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REVIEW ARTICLE

Corneal Physiology and Responses to Cross-Linking and Intracorneal Rings in Keratoconus

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ABSTRACT

Keratoconus is a noninflammatory, usually, bilateral progressive disease. It is a pathology characterized by a progressive thinning and ectasia of the stroma that results in cone-shaped cornea. In advanced keratoconus with corneal opacities, keratoplasty, can be the only surgical alternative for a long time. Recently, new treatment alternatives were developed in keratoconus treatment, such as intracorneal ring segments and corneal cross-linking. Intracorneal ring segment acts as spacer elements between the bundles of corneal lamellae producing a shortening of the central arc length. Corneal cross-linking treatment increases the stiffness of the cornea. Several studies reported that collagen cross-linking can delay or stop keratoconus progression and produce better quality of vision.

Keywords: Keratoconus, Cross-linking, Cornea, Ectasia, Intracorneal ring.

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INTRODUCTION

The shape of the cornea and its ability to maintain a constant optical form is very important for the quality of vision. Neither the etiology nor the pathogenesis of keratoconus is known in detail but the progressive alteration of the keratoconic corneal shape may be based on elastic deformation.

Theoretically, increased intraocular pressure, decreased corneal tissue strength, decreased corneal tissue mass, or a combination, may be pathogenetic factors. We can see that there is a direct relationship between the radius of the eye and the tensile stress, i.e. in a myopic eye, the tensile stress will be higher than in a smaller eye with the same intraocular pressure (IOP).[1] Laplace law, P = 2T/r, relates the pressure (P) inside a hollow sphere with its radius (r) and the tension (T) in its wall.[2] This report clearly defined potential correlations between the IOP, axial length and scleral thickness. Ernst et al reported that keratoconic eyes have on average longer axial lengths that are primarily because of longer posterior segment lengths than emmetropic eyes.[3] So, there is a potential correlation between the IOP, axial length, scleral thickness and corneal thickness. In myopic eyes, scleral and corneal tension is higher than in eyes with a shorter axis even IOP is the same according to Laplace law. In terms of the cornea, the load is supplied by the IOP, which generates stress in the cornea and produces strain or stretching, of the lamellae from the relaxed, unloaded state. Since, stress is defined as load divided by cross-sectional area, a thinner cornea will experience greater stress under the same load. In addition, the cornea does not have uniform thickness, which also means that the stress is not uniform. The thinner regions of the cornea experience greater stress than the thicker regions, as illustrated in Figure 1. This is further complicated, since the cornea is not a linear elastic material. The modulus of elasticity is lower at lower stresses and higher at higher stresses. Therefore, as the IOP increases, the cornea's resistance to deformation also increases.[4]

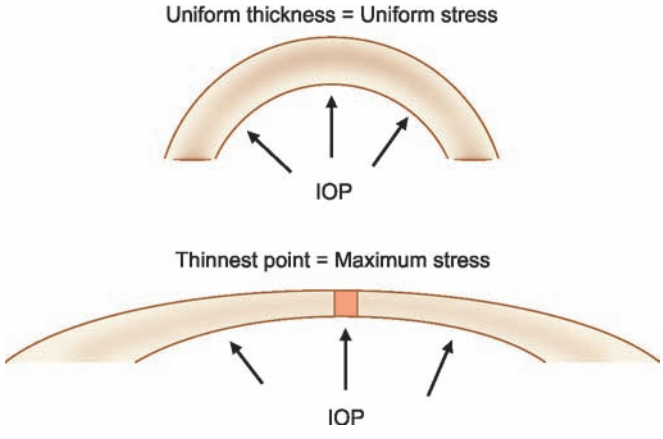

Fig. 1: A uniform corneal thickness produces a uniform stress distribution (reprinted with permission from Colin J, Ertan A. Intracorneal ring segments and alternative treatments for corneal ectatic diseases. Ankara: Kudret Eye Hospital 2007:160.)

EPITHELIUM

The epithelial surface of the cornea creates the first barrier to the outside environment. It is composed of nonkeratinized, stratified squamous epithelium that is 4 to 6 cell layers thick (40 to 50 microns) in normal eyes.[5] The epithelium is covered with tear film, which is optically important in smoothing out microirregularities of the anterior epithelial surface. Corneal epithelial cells have an average lifespan of 7 to 10 days and routinely undergo orderly involution, apoptosis and desquamation.[6] Surface cells maintain tight junctional complexes between their neighbors, which prohibit tears from entering the intercellular spaces. The epithelial basement membrane approximately 0.05 microns thick, comprises type IV collagen and laminin secreted by

the basal cells. If damaged, fibronectin levels increase and the process of healing can take up to 6 weeks. During this time, the epithelial bond to the underlying newly laid basement membrane tends to be unstable and weak.[7]

Reinstein et al[8] propose the use of epithelial thickness profile maps as a new adjunctive diagnostic tool, with the aim to provide to diagnose early keratoconus (Fig. 2).

Total corneal thickness changes associated with keratoconus are due to changes in both stromal thickness and epithelial thickness.[9] The corneal epithelium has the ability to alter its thickness profile to re-establish a smooth, symmetrical optical surface and either partially or totally mask the presence of an irregular stromal surface from front surface corneal topography. In keratoconus, the epithelium is known to be thin in the area overlying the cone, and in advanced keratoconus, there may be excessive epithelial thinning leading to a breakdown in the epithelium. Epithelial thinning over the cone has been demonstrated using histopathologic analysis of keratoconic corneas[10] and the central epithelial thickness was found to be thinner in keratoconus than in normal corneas using OCT.[11] The average epithelial thickness map showed an epithelial doughnut pattern, characterized by a localized central zone of thinning, surrounded by an annulus of thick epithelium in keratoconic eyes. The thinnest epithelial point was located in the inferotemporal quadrant for 74% of eyes. The epithelium at the thinnest point was on average 7.5 microns thinner than at the corneal vertex, which was a statistically significant difference ($p \leq 0.001$). At the thinnest location, Reinstein et al found an average epithelial thickness of 38.2 ± 42.5 microns. Eyelid blinking and friction onto the corneal surface may regulate the epithelial thickness profile.[12] The eyelid might effectively be chafing the surface epithelium during blinking and the posterior surface of the semi-rigid tarsus provides a template for the outer shape of the epithelial surface. In keratoconus, because the cone is protruding, the apex would be the first point of contact with the eyelid, resulting in increased chafing and therefore thinning of the epithelium at the apex of the cone. As the cone becomes more pronounced, it makes sense that the epithelium at the apex of the cone becomes thinner.

The epithelial response to the insertion of intracorneal ring segments provides a similar example of this phenomenon: The epithelium becomes thinner where the intracorneal ring segment causes a ridge in the anterior stromal surface.[13]

The difference between the thinnest and thickest epithelium showed a stronger correlation with the steepest keratometry than the thinnest epithelium or thickest epithelium alone.[9]

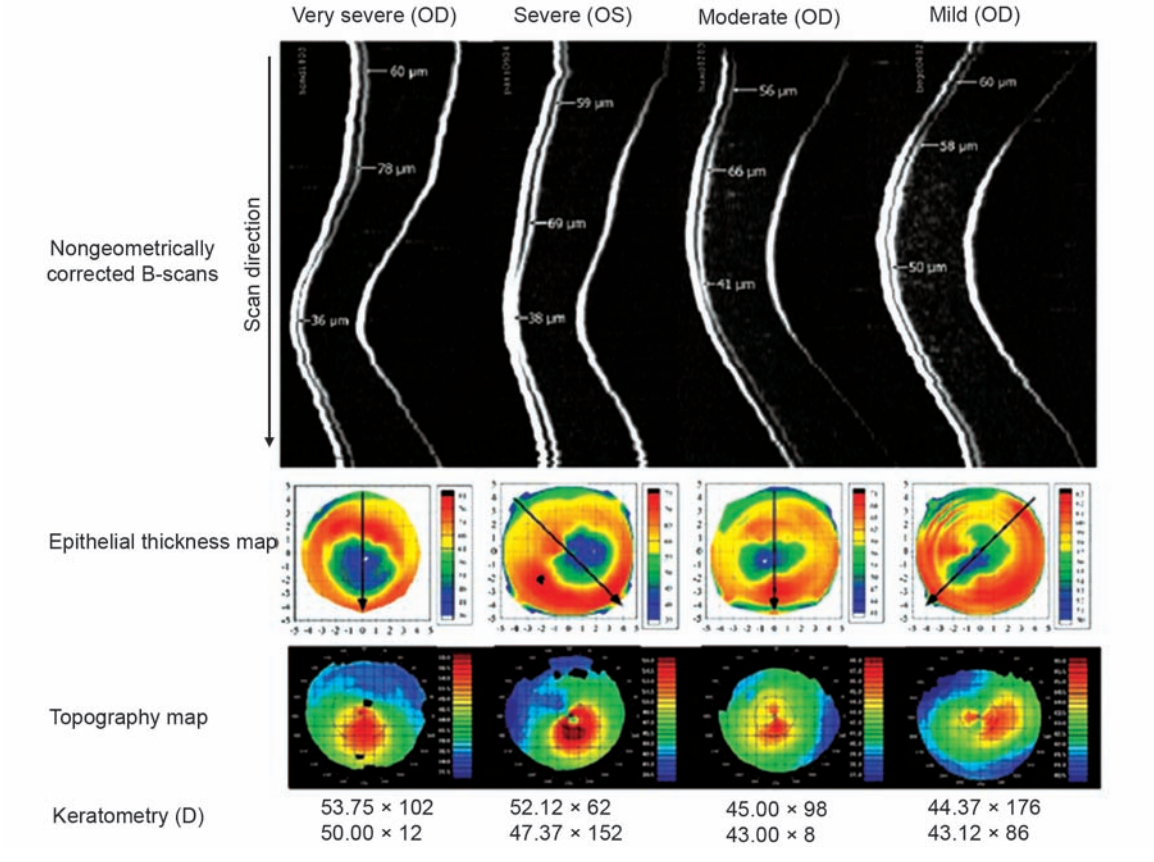

Fig. 2: Artemis epithelial thickness profile maps (Reinstein DZ, Archer TJ, Gobbe M. Corneal epithelial thickness profile in the diagnosis of keratoconus. J Refract Surg 2009;25:604-610)

CXL AND EPITHELIUM

Permeability

In human cornea, there are 5 to 7 layers of epithelial cells, each connected by tight junctions, which is expected to provide a large barrier to anything but small, lipophilic compounds. If permeability of full cornea was found to be smaller than that of deepithelialized cornea, it would suggest that the epithelium presents a significant barrier to transport. In contrast, if the permeability of full cornea was found to be equal to that of deepithelialized cornea, it would suggest that the epithelium does not present a significant barrier to transport.[14]

When a mechanical force creates a break in the epithelial barrier, cells at the edge of the abrasion begin to cover the defect within minutes by a combination of cell migration. This early nonmitotic wound coverage phase can proceed at the remarkable rate of 60 to 80 microns per hour.[15]

Epithelium is important point in cross-linking (CXL) treatment. Riboflavin is a hydrophilic compound and cannot easily cross the intact epithelial barrier. To efficient permeate the corneal stroma, the central corneal epithelium must be debrided.[16] The epithelial debridement can cause severe pain and visual loss during first few days after treatment until complete regrowth of the epithelium occurs.[17] The theoretical basis for transepithelial CXL lies in the possibility of penetration of a hydrophilic macromolecule such as riboflavin through corneal epithelium. Epithelial permeability can be enhanced by several topical drugs, including benzalkonium chloride, ethylenediaminetetraacetic acid (EDTA), and gentamicin, at concentrations normally used in industrial preparations.[18,19] The effect in human eyes was estimated to be approximately one-fifth of conventional CXL with epithelial debridement, but at the moment it is unclear whether the full effect of CXL with epithelial debridement is needed to stop the progression of keratoconus. Leccisotti et al[20] reported a favorable effect of transepithelial CXL on keratoconic eyes, without complications. They concluded this effect appeared to be less significant than that was described in the literatüre after CXL with deepithelialization, although comparisons were uncertain, because of the varied quality of published studies. Transepithelial CXL was designed to avoid the early postoperative pain and temporary worsening of vision associated with the classic CXL technique. It is based on the use of a specially formulated riboflavin solution, Ricrolin TE (Sooft Italia SpA). To this formulation, two enhancers were added, i.e. trometamol and sodium ethylenediaminetetraacetic acid (EDTA). The enhancers help riboflavin penetrate the corneal stroma through an intact epithelium, thereby avoiding the need for epithelial debridement.[21] A limited but favorable effect of transepithelial CXL can be noted on keratoconic eyes, without complications.[20]

Stroma

Corneal shape and curvature are governed by the intrinsic biomechanic structure and extrinsic environment. Anterior corneal stromal rigidity appears to be particularly important in maintaining the corneal curvature.[22] Organizational differences in collagen bundles of the anterior stroma may contribute to a tighter cohesive strength in this area and may also explain why the anterior curvature resist change to stromal hydration much more than the posterior stroma, which tends to more easly develop folds.[23]

The corneal stroma provides the bulk of the structural framework of the cornea and comprises roughly 80 to 85% of its thickness. The collagen fibers, arranged in parallel bundles called fibrils, are packed in parallel arranged layers of lamellae. The stroma of human eye contains 200 to 250 distinct lamellae, each layer arranged at right angles relative to fibers in adjacent lamellae.[24] Deeper layers of stroma are more strictly organized than superficial layers, and this difference accounts for the greater ease of surgical dissection in a particular plane.[25]

Keratocytes are the major cell type of the stroma and most of these keratocytes reside in the anterior stroma. Keratocytes contain corneal 'cristallins' representing 25 to 30% of soluble protein in the cells. Crystallins appear to be responsible for reducing backscatter of light from the keratocytes and maintaining corneal transparency.[26]

RESPONSE TO CXL AND INTRACORNEAL RING

Permeability

When the stromal layer of cornea is isolated, its permeability shows no apparent dependence on distribution coefficient and a strong dependence on molecular radius as expected for its anatomical structure (i.e. hydrophilic and fibrous). Because whole cornea and corneal stroma have such different permeability properties, it, at first appears that the stroma is not a rate-limiting barrier within the cornea. However, comparing the permeabilities for small compounds across the stroma and full cornea, the full range of stromal permeabilities falls within the upper range of corneal permeabilities. This indicates that while the stroma may not limit compounds that show a small corneal permeability (e.g. hydrophilic compounds), the stroma provides a barrier to lipophilic compounds that is similar to that of the whole cornea. If a molecule is sufficiently lipophilic to readily cross the epithelium, the barrier presented by stroma appears to become important.[26]

CXL

Although the epithelial debridement can cause a necrosis of the keratocytes at a depth of 50 to 60 microns,[27] the significant cytotoxic effect of cross-linking seems concentrated in the anterior part of the cornea because of the high absorption of UVA by riboflavin. This behavior prevents UVA from reaching deeper levels, thus preserving the endothelium and lens. Mazzotta et al[28] results show for the first time *in vivo* in humans that the cytotoxic effects of corneal collagen cross-linking used to treat keratoconus are significant but concentrated in the first 350 microns of the stroma. Real time HRT II confocal microscopy *in vivo* detects an incomplete disappearance of keratocytes in the anterior and intermediate corneal stroma immediately after the operation. This study showed that activated keratocytes repopulate the stroma from the deeper layers, starting at 2 to 3 months after the operation. Six months after the operation, stromal repopulation was almost complete.

In clinical setting, a typical corneal haze is a typical response after CXL.[29] Haze after CXL is different in clinical character from haze after other procedures, such as excimer laser photorefractive keratomy. The former is a dust-like change in the corneal stroma or midstromal demarcation line, whereas the latter has a more reticulated subepithelial appearance.[30] A significant decrease in CXL-associated corneal haze was not observed in the keratoconus groups until 6 months.[31]

Intracorneal Rings

The significant stromal wound healing process consists of three stages: Repair, regeneration and remodeling.[32] The activation and migration of stromal keratocytes are the first responses to a stromal injury, such as intracorneal ring implantation, can occur within hours.

The placement of intracorneal segments generates both an immediate response that interrupts the biomechanical disease progression in keratoconus and a time-dependent biomechanical response that allows subsequent improvement of vision over 6 months (Fig. 3).[33] The immediate response is governed by the elastic properties and the long-term response is governed by the viscoelastic properties.

Within 1 to 2 weeks of the procedure, myofibroblasts with contractile properties enter the injured area and become involved in the stromal remodeling. These cells use increased expression of matrix metalloproteinases (MMPs), which are a family of proteolytic enzymes responsible for extracellular matrix remodeling, cell-matrix interaction, inflammatory cell recruitment and cytokine activation.[34] The interaction between these cytokines, MMPs and other

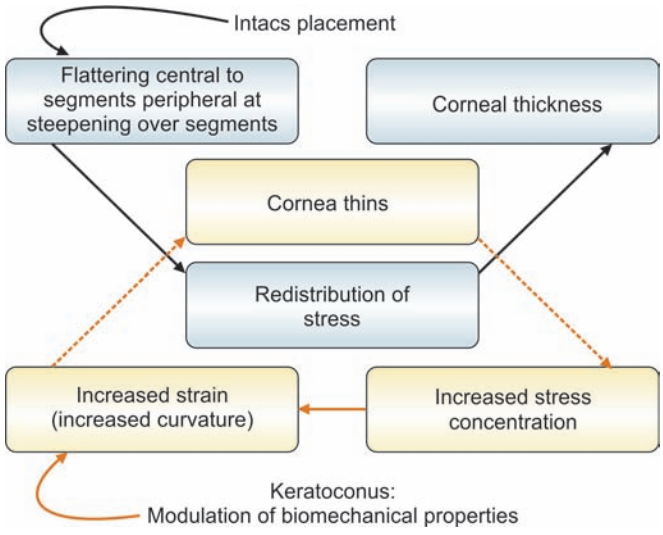

Fig. 3: The red biomechanical cycle reflects disease progression in keratoconus. The blue biomechanical cycle reflects the impact of Intacs placement. Once the segments are inserted, the curvature is decreased centrally, including the region of the cone. As curvature is decreased in this region, the stress is redistributed, and the decompensatory biomechanical cycle of keratoconus is broken (reprinted with permission from Colin J, Ertan A. Intracorneal ring segments and alternative treatments for corneal ectatic diseases. Ankara: Kudret Eye Hospital 2007:164)

mediators, rather than the mere presence or absence of these proteins, often plays a decisive role in regulating the complex remodeling process during corneal wound healing. This process can take months or even years to complete; the end result sometimes reduces corneal clarity long after primary wound healing has occurred.[35]

An important question that arises is whether the curvature and stress redistribution, that occurs with intracorneal ring segment placement, actually modulates biomechanical properties or whether the new, more balanced biomechanical environment simply interrupts the decompensatory cycle in keratoconus. Preliminary data over the short-term postoperative period indicates that fundamental biomechanical properties are not altered with ring placement.[36] This leads to the conclusion that the stromal pathology is not altered, which is expected, even though the biomechanical environment is optimized with ring segment placement. Longer follow-up is needed to determine the long-term affect on biomechanical properties.

Endothelium

Endothelial cell density and topography continue to change throughout life. Endothelial cells have no mitotic activity *in vivo*; however, humans are born with a significant reserve. Cell density is approximately 3500 cells/mm^2 at birth, this number decreases gradually throughout life at approximately 0.6% per year.[37] Endothelial cells do not appear to undergo mitosis *in vivo*, which means the damaged

cells are rapidly replaced by enlargement of the surrounding cells and their centripetal migration into the injured region. With more severe trauma, as a result of keratoconus and as a possible complication of incisional anterior segment surgery, the underlying descement membrane may be torn or ruptured. If this occurs, migrating endothelial cells are required to produce new descement membrane. As seen with acute hydrops, focal corneal edema may be seen early, which resolves when the break is repaired.[7]

The photopolimerization process inducing additional cross-links in the corneal stroma is believed to be carried out by free radicals mediated by the riboflavin irradiated with UV light. Such radicals can create cell damage that may be accepted at the keratocytes but not at the corneal endothelium.[37] A cytotoxic effect of the combined riboflavin/UVA treatment on corneal endothelial cells is to be expected with a corneal thickness of less than 400 microns. Therefore, pachymetry should be routinely performed before riboflavin/UVA treatment to exclude patient at risk.[38]

Azar et al[39] evaluate the 2-year effects of intrastromal corneal ring segments (Intacs) on the corneal endothelium. Endothelial cell density changes at 2 years after Intacs implantation were not clinically significant and endothelial cell remodeling was present.

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