure 11g shows the gel electrophoresis

for real-time PCR of the extracted stromal lenticules after cryopreservation, wherein KERA refers to keratoctyes, ALDH31A refers to Aldehyde dehydrogenase 31A, GAPDH refers to glyceraldehyde-3-phosphate dehydrogenase, and the leftmost band of the gel electrophoresis being a marker. In Figure 11g, the presence of keratocyte mRNA is observed.

[0097] Using TEM and cell culture, presence of keratoctyes before and after cryopreservation are observed and that they were viable. Viability is shown both with 4',6-diamidino-2-phenylindole (DAPI)/TUNNEL assay and also cell culture. Cell phenotype is shown on real-time PCR to verify that they are keratocytes.

[0098] The object of various embodiments is to store the lenticule following extraction for an indefinite period (or for at least the lifetime of a patient) in a freezing manner.

[0099] Freezing may include all forms of cryobiology from fast freezing to vitrification. Following preservation, the lenticule is stored by freezing. Storage is required so that re-implantation of the lenticule at a later date can be carried out. The re-implantation is able to correct the original refractive error that has been removed by the lenticule; thereby reversing the procedure, or to enable further adjustment of the refractive errors, including restoration of near vision, This allows for solving presbyopia at a subsequent date, or to treat the potential complication of keratectasia due to, for example, inadequate stromal bed thickness and strength, which may be associated with collagen cross-linking of the lenticule and/or stromal recipient bed. This makes the ReLEx procedures reversible forms of corneal refractive surgery, which offers considerable advantages over current techniques.

[00100] The reversible refractive procedure allows for (i) the reimplantation of the lenticule if needed to treat future presbyopia, or (ii) the correction of the refractive error if the patient wants the procedure reversed, or (iii) the treatment of keratectasia, or (iv) more accurate calculation of intraocular lens power for cataract surgery if the patient needs this as he/she gets older. Ultimately, it is desirable that the lenticules are allowed to be stored for the lifetime of the patient so that it may be replaced at anytime.

[00101] The lenticules may also be modified by surgical or excimer laser techniques to alternative refractive powers. Various clinical scenarios are:

- Scenario 1: Total reversal of refractive procedure in the same patient

  The unmodified lenticule is re-implanted back into the same patient either through the original flap or via a new femtosecond ablation procedure, potentially for treatment of keratectasia.

- Scenario 2: Reimplantation into the same patient to treat subsequent alterations in refractive error

  The lenticule may be modified (e.g. by excimer ablation) to suit the required refractive error, e.g. hyperopia or astigmatism.

- Scenario 3: Reimplantation to treat onset of presbyopia in the same patient

  The lenticule may be modified to a low hyperopic power (e.g. about 1.00D or about 1.50D) and reimplanted in the non-dominant eye of a presbyopic patient to effect monovision correction.

- Scenario 4: Allograft lenticule insertion

  The lenticule may be donated by the patient (with appropriate informed consent and donor serology testing) to be used as a lamellar corneal stromal allograft transplant to effect refractive treatments for other patients in the same situations as Scenarios 1 to 3 above.

- Scenario 5: Allograft lenticule insertion

  The lenticules may be donated by the patient (with appropriate informed consent and donor serology testing) to be used as a lamellar corneal stromal allograft transplant for treating a variety of other corneal refractive, tectonic or therapeutic conditions, such as keratoconus, corneal thinning and irregularities, descemetoceles, and corneal perforations.

- Scenario 6: Allograft lenticule insertion as a standardized presbyopic biological implant

  The lenticules may be donated by the patient (with appropriate informed consent and donor serology testing) to be used as a lamellar corneal stromal allograft transplant for treating presbyopia in other patients by standardized modification of the lenticules to presbyopic designs, such as multifocal designs,

wavefront guided designs, diffractive designs, aspheric profile designs, and a small (e.g. about 3-5 mm) "centre near" presbyopic implant to effect a bifocal cornea effect.

[00102] A lenticule banks linked to the ReLEx procedure with storing them on a RGP contact lens or similar devices or a mounting structure which has a curved surface replicating the corneal surface radius to ensure correct shape for storage. The patients' lenticules may be stored in such a bank underwritten costs by the patient for possible reimplantation at a future date.

[00103] While the invention has been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. The scope of the invention is thus indicated by the appended claims and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

CLAIMS

1. A method for performing a reversible refractive procedure by reimplanting a refractive lenticule that has been obtained by a refractive procedure on the eye, the method comprising the steps of:

preparing the refractive lenticule for reimplantation;

creating a corneal flap in an eye of an acceptor;

transferring the refractive lenticule under the corneal flap; and

repositioning and covering the acceptor's corneal flap; thereby reimplanting the refractive lenticule.

2. The method as claimed in claim 1, wherein the refractive lenticule has been stored at a temperature below 0 °C.

3. The method as claimed in claim 1 or 2, wherein the step of preparing the refractive lenticule for reimplantation comprises thawing the refractive lenticule in a water bath of about 37 °C.

4. The method as claimed in claim 3, wherein upon thawing, the refractive lenticule is washed in a buffer solution.

5. The method as claimed in claim 4, wherein the buffer solution is selected from the group consisting of morpholinepropanesulfonic acid, a Phosphate Buffered Solution (PBS), a trehalose buffer, and a sucrose buffer.

6. The method as claimed in any one of claims 1 to 5, wherein the step of preparing the refractive lenticule for reimplantation further comprises enzymatically digesting the thawed refractive lenticule for culture of corneal stromal fibroblast.

7. The method as claimed in any one of claims 1 to 6, wherein the step of preparing the refractive lenticule for reimplantation further comprises adjusting refractive

errors of the refractive lenticule to restore near vision, or to correct presbyopia, or to treat complications of keratectasia.

8. The method as claimed in any one of claims 1 to 7, wherein the step of repositioning and covering the acceptor's corneal flap comprises placing a bandage contact lens over the repositioned corneal flap.

9. The method as claimed in any one of claims 1 to 8, wherein the refractive lenticule for reimplantation has been obtained by a method comprising the steps of:

creating an incision in an eye of a donor; and

extracting the refractive lenticule through the incision.

10. The method as claimed in claim 9, wherein the incision is made using a one-laser refractive surgery.

11. The method as claimed in claim 10, wherein the one-laser refractive surgery is Refractive Laser Extraction (ReLEx).

12. The method as claimed in claim 11, wherein the ReLEx comprises Small Incision Lenticule Extraction (SMILE).

13. The method as claimed in claim 10, wherein the incision comprises one or two or three or four stromal incisions.

14. The method as claimed in claim 13, wherein the four stromal incisions further create a corneal flap in the donor's eye.

15. The method as claimed in claim 11, wherein the ReLEx is Femtosecond Lenticule Extraction (FLEx).

16. The method as claimed in any one of claims 9 to 15, wherein the donor and

the acceptor are the same subject.

17. The method as claimed in any one of claims 9 to 15, wherein the donor and the acceptor are different subjects.

18. A refractive lenticule obtained by a refractive procedure on the eye of a donor for use in a method for subsequent reimplantation in a reversible refractive procedure on an acceptor, wherein the refractive lenticule is stored at a temperature below 0°C.

19. The refractive lenticule for use as claimed in claim 18, wherein for storage, the refractive lenticule is snap-freezed in liquid nitrogen for about 10 minutes.

20. The refractive lenticule for use as claimed in claim 18 or 19, wherein the refractive lenticule is stored at about -80 °C.

21. The refractive lenticule for use as claimed in claim 18, wherein the refractive lenticule is cryopreserved.

22. The refractive lenticule for use as claimed in claim 21, wherein for the cryopreservation, the refractive lenticule is

suspended in a serum-containing medium in a vessel;

a freezing solution of about 10% serum and about 20% organic solvent is added in a dropwise manner into the vessel to form a mixture of about 10% serum and about 10% organic solvent;

the vessel containing the mixture is placed in a -80 °C freezer for at least 4 hours; and

the frozen mixture is transferred into liquid nitrogen for storage.

23. The refractive lenticule for use as claimed in claim 22, wherein the organic solvent is selected from the group consisting of organic solvent is selected from the group

consisting of hexane, heptane, cyclohexane, benzene, pyridine, dichloromethane, chloroform, carbon tetrachloride, carbon disulfide, tetrahydrofuran, dioxane, diethyl ether, diisopropylether, ethylene glycol monobutyl ether, methyl ethyl ketone, methyl isobutyl ketone, acetone, cyclohexanone, ethyl acetate, isobutyl isobutyrate, ethylene glycol diacetate, dimethylformamide, acetonitrile, N,N-dimethyl acetamide, nitromethane, acetonitrile, N-methylpyrrolidone, dimethylsulfoxide water, methanol, ethanol, butyl alcohol and formic acid.

24. The refractive lenticule for use as claimed in claim 22 or 23, wherein the serum is a fetal bovine serum.

25. The refractive lenticule for use as claimed in any one of claims 18 to 24, wherein the refractive lenticule is obtained by creating an incision in an eye of the donor; and extracting the refractive lenticule through the incision.

26. The refractive lenticule for use as claimed in claim 25, wherein the incision is created using a one-laser refractive surgery.

27. The refractive lenticule for use as claimed in claim 26, wherein the one-laser refractive surgery is Refractive Laser Extraction (ReLEx).

28. The refractive lenticule for use as claimed in claim 27, wherein the ReLEx comprises Small Incision Lenticule Extraction (SMILE) or Femtosecond Lenticule Extraction (FLEx).

29. The refractive lenticule for use as claimed in any one of claims 18 to 28, wherein the donor and the acceptor are the same subject.

30. The refractive lenticule for use as claimed in any one of claims 18 to 28, wherein the donor and the acceptor are different subjects.

31. The refractive lenticule for use as claimed in any one of claims 18 to 30, wherein prior to the subsequent reimplantation, the stored refractive lenticule is processed to adjust its refractive errors to restore near vision, or to correct presbyopia, or to treat complications of keratectasia.

Method for Performing a Reversible Refractive Procedure

ABSTRACT

The present invention is directed to a method for performing a reversible refractive procedure by reimplanting a refractive lenticule that has been obtained by a refractive procedure on the eye, the method comprising the steps of preparing the refractive lenticule for reimplantation, creating a corneal flap in an eye of an acceptor, transferring the refractive lenticule under the corneal flap, and repositioning and covering the acceptor's corneal flap; thereby reimplanting the refractive lenticule. The present invention is also directed to a refractive lenticule obtained by a refractive procedure on the eye of a donor for use in a method for subsequent reimplantation in a reversible refractive procedure on an acceptor, wherein the refractive lenticule is stored at a temperature below 0°C.

Figure 6a

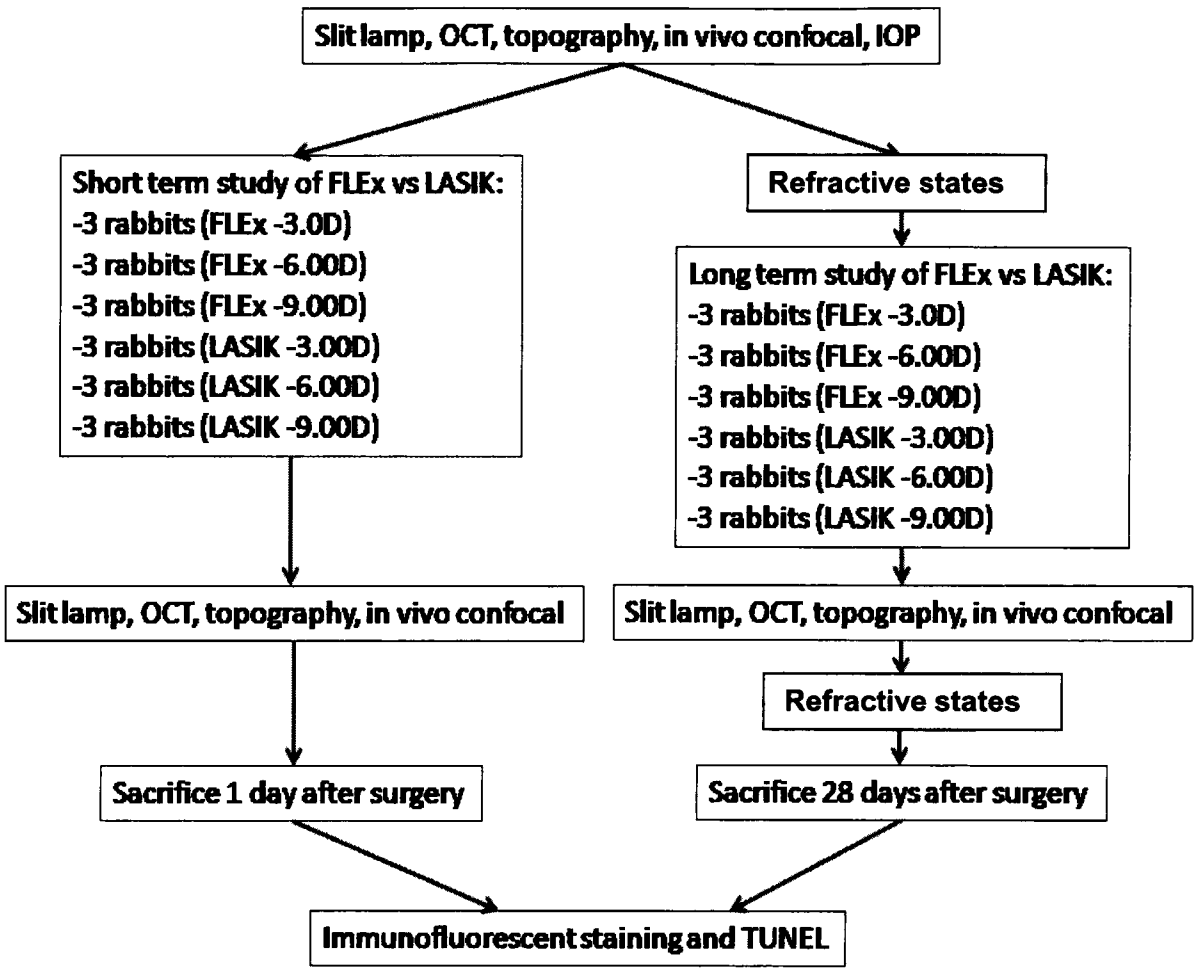
Slit lamp, OCT, topography, in vivo confocal, IOP
Short term study of FLEx vs LASIK:
-3 rabbits (FLEx -3.0D)
-3 rabbits (FLEx -6.00D)
-3 rabbits (FLEx -9.00D)
-3 rabbits (LASIK -3.00D)
-3 rabbits (LASIK -6.00D)
-3 rabbits (LASIK -9.00D)
Refractive states
Long term study of FLEx vs LASIK:
-3 rabbits (FLEx -3.0D)
-3 rabbits (FLEx -6.00D)
-3 rabbits (FLEx -9.00D)
-3 rabbits (LASIK -3.00D)
-3 rabbits (LASIK -6.00D)
-3 rabbits (LASIK -9.00D)
Slit lamp, OCT, topography, in vivo confocal
Slit lamp, OCT, topography, in vivo confocal
Refractive states
Sacrifice 1 day after surgery
Sacrifice 28 days after surgery
Immunofluorescent staining and TUNEL

Figure 1

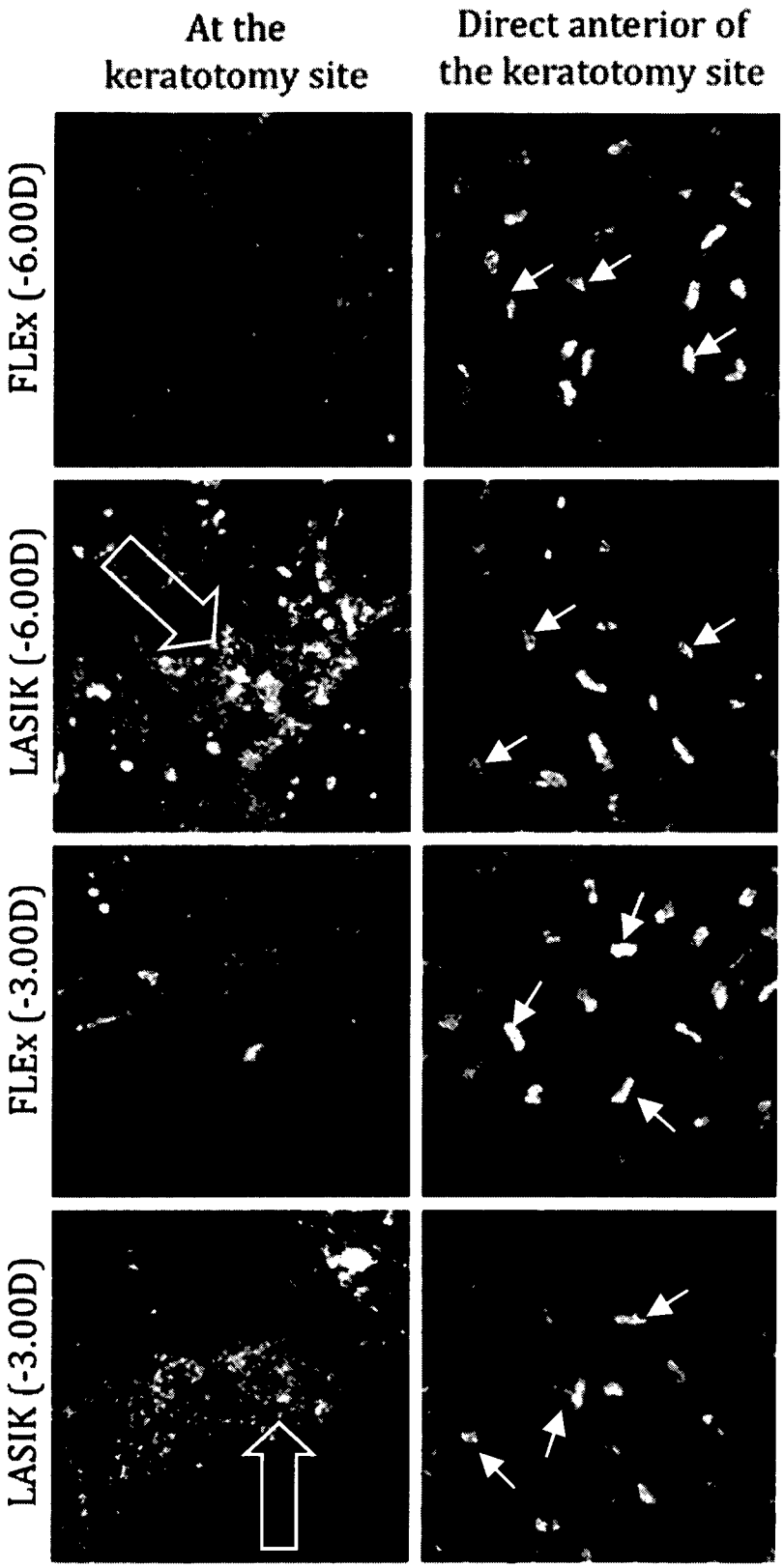
At the keratotomy site
Direct anterior of the keratotomy site
FLEx (-6.00D)
LASIK (-6.00D)
FLEx (-3.00D)
LASIK (-3.00D)

Figure 2

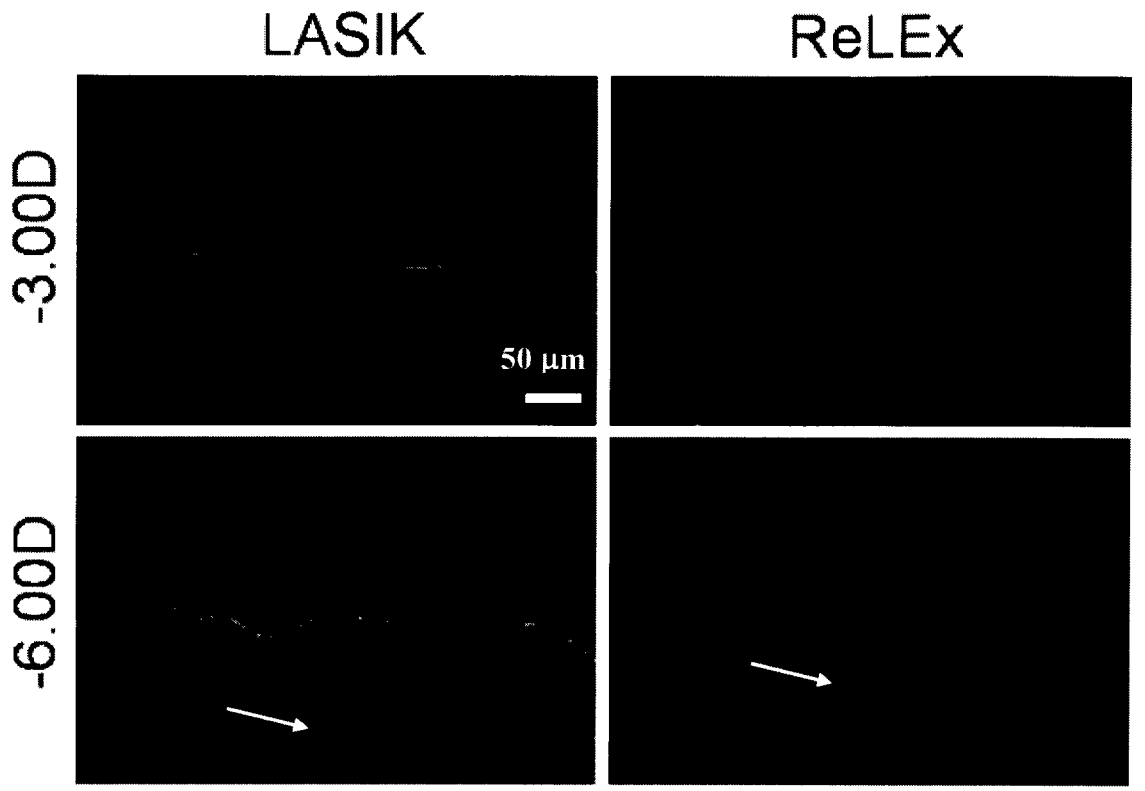

Figure 3a

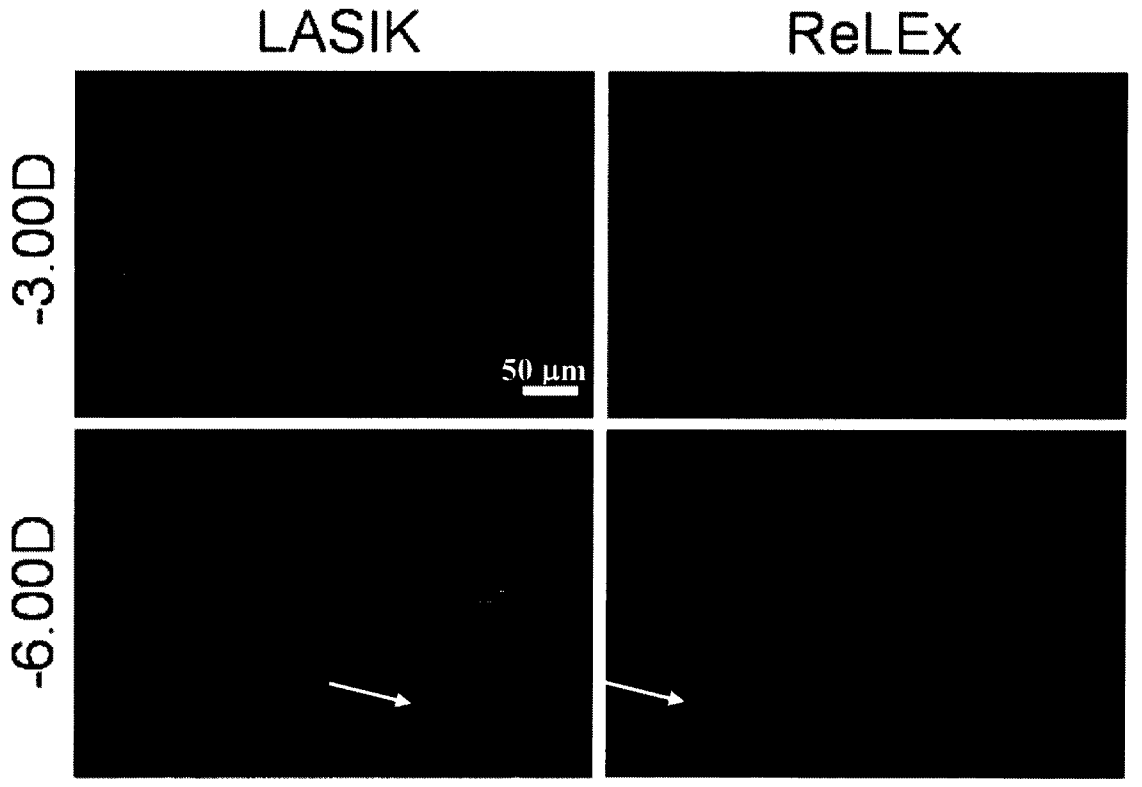

Figure 3b

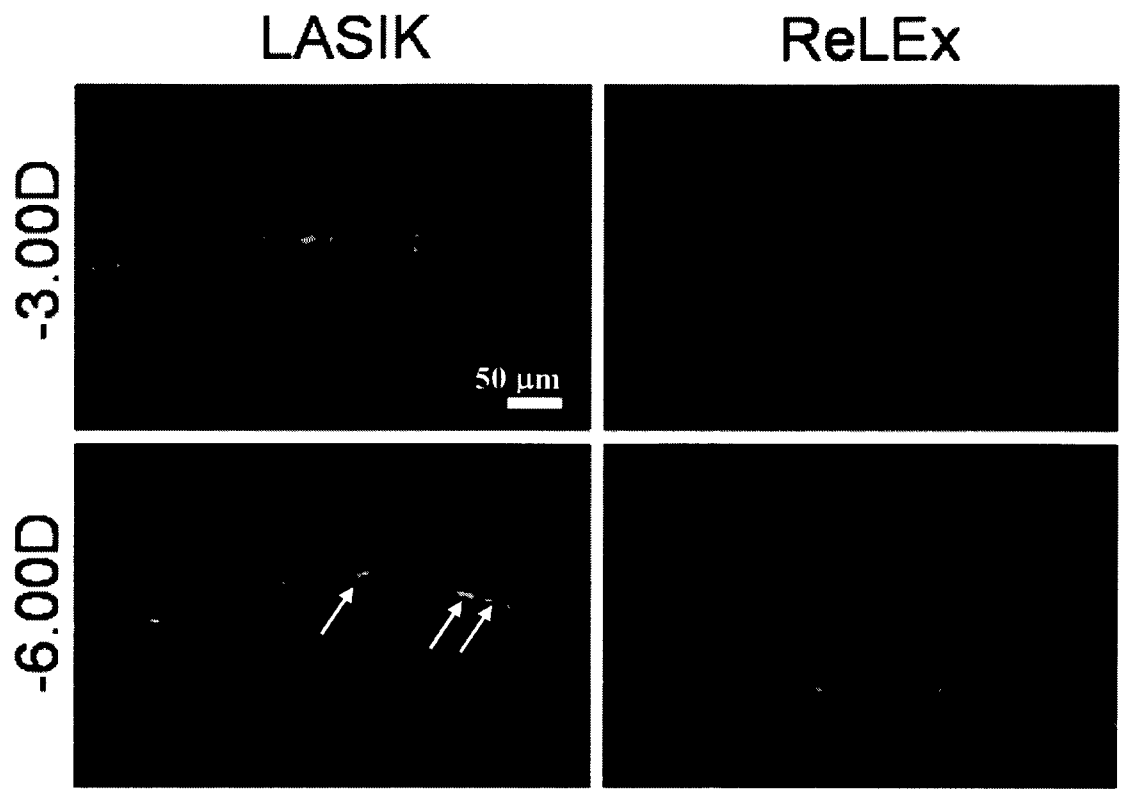

Figure 4a

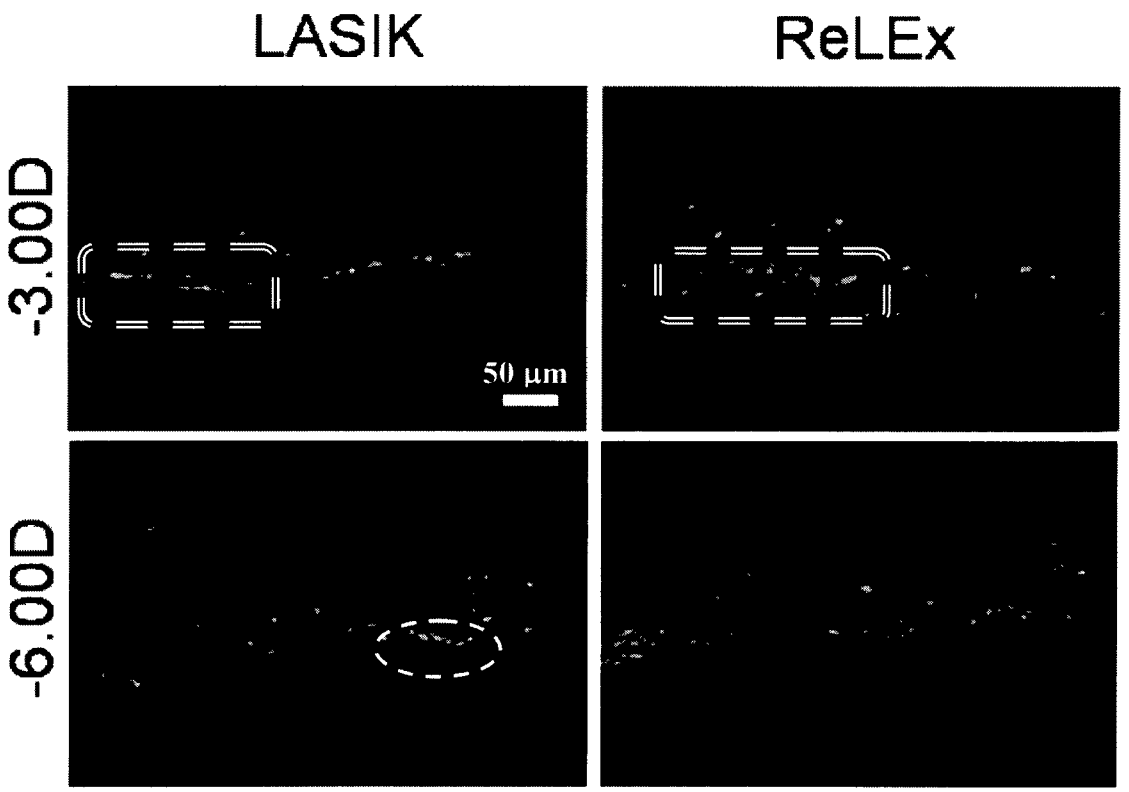

Figure 4b

Figure 5a

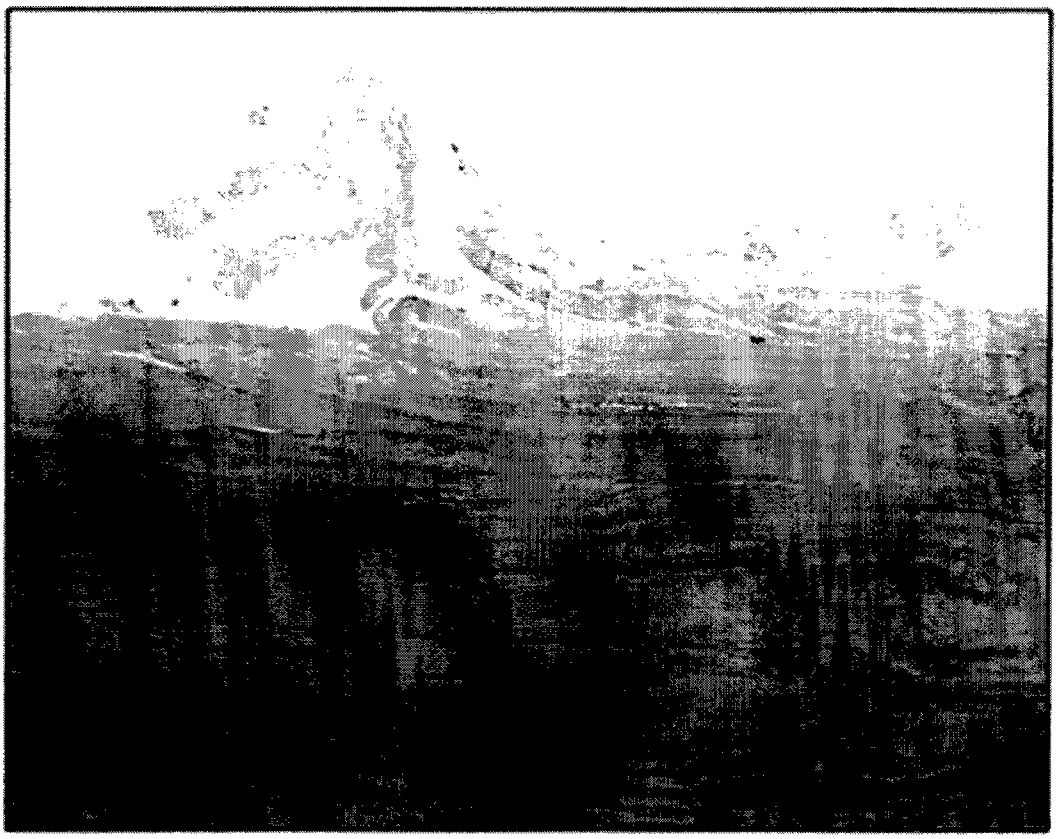

Figure 5b

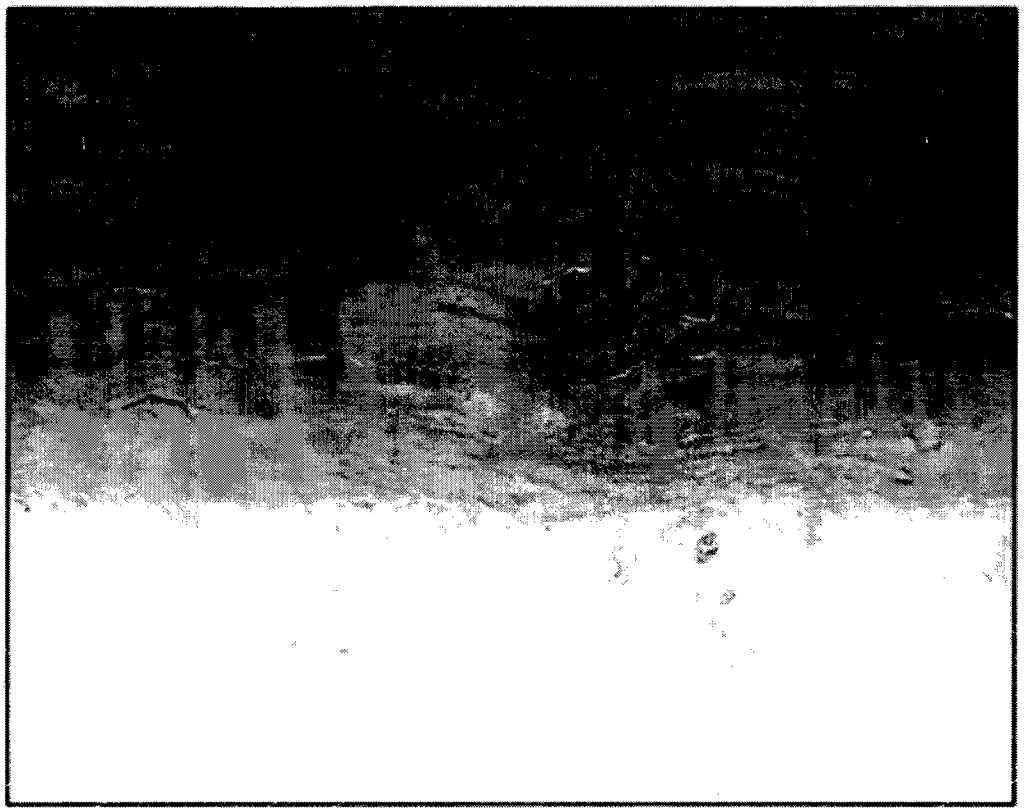

Figure 5c

Figure 5d

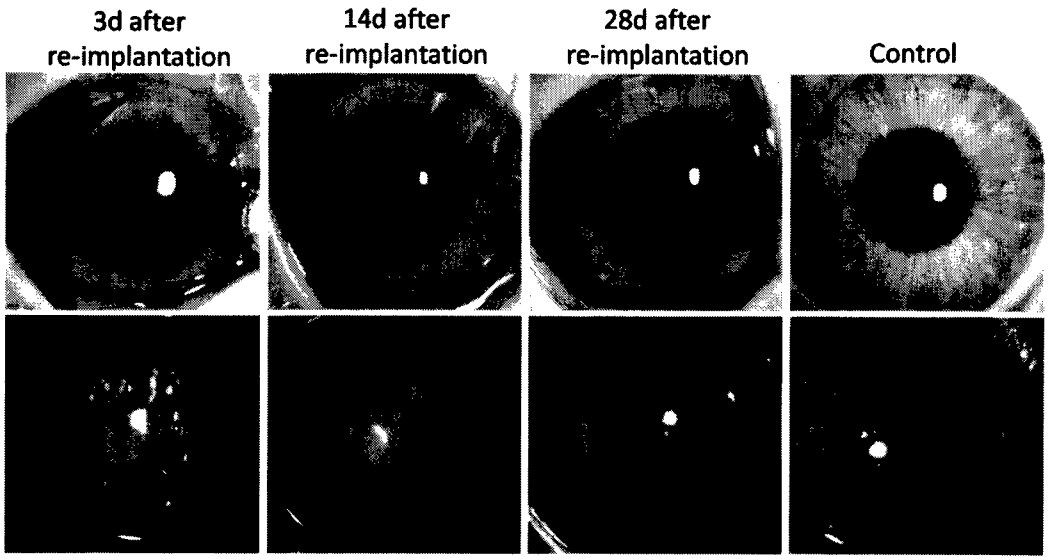

Figure 6a

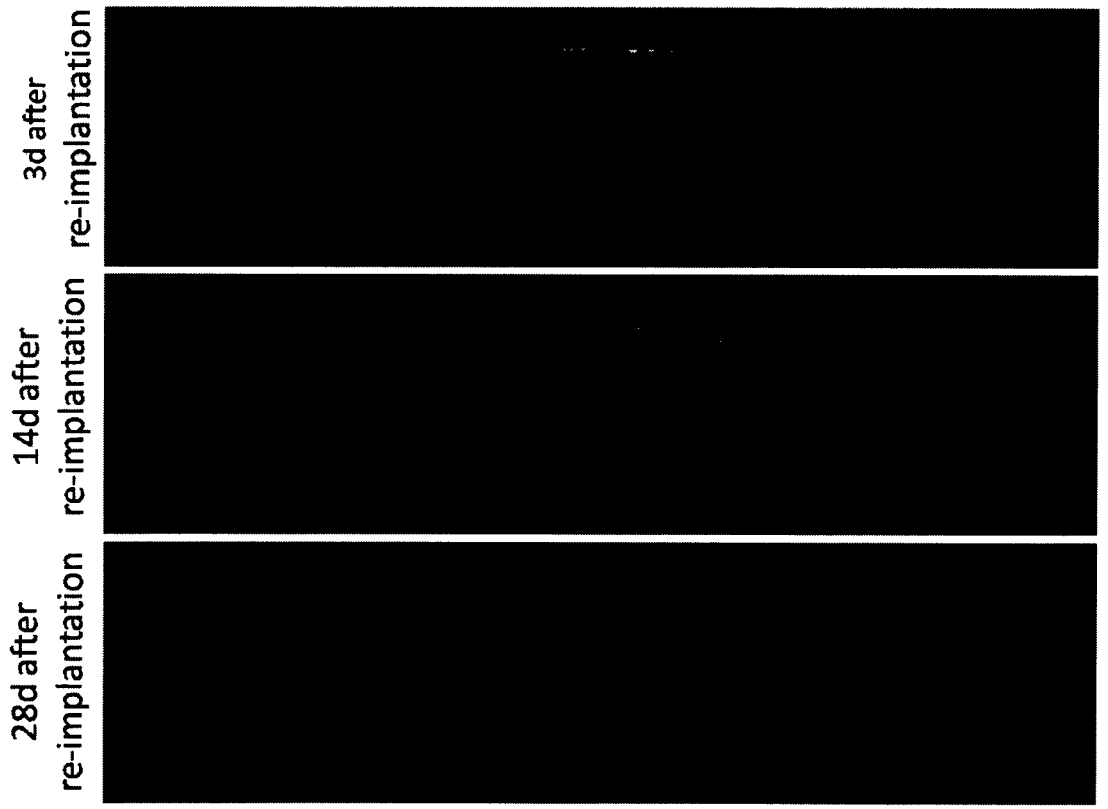

Figure 6b

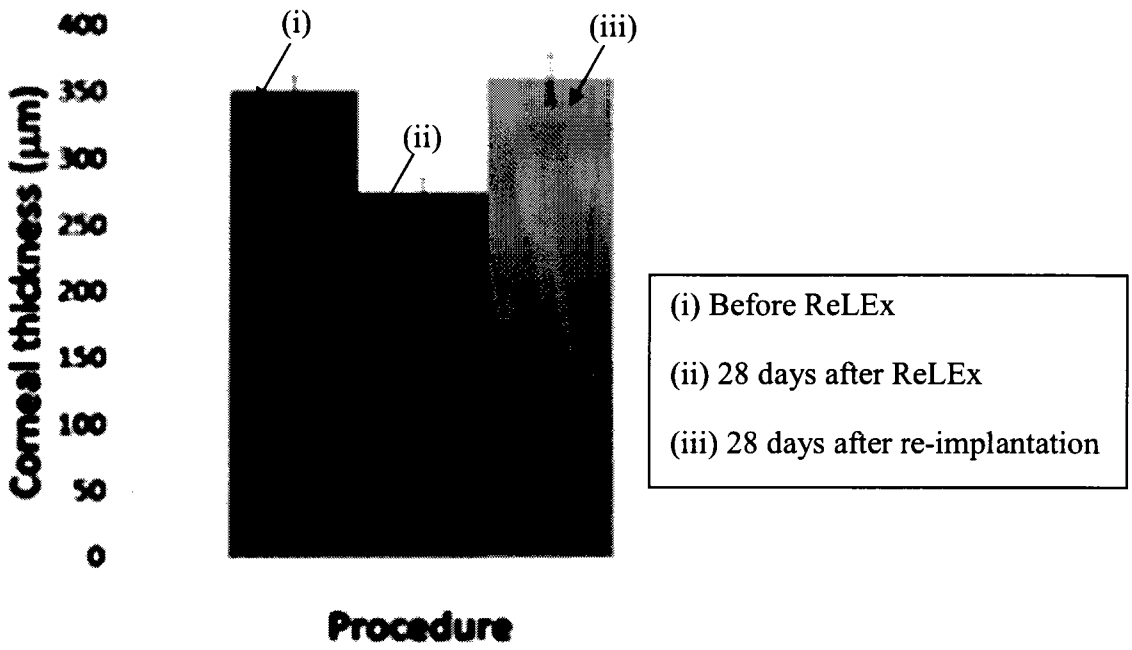
Corneal thickness (µm)
400
350
300
250
200
150
100
50
0
(i)
(ii)
(iii)
Procedure
(i) Before ReLEx
(ii) 28 days after ReLEx
(iii) 28 days after re-implantation

Figure 6c

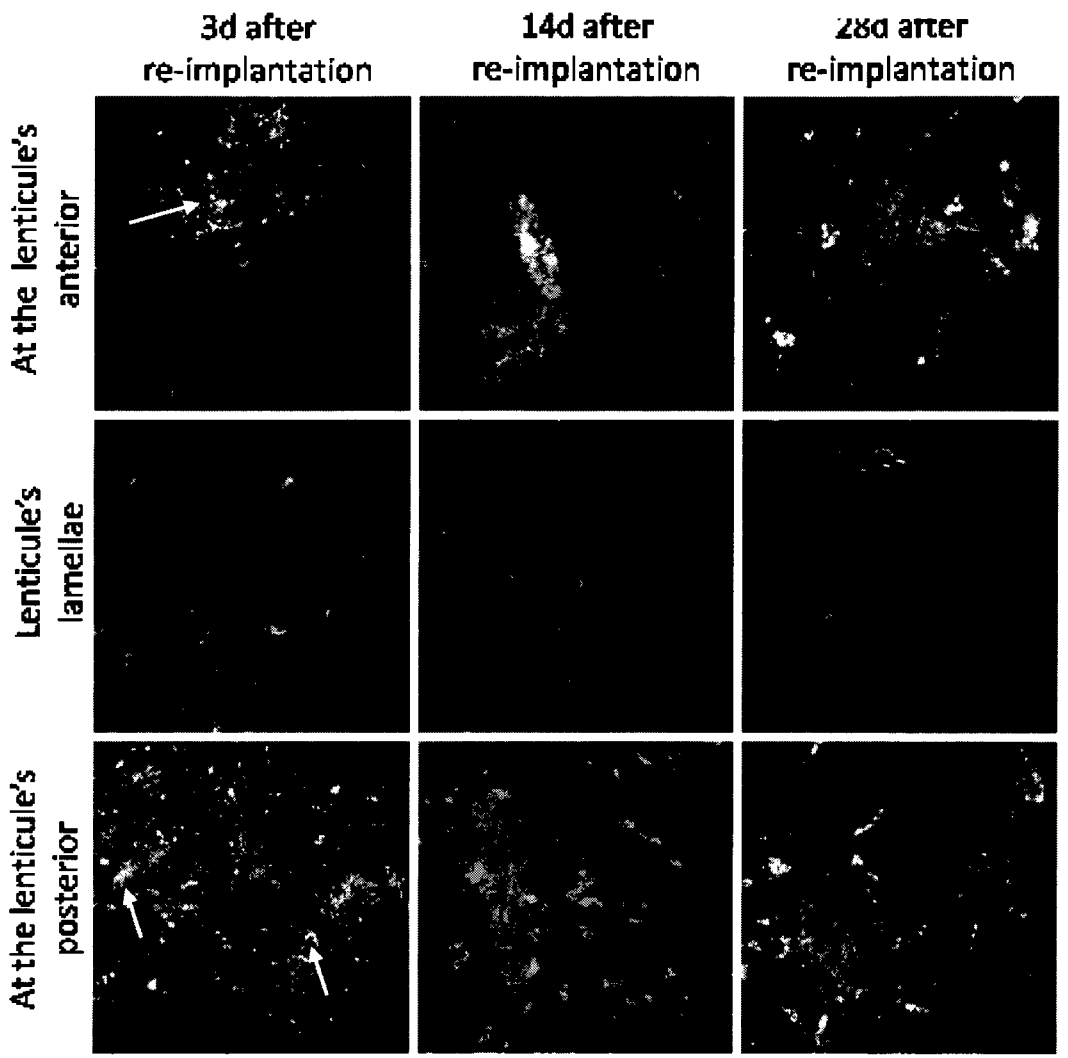
3d after re-implantation
14d after re-implantation
28d after re-implantation
At the lenticule's anterior
Lenticule's lamellae
At the lenticule's posterior

Figure 7

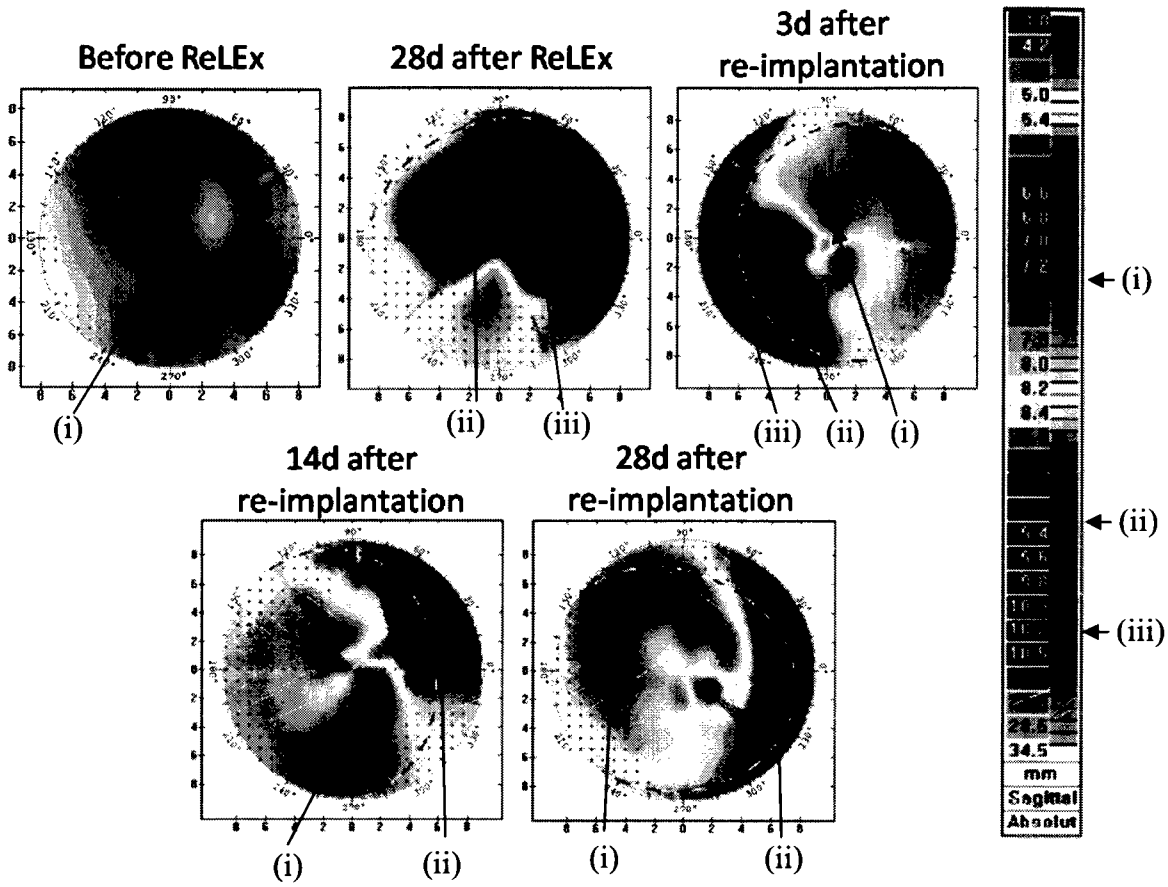
Before ReLEx
28d after ReLEx
3d after re-implantation
14d after re-implantation
28d after re-implantation
(i)
(ii)
(iii)
5.0
5.4
8.0
8.2
8.4
34.5
mm
Sagittal
Absolut

Figure 8

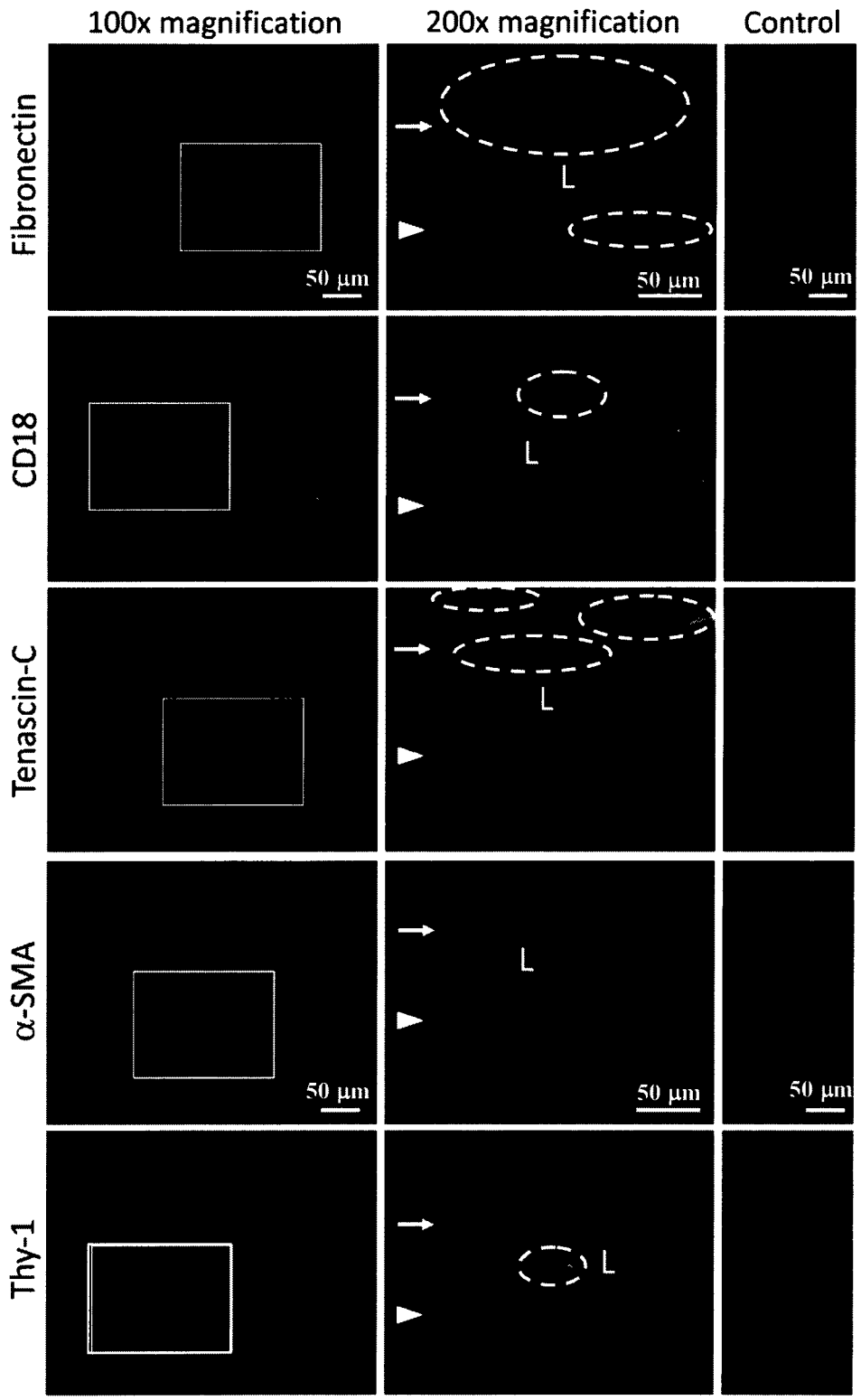
100x magnification
200x magnification
Control
Fibronectin
CD18
Tenascin-C
α-SMA
Thy-1
L
50 µm

Figure 9

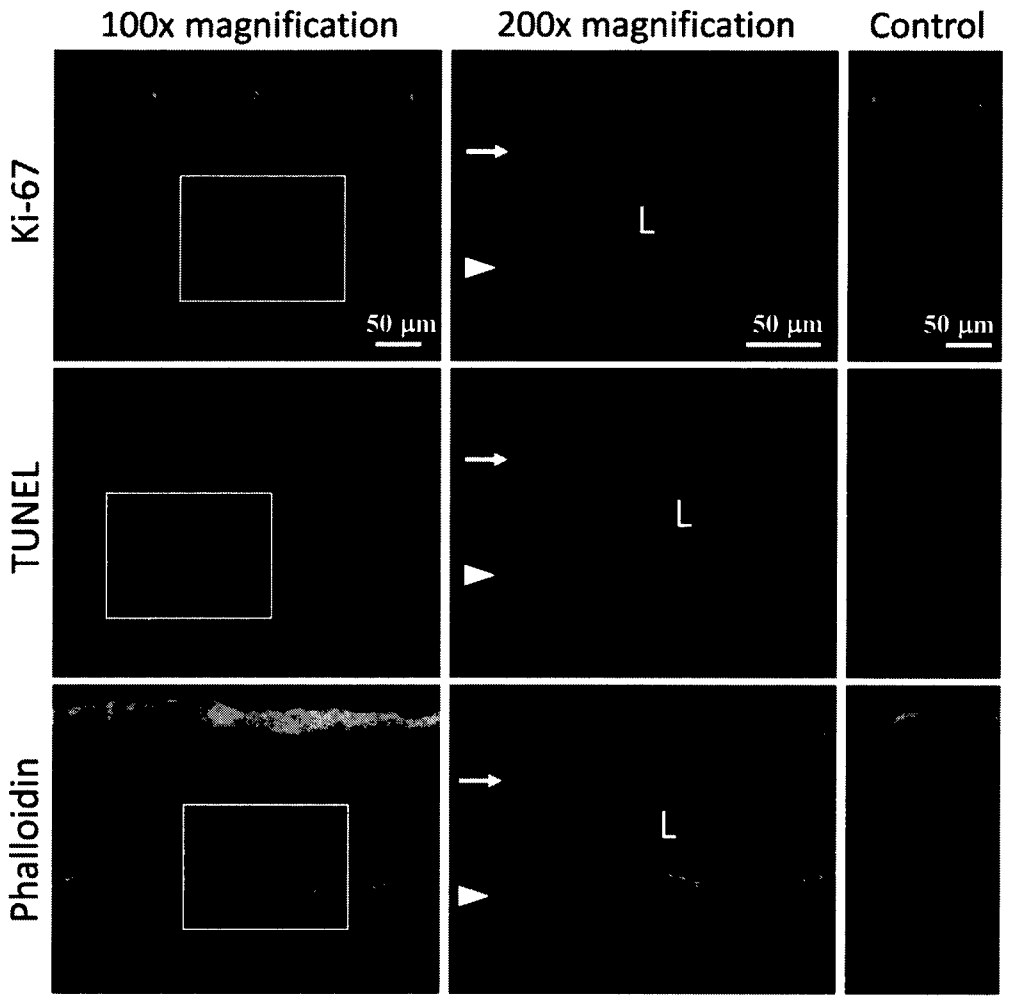
100x magnification
200x magnification
Control
Ki-67
TUNEL
Phalloidin
L
50 µm

Figure 10

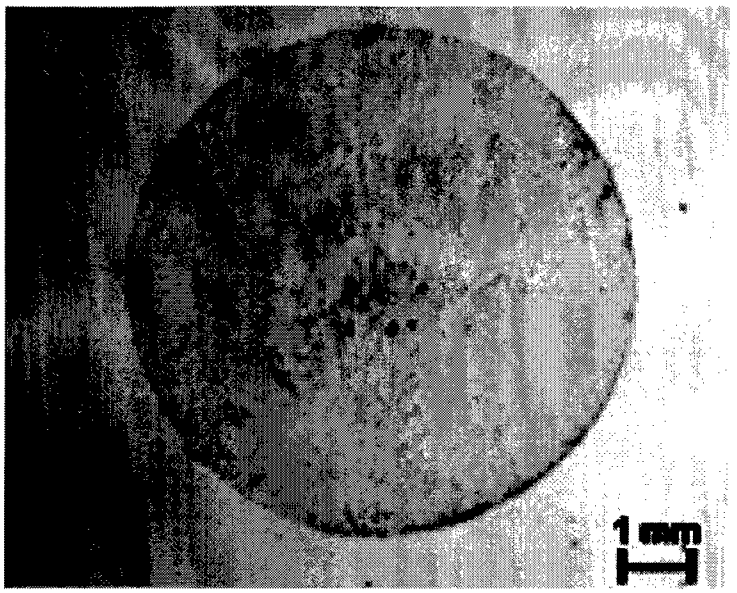

Figure 11a

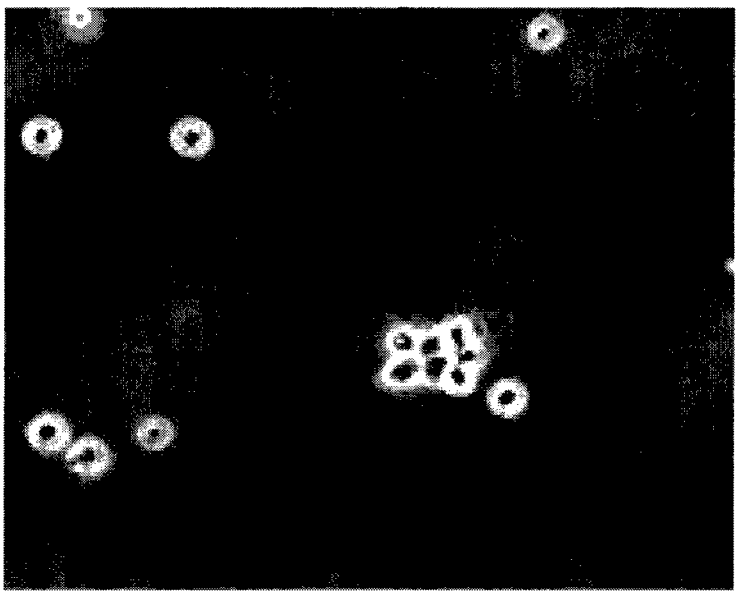

Figure 11b

Figure 11c

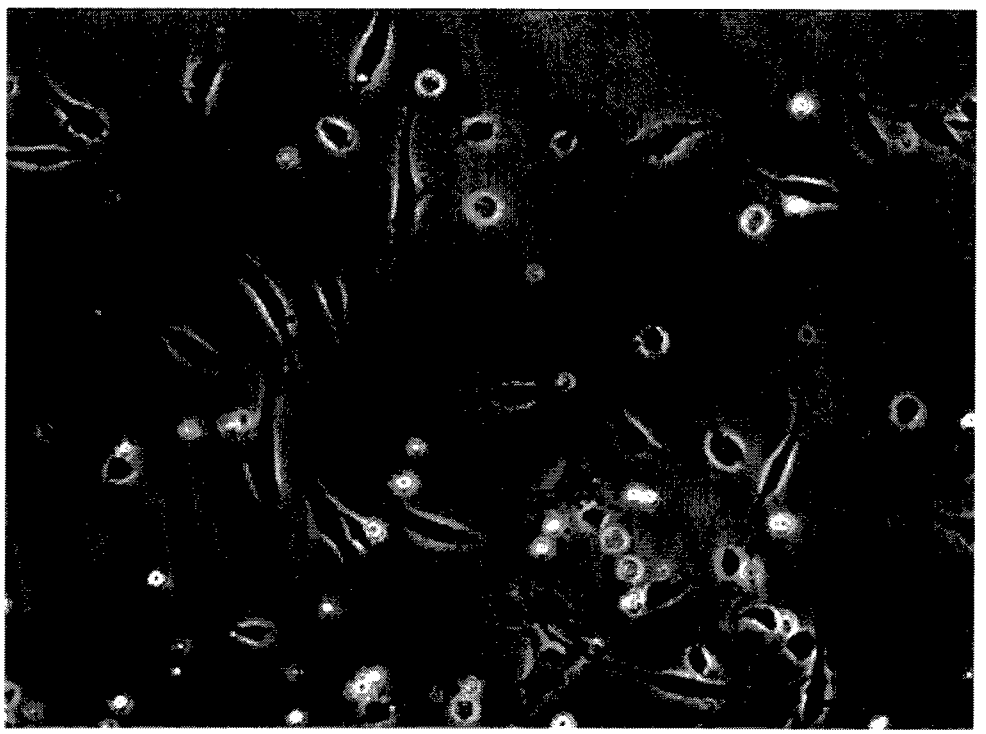

Figure 11d

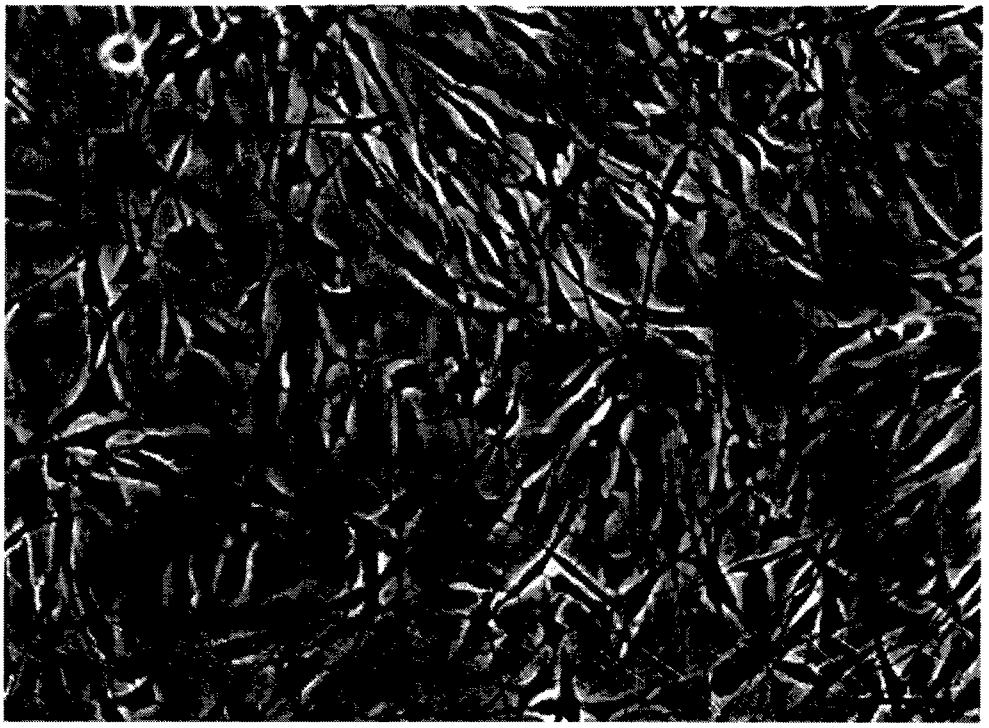

Figure 11e

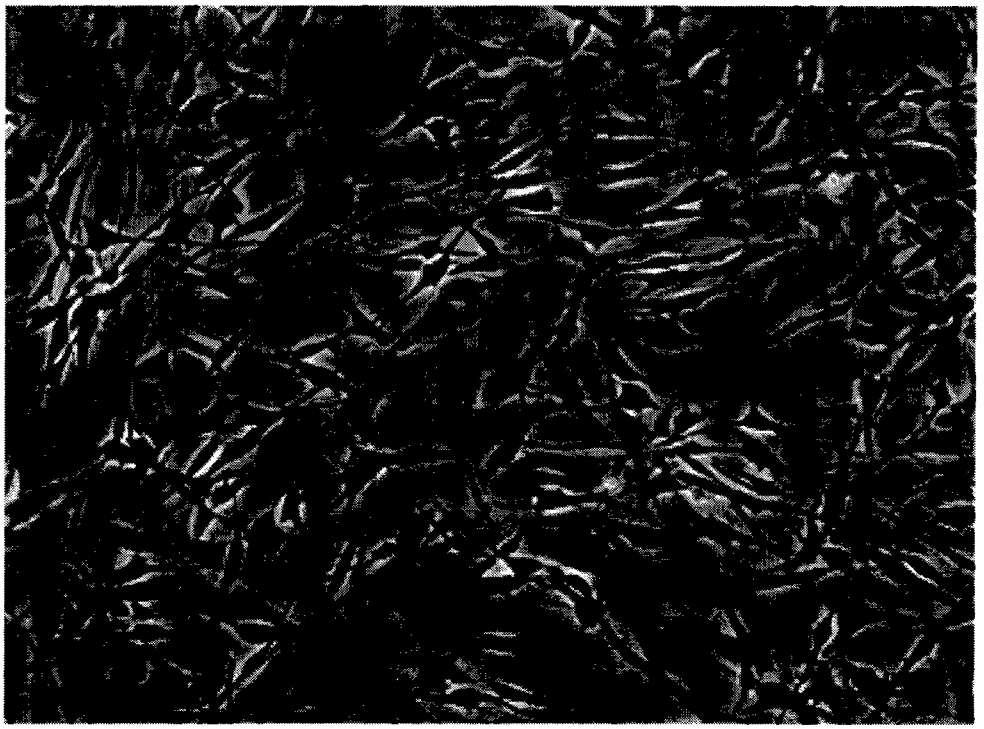

Figure 11f

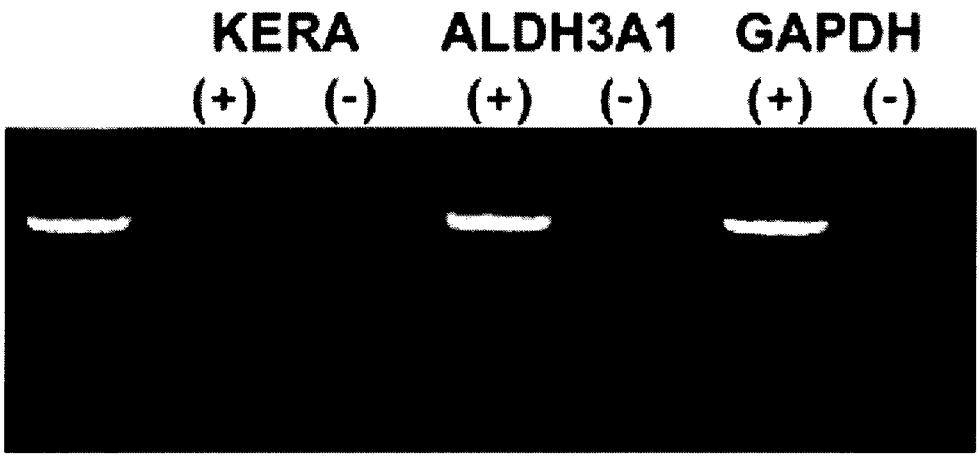

Figure 11g

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2011/002125

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61F 9/01 (2006.01) ***A61F 9/013*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC, WPI: IPC and EC: A61F 9/- and keywords: laser, ultra fast, femto, relex, smile, flex, cryo freeze, cryo lathe, corneal press, reposition, reimplant, reverse, incise, shape, carve, pre carve, sculpt, extract, graft, refractive surgery, optical power, thaw, liquid nitrogen, dehydrate, rehydrate, epithelial, lyophilize, donor, lenticule; and like terms

PATENT LENS, GOOGLE PATENTS Keywords: Refractive lenticule, Corneal flap, donor, acceptor, allograft, xenograft, autograft, recipient, anterior, curvature, tissue lens, stromal bed, stromal fibroblast, serum, fetal bovine, buffer, morpholinepropanesulfonic, phosphate, PBS, trehalose, sucrose

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0326650 A1 (ZICKLER et al.) 31 December 2009 Abstract, Figure 4, paragraphs [0005], [0008], [0009], [0020], [0021], [0025], [0030], [0038], [0051], [0054], [0056], [0060]	1-31
A	US 6989008 B2 (PEYMAN) 24 January 2006 column 9, lines 24-25	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2012	31January 2012
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **ASHOK KRISHNAN** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2190

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2011/002125

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5891617 A (WATSON et al.) 6 April 1999 column 4, lines 16-19	
A	US 2009/0017438 A1 (ROY et al.) 15 January 2009 paragraphs [0084], [0094]	
A	US 6630001 B2 (DURAN et al.) 7 October 2003 column 6, lines 12-23	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/IB2011/002125

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
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		BR	0008601	BR	0008624	BR	9809289
		CA	2286718	CA	2364038	CA	2366022
		CN	1253484	CN	1342095	CN	1360486
		EP	1014872	EP	1158936	EP	1159033
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		MX	PA01008759	MX	PA01008760	MX	PA05004067
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		MX	9705540	NO	973463	NO	961048
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX