

Collagen crosslinking in the management of ectatic conditions of the globe

Essay

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Abstract

Collagen crosslinking is in widespread use in polymer industry. It has been shown in laboratory studies to increase the biomechanical strength of the cornea and sclera. It is now used as a new method for treating keratoconus by the use of combined riboflavin and ultraviolet-A radiation. It arrests the progression of ectasia by increasing the stiffness of the cornea. It is considered a minimally invasive procedure with little side effects. It has been also used to treat iatrogenic keratectasia. It is now under trial for the treatment of pathological myopia

Key words: collagen crosslinking, corneal stiffening, keratoconus, iatrogenic keratectasia, myopia, contact lenses, INTACS, keratoplasty.

List of contents

Title	page
List of abbreviations	i
List of figures	ii
List of tables	iii
Introduction	1
Anatomy of the cornea	4
Biochemistry of the cornea	13
Keratoconus	24
Collagen Crosslinking principle and laboratory studies	52
Clinical studies in Collagen Crosslinking	71
Collagen Crosslinking for iatrogenic keratectasia	82
Collagen Crosslinking for progressive myopia	86
Summary	91
References	96

List of abbreviations:

AGEs	Advanced glycation end products
BSCVA	Best spectacle corrected visual acuity
DALK	Deep anterior lamellar keratoplasty
DM	Descemet's membrane
DNA	Deoxyribonucleic acid
GAG	Glycosaminoglycans
ICR	Intrastromal corneal rings
ICRS	Intrastromal corneal ring segments
IgG	Immunoglobulin G
IOP	Intra ocular pressure
LASIK	Laser assisted in situ keratomileusis
LED	Light-emitting diodes
MEM	Modified Eagle's medium
MW	Molecular weight
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
OCT	Optical coherence tomography
Pa	Pascal
PK	Penetrating keratoplasty
PMMA	Polymethyl methacrylate
PRK	Photorefractive keratectomy
RGP	Rigid gas permeable
ROS	Reactive oxygen species
SAI	Surface asymmetry index
SimK	Simulated keratometry
SRI	Surface regularity index
SSCVA	Sphere spectacles corrected visual acuity
TCA	Tricarboxylic acid
TGF	Transforming growth factor
TUNEL	terminal deoxynucleotidyl transferase deoxy-UTP-nick-end labeling
UCVA	Uncorrected visual acuity
UVA	Ultra violet-A

List of figures:

Number	Title	Page
Figure 1	In the anterior aspect the cornea is transversely ellipsoid, whereas its posterior aspect is circular	4
Figure 2	Surface zones of the cornea	6
Figure 3	Histological view of the corneal layers	7
Figure 4	Steps of collagen biosynthesis	17
Figure 5	Stromal microstructure	18
Figure 6	Collagen fibrils in a region where a lamella splits into two separate lamellae.	27
Figure 7	Perl's stain for iron demonstrates the epithelial positivity in the region of the Fleischer ring	29
Figure 8	Histological section showing a) Typical keratoconus b) Atypical keratoconus	30
Figure 9	Histologic section through the center of the cone shows corneal thinning, stromal scarring and breaks in Bowman's membrane	31
Figure 10	Stroma scarring with irregular corneal thickness and rupture of the Descemet's membrane	31
Figure 11	Topographic pattern of mild Keratoconus	34
Figure 12	Topographic pattern of moderate Keratoconus	35
Figure 13	(a) Rizzuti sign (b) Munson's sign	36
Figure 14	Topographic pattern of advanced Keratoconus	37
Figure 15	Keratography of normal cornea with regular astigmatism	38
Figure 16	Topographic pattern of Keratoconus suspect	39
Figure 17	Videokeratography of keratoconus showing inferior steepening	40
Figure 18	Topographic map of Orbscan showing keratoconus	41
Figure 19	Photokeratoscope image showing crowding of the mires inferiorly	43
Figure 20	Appearance following DALK	49
Figure 21	Difference in strength between crosslinked & non crosslinked collagen matrix	52
Figure 22	Mechanism of collagen crosslinking using UVA and the photosensitizer riboflavin	54
Figure 23	schematic of the biomechanical measuring technique	56
Figure 24	stress-strain measurements in porcine corneal flaps	60
Figure 25	depth dependent keratocytes loss according to the irradiance level	64
Figure 26	Measurement of collagen fiber diameter using morphometric computer software	67
Figure 27	(a) Crosslinked corneas (b) Control corneas 6 days after collagenase treatment	68

Figure 28	Mushroom-like shape at 70°C (left) and cylinder shape at 75°C (right)	69
Figure 29	Treatment of the central 7 mm of the centrally abraded cornea with riboflavin drops and two UVA diodes	72
Figure 30	Corneal topography of a treated patient	73
Figure 31	Topography immediately before treatment (a), 18 months (b), 40 months (c) following crosslinking	74
Figure 32	Summary of surgical procedure and timing of UVA and riboflavin-Dextran administration.	78
Figure 33	Preoperative topography	83
Figure 34	Topography 10 months after LASIK	83
Figure 35	Schematic of the crosslinking treatment procedure.	84
Figure 36	Topography 12 and 18 months after collagen crosslinking.	85

List of tables

Number	Title	Page
Table (1)	Classification of keratoconus according to K-reading	42
Table (2)	Treatment groups and type of given treatment	55
Table (3)	Treatment groups, surface epithelial and endothelial UVA irradiance, and endothelial cytotoxicity in rabbits	61
Table (4)	Depth of Keratocyte Loss in Relation to UVA Irradiance	63

Introduction

Collagen, which makes up to 71 percent of the dry weight of the cornea, is the most abundant protein in the body. It provides the structural backbone of many tissues (e.g., cornea, cartilage, skin, and tendon) by making up, along with the proteoglycan matrix, the bulk of the connective tissue between the cells. In cornea, collagen is present in the subepithelial basement membrane, Bowman's layer, the lamellae of the stroma, and Descemet's membrane.¹

A new technique of collagen crosslinking by the photosensitizer riboflavin and UVA similar to photopolymerization in polymers has been developed. Extensive experimental studies in rabbit and porcine eyes, including biomechanical stress-strain measurements showed a significant increase in corneal rigidity by approximately 70% in untreated versus treated corneas after collagen crosslinking.²

Keratoconus is a noninflammatory conelike ectasia of the cornea, which is usually bilateral and progresses over time. Its reported frequency is approximately 1 in 2,000 in the general population. Usually, the condition starts at puberty, progressing in approximately 20% to such an extent that penetrating keratoplasty becomes necessary.³

Besides penetrating keratoplasty, hard contact lenses are the major treatment modality for keratoconus. In rare cases, epikeratoplasty, photorefractive keratectomy, or intracorneal rings can be considered. However, all of these techniques only correct the refractive errors of keratoconus but do not treat the cause underlying the corneal ectasia and therefore cannot stop the progression of keratoconus.⁴

In keratoconus the tensile strength of the cornea's lamellar fibers diminish to about half of their normal values, thus stiffening the cornea with crosslinking may stop the progression of the disease and turn it into forme fruste.⁵

Keratectasia is one of the most severe complications after refractive laser surgery. Usually penetrating keratoplasty is the treatment of choice to achieve an optical rehabilitation in such cases. Collagen cross-linking leads to a

stiffening of the anterior parts of the corneal stroma. The increase of biomechanical stability can stop the progression of a keratectasia after LASIK by means of a simple procedure.⁶

The scleral strength and the degree of collagen crosslinking in the sclera seem to play a vital role in the development of myopia. Therefore, scleral collagen might be a good target for the crosslinking treatment in progressive myopia and should be tried clinically and experimentally.⁷

Photochemical crosslinking of the cornea using riboflavin and UVA results in a markedly increased resistance versus collagen digesting enzymes. The findings support the use of the new method in the treatment of corneal ulcers.⁸

Aim of the work:

To review the literature about this recent modality in the treatment of ectatic conditions of the globe.

References:

- 1) **Linsenmayer, TE.** Collagen. In E. D. Hay (ed.), *Cell Biology of the Extracellular Matrix*. New York: Plenum, 1981. Pp. 5-37.
- 2) **Spoerl E, Huhle M, Seiler T.** Induction of cross-links in corneal tissue. *Exp Eye Res* 1998;66:97-103.
- 3) **Tuft SI, McCauley LC, Gregory WM, Davison CR, Buckley RI.** Prognostic factors for the progression of keratoconus. *Ophthalmology* 1994;101:439-447.
- 4) **Rabinowitz YS.** Keratoconus. *Surv Ophthalmol* 1998;42: 297-319
- 5) **Wollensak G, Spoerl E, Seiler T.** Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. *Am J Ophthalmol.* 2003 May;135(5):620-7.
- 6) **Kohlhaas M, Spoerl E, Speck A, Schilde T, Sandner D, Pillunat LE.** A new treatment of keratectasia after LASIK by using collagen with riboflavin/UVA light cross-linking. *Klin Monatsbl Augenheilkd.* 2005 May;222(5):430-6.
- 7) **Wollensak G, Spoerl E.** Collagen crosslinking of human and porcine sclera. *J Cataract Refract Surg.* 2004 Mar;30(3):689-95.
- 8) **Spoerl E, Wollensak G, Seiler T.** Increased resistance of crosslinked cornea against enzymatic digestion. *Curr Eye Res* 2004; 29:35-40.

Anatomy of the cornea

The cornea is a transparent avascular tissue with a smooth, convex surface and concave inner surface, which resembles a small watch-glass. The main function of the cornea is optical; it forms the principal refractive surface, accounting for some 70% (40-45 dioptres) of the total refractive power of the eye. Refractive requirements are met by the regular anterior curvature of the cornea and the optically smooth quality of the overlying tear film. The resistance of the cornea, which provides a protective layer and resists the ocular pressure, is due to the collagenous components of the stroma. Transparency of the corneal stroma is achieved by the regularity and fineness of its collagen fibrils and the closeness and homogeneity of their packing. Water is constantly pumped out of the cornea by its posterior layer, the endothelium. This maintains the optical homogeneity of the corneal layers and prevents swelling and clouding. The cornea is thus an evolutionary compromise, being a multicomponent, thick, tough avascular tissue with a smooth surface and uniform curvature.¹

Dimensions:

In front the cornea appears elliptical, being 11.7 mm wide in the horizontal meridian and 10.6 mm in the vertical in adults.² The posterior surface of the cornea appears circular, about 11.7 mm in diameter. This difference is due to the greater overlap of sclera and conjunctiva above and below than laterally. The axial thickness of the cornea is 0.52 mm with a peripheral thickness of 0.67 mm.¹

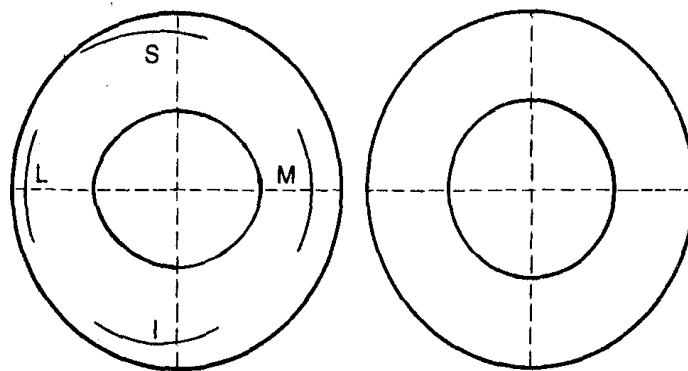


Fig (1). In the anterior aspect the cornea is transversely ellipsoid, Whereas its posterior aspect is circular. From (Bron et al. 1997).¹

The cornea forms part of what is almost a sphere, but it is usually more curved in the vertical than the horizontal meridian, giving rise to astigmatism 'with the rule'. In its central third, the optical zone, the radius of curvature of the anterior surface is about 7.8 mm and that of the posterior 6.5 mm, in adult males. The natural and normal cornea is generally prolate, with steeper curvature centrally and relatively flatter peripherally.¹

Surface zones of the cornea:

The corneal surface can be divided into four anatomical zones: the central (optical) zone, the paracentral zone, the peripheral zone and the limbal zone.³

Central zone: also called optical zone of the cornea. It is 2.4 mm in diameter and overlies the entrance of the pupil where it represents the most spherical area of the cornea and determines the high-resolution image formation on the fovea.³

Paracentral zone: also called mid, intermediate or mid peripheral zone. It is 6-8 mm in diameter.³

Peripheral zone: it is also called transitional zone. It is 7-11 mm in diameter.³

Limbal zone: it is 11.5-12 mm in diameter. It is the ring of cornea about 0.5 mm wide that contains the capillary arcade and stem cells.³

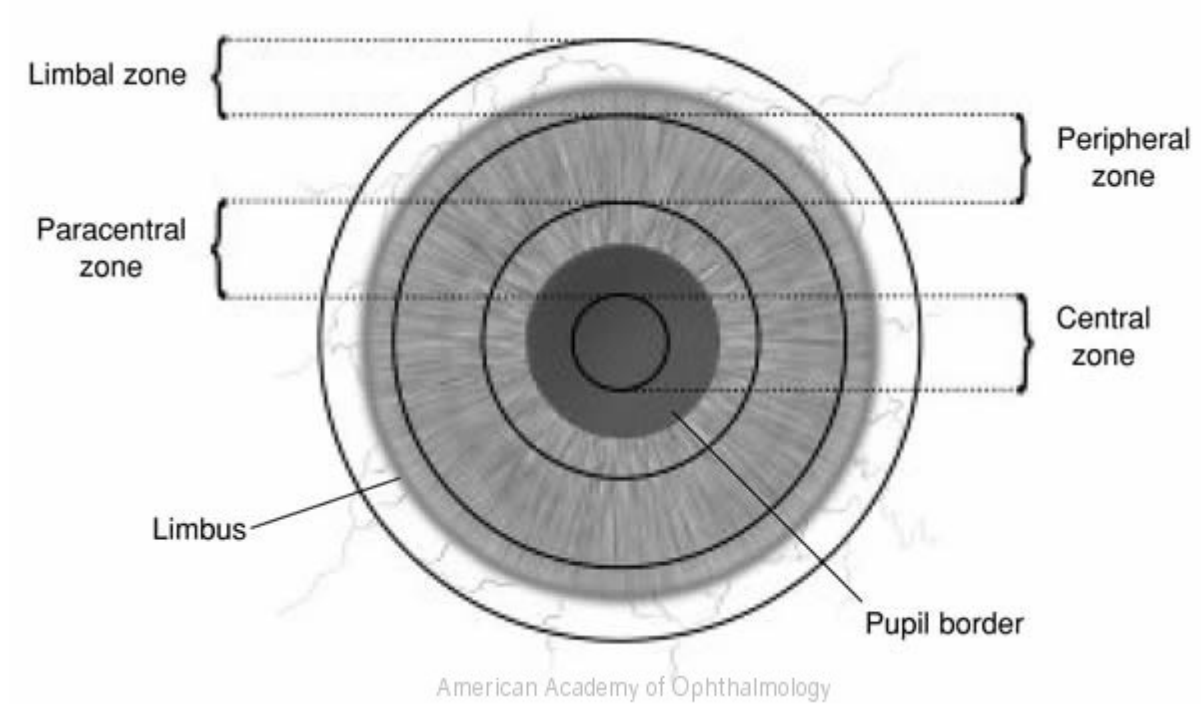


Fig (2). Surface zones of the cornea

From **American academy of ophthalmology (2003)**⁴

Structure:

Behind the precorneal tear film are five tissue layers:

1. Epithelium;
2. Bowman's layer;
3. Stroma;
4. Descemet's membrane;
5. Endothelium.