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The Regional Influence of Different Corneal Collagen Cross-linking Methods on the Biomechanical Behavior of Rabbit Cornea

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Abstract

Purpose: To investigate the regional effect of different corneal collagen crosslinking methods on the biomechanical behavior of rabbit cornea.Methods: 12 white Japanese rabbits were randomly divided into two groups(n=6 each). The treated(right) eyes of the two groups were treated with riboflavin for 3mw/cm² and 30min, 30mw/cm² and 3min respectively (5.4 J/cm²). The control (left) eyes were only treated with riboflavin. After feeding for one month, the corneas were removed and marked. The cornea is divided into three regions, and the deformation curves of different regions are obtained. The modulus of each region is obtained by reverse modeling.Results: The modulus of the treated eyes were higher than that of the control eyes. The 3mw group increased by 123.41% in the central region, 80.93% in the paracentral region, and 59.47% in the peripheral region .The 30mw group increased by 43.88%, 36.93% and 32.95%. Conclusions: The total energy is the same, the irradiation is different, the cross-linking effect is also different, the Bunsen-Roscoe law of reciprocity is not always suitable for corneal cross-linking; compared with 30mw group, the 3mw group had better effect in all regions, especially in central region, and the effect of cross-linking was different in the three regions of cornea, central region was the best. The better therapeutic effect may be achieved by changing irradiating energy or irradiating degree to make up for the deficiency of corneal mechanical properties.

Keywords

corneal collagen crosslinking; biomechanics; regional.

1. Introduction

Keratoconus(KC) is one of the most common non-inflammatory degenerative keratopathy, which occurs in both sides of the cornea, the arrangement of collagen fibers in the corneal stromal layer becomes disordered, the reduction of fiber and the degradation of extracellular matrix lead to regional thinning[1], which can produce highly irregular myopic astigmatism and different degrees of visual impairment[2], which are common causes of corneal transplantation [3]. KC usually starts in adolescence and is relatively common in Asians [4], with prevalence ranging from 17.3 to 2300 per 100,000 people [5,6]. The occurrence and development of KC are closely related to the regional changes in corneal biomechanical properties [7,8]. Most of the pathological changes occur in the inferior temporal lobe of the cornea with thinning thickness and weak mechanical properties [9]. Under the action of intraocular pressure, KC gradually expands outward, leading to the occurrence of diseases [7,8], which seriously affects the patients' vision and quality of life [10].

Corneal collagen cross-linking [11] can enhance the mechanical properties [12] of cornea and is widely used to prevent KC [13]. Corneal collagen cross-linking was performed by using riboflavin under 370nm UVA irradiation to induce new covalent bonds in and between collagen fibers of corneal matrix, which can improve corneal mechanical properties, and slow down or

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even prevent the progression of lesions. After years of development, CXL has gradually developed from the classical cross-linking with long time and strong discomfort in the early stage to the rapid cross-linking and transepithelial cross-linking scheme with shorter treatment time and less discomfort, and the methods have gradually diversified [14,15]. Although experimental and clinical studies have preliminarily proved the mechanism and efficacy of CXL, as an advanced treatment method, CXL still has some shortcomings. How to evaluate and find the best personalized CXL scheme becomes the key to the further development and further research of CXL.

At present, the long-term clinical effect, development and application prospect of CXL technology is the forefront and hot spot of international research on the treatment of keratoconus, especially in Europe and the United States. Many institutions and scholars have started to explore more optimized treatment parameters and treatment plans. In the evaluation of corneal biomechanics after CXL operation, Wollensak [16], Chai [17], Wernli [18] and Hammer [19]et al used axial tensile method to measure the changes of corneal biomechanics in pig eyes and in vitro human cornea before and after CXL, the results showed that elastic modulus of porcine and human corneas were higher than that before CXL operation. On the laboratory platform of in vitro corneal swelling, Knox [20], Dias [21] and Kling [22] used interferometer, atomic force microscope and Ray Tracing to measure the deformation of the cornea under the effect of hydraulic pressure respectively. The results showed that stiffness of the cornea increased after CXL, and the anterior corneal stroma is more obvious than that of the posterior stroma. The equipment of the axial stretching experiment platform[22,23] is simple and easy to operate, but it also has obvious disadvantages, such as destroying the angular sclera physiological arc and ignoring the difference of the thickness of the eye tissues in each region [24,25], which cannot accurately measure the variation degree of corneal mechanical properties (constitutive parameters) in different regions, and can't make up for the deficiency of corneal mechanical properties by irradiation energy regional differentiation CXL. The corneal inflation platform [26,27] can keep the surface appearance and the way of pressure is closed to the physiological state of eyeball, which has better accuracy and repeatability. Therefore, this study conducted multi-area measurement of corneal biomechanical properties through corneal inflation platform, verified the regional applicability of reciprocity rate in the process of CXL, and discussed the regional differences in the influence of CXL on corneal biomechanical properties, so as to provide basis for subsequent individualized regional differences in coital therapy.

2. Materials and Methods

2.1. Animals

Twelve white Japanese rabbits (2 to 3 kg, aged 3 to 4 months) were obtained from the Animal Breeding Unit of Wenzhou Medical University and randomly divided into two groups of 6 rabbits each. The rabbits were observed for 1 month before commencing the experimental study. All animals were treated in agreement with the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research and with the approval of the Animal Care and Ethics Committee of the Eye Hospital, Wenzhou Medical University.

2.2. CXL Treatment

General anesthesia was administered by intramuscular injection of pentobarbital sodium (Merck KGaA, Darmstadt, Germany; 30mg/kg) and SU-MIAN-XIN (Veterinary Institute at University of Munitions, Changchun, China) (0.2 mL/kg) and a wire eyelid speculum was positioned in the right eye of each rabbit. Prior to UVA irradiation, the epithelium was removed

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from the left eyes using a hockey knife and the corneas were saturated with 0.22% riboflavin drops (VibeX Xtra; Avedro, Inc., Waltham, MA) with 3-minute intervals over a total period of 30 minutes. The CXL (3mw/cm^2 and 30min, 30mw/cm^2 and 3min) procedure was then performed using a UVA irradiation system (CL-01; SiHaiTong Co., Suzhou, China). Irradiation was applied on the central 9-mm zone of the cornea and provided exposure to a total dose of 5.4 J/cm2.

The right eyes received tobramycin ophthalmic ointment (Tobrex; Alcon Laboratories, Inc., Fort Worth, TX) immediately after CXL, and continued to receive tobramycin ophthalmic drops and deproteinized calfblood extract eye gel (Xingqi; Shenyang Xingqi Pharmaceutical Co., Ltd., Shenyang, China) three times a day for 1 month to help the new epithelium form completely. At this point, the rabbits were killed by intravenous injection of pentobarbital sodium overdose of 100 mg/kg and the left eyes were immediately enucleated. The corneas were separated along with a 3-mm wide ring of scleral tissue and all other ocular components were removed. Twelve corneas from each group were prepared for inflation testing, whereas the other six were used in histological measurements.

2.3. Biomechanical Inflation Testing

The mechanical properties of the modified corneas were evaluated using biomechanical inflation testing. Before this, the cornea needs to be demarcated with speckle (Figure 1) and divided into three regions (Figure 2), central region was defined 0-1 mm off-center, paracentral region was defined from 1 mm off-center to 2.5 mm and peripheral region was defined from 2.5 mm off-center to 4 mm. After that, the corneas were mounted onto a custom-built pressure chamber used for corneal inflation testing. The pressure chamber was filled with phosphate buffered saline solution (Maixin, China) and connected to a syringe pump, which in turn was connected to a motor whose movement was controlled by bespoke LabView software. The pressure was controlled by the movement of the motor and continuously monitored using a pressure transducer (DMP-HS, Hangzhou, China) that connected with the pressure chamber. Side images of the corneal profile were recorded with digital cameras (EOS 60D, Canon, Inc., Tokyo, Japan) positioned in the three directions (120 degrees apart), which was used to obtain deformation curves in different regions. The initial profiles and values of corneal diameters and thicknesses measured at 2 mmHg pressure were used to construct a numerical model of each corneal specimen (FIgure 2). To ensure a fully inflated and wrinkle-free corneal surface, all specimens were subjected to an initial inflation pressure of 2 mm Hg. Connected to a personal computer to record the data automatically, a charge-coupled device laser displacement sensor (LK series; Keyence, Ithasca, IL) was used to monitor the displacement at the corneal apex continually. Each specimen was tested within 3 hours postmortem. To condition and stabilize the behavior, three cycles of loading and unloading up to a pressure of 32 mm Hg were applied at a rate of 0.4 mm Hg/sec. A recovery period of 180 seconds was allowed between each of the two loading cycles to ensure the behavior was not affected by the strain history of loading cycles. Finally, the specimens were subjected to a fourth loading cycle, the deformation curves (FIgure3) of each region were used in a subsequent inverse analysis.

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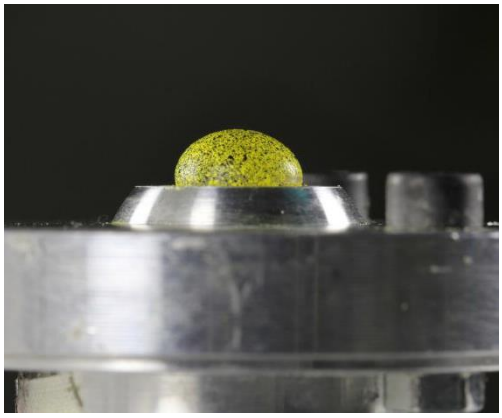

Figure 1. Speckle on the cornea

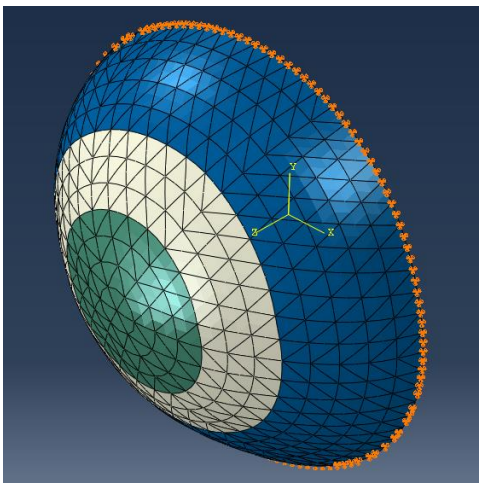

Figure 2. Finite element model (different colors represent different material properties)

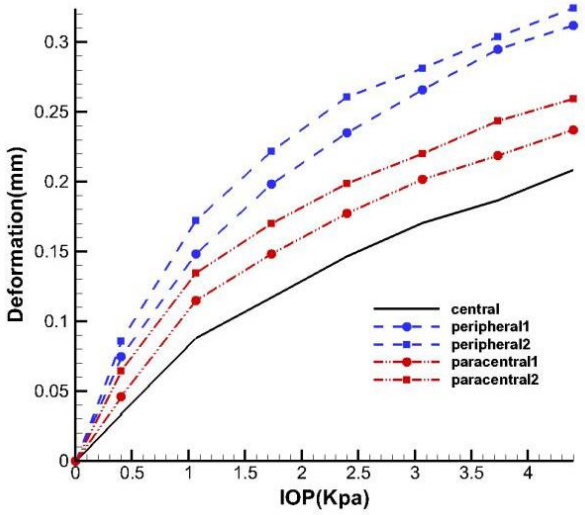

Figure 3. The deformation curves of each region

2.4. Inverse Analysis

An inverse analysis process was used to evaluate the material's mechanical properties of corneal tissue based on the pressure-deformation experimental results. As described in a previous study, the finite element solver Abaqus (Dassault Systèmes Simulia Corporation, Forest Hill, MD) and optimization software package LS-OPT (Livermore Software Technology Corporation, Livermore, CA) were used to implement the iterative process of the inverse analysis procedure. An encastre connection was as summed along the limbus to simulate

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connection to the mechanical clamps. A first-order hyperelastic Ogden model was used to represent corneal material behavior.

$$W = \frac{2\mu}{\alpha^2}\left(\overline{\lambda_1}^{\alpha} + \overline{\lambda_2}^{\alpha} + \overline{\lambda_3}^{\alpha} - 3\right) + \frac{1}{D}(J-1)^2$$

where W represents the strain energy per unit volume, $\overline{\lambda_k}$ the deviatoric principal stretches = $J^{-1/3} \times \lambda_k$ (k=1, 2, 3), λ_1, λ_2, λ_3 the principal stretches, J = $\lambda_1\lambda_2\lambda_3$. Material parameters μ and α are the strain hardening exponent and the shear modulus, respectively. D is a compressibility parameter $= \frac{3(1-2\nu)}{\mu(1+\nu)}$ calculated assuming corneal tissue was nearly incompressible with a Poisson's ratio, ν, of 0.48

3. Results

Comparing the tangent modulus of treated corneas and normal corneas, it was found that the tangent modulus of corneas increased gradually from the central region to the peripheral region. From the center to the peripheral region, the tangent modulus of the treated eyes in the 3mw group decreased from 0.4781±0.1558 Mpa to 0.1137±0.0434 Mpa, and the control eyes reduced from 0.2140±0.0446 Mpa to 0.0713±0.0183 Mpa. The tangent modulus of the treated eyes in the 30mw group decreased from 0.2492±0.0717 Mpa to 0.0916±0.0432 Mpa, and the control eyes reduced from 0.1732±0.0376 Mpa to 0.0689±0.0102 Mpa. The tangent modulus of the treated eyes was higher than that of the control eyes. The 3mw group increased by 123.41% in the central region, 80.93% in the paracentral region, and 59.47% in the peripheral region. The 30mw group increased by 43.88%, 36.93% and 32.95%. The detailed data results are listed in Table 1.

Table 1. The tangent modulus of two groups

Tangent modulus	Central 0mm-1.5mm	Paracentral 1.5mm-2.5mm	Peripheral 2.5mm-4mm
3mw Treated (R)	0.4781±0.1558	0.2068±0.0666	0.1137±0.0434
3mw Control (L)	0.2140±0.0446	0.1143±0.0385	0.0713±0.0183
R/L	2.2341	1.8093	1.5947
30mw Treated (R)	0.2492±0.0717	0.1357±0.0330	0.0916±0.0432
30mw Control (L)	0.1732±0.0376	0.0991±0.0175	0.0689±0.0102
R/L	1.4388	1.3693	1.3295

4. Discussion

Corneal collagen cross-linking was performed by using riboflavin under 370nm UVA irradiation to induce new covalent bonds in and between collagen fibers of corneal matrix, which can improve corneal mechanical properties, and slow down or even prevent the progression of lesions. After years of development, CXL has gradually developed from the classical cross-linking with long time and strong discomfort in the early stage to the rapid cross-linking and

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transepithelial cross-linking scheme with shorter treatment time and less discomfort, and the methods have gradually diversified. This paper evaluated the regional effectiveness of different CXL protocols for KC and found that the total energy is the same, the irradiation is different, the cross-linking effect is also different, the Bunsen-Roscoe law of reciprocity is not always suitable for corneal cross-linking; compared with 30mw group, the 3mw group had better effect in all regions, especially in central region, and the effect of cross-linking was different in the three regions of cornea, central region was the best. The better therapeutic effect may be achieved by changing irradiating energy or irradiating degree to make up for the deficiency of corneal mechanical properties. These results provide a reference for individualized regional differential coital therapy and safe implementation. The regional research process of CXL is complex, and the regional solution method is mastered in this paper. The accuracy of the experimental results and the reliability of the method need to be further studied.

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