HHS Public Access

Author manuscript

Cornea. Author manuscript; available in PMC 2019 November 01.

Published in final edited form as:

Cornea. 2018 November ; 37(11): e49–e50. doi:10.1097/ICO.0000000000001685.

Just what DO we know about corneal collagen turnover?

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Keywords

cornea; corneal cross-linking (CXL); riboflavin-UVA; keratoconus; collagen; protein turnover

Letter to the Editor

Mazzotta et al in a recent article in CORNEA reported 10-year results in pediatric keratoconus patients following riboflavin-UVA photochemical corneal cross-linking (i.e. CXL).[1] The authors found an increased rate of keratoconus progression in this population requiring either CXL retreatment or deep anterior lamellar keratoplasty and note that the therapeutic regression was found in patients aged 10–15 years old at the time of cross-linking. In the discussion, the authors cite estimates of collagen turnover rates based on available literature and make recommendations regarding cross-linking in pediatric patients to which we would like to make comments.

Although protein turnover (and collagen turnover specifically) has been of interest to a broad spectrum of scientists (from nearly 60 years ago) that include collagen biochemists, cross-link chemists, and researchers studying aging and age-related degenerative diseases, the relevance of corneal collagen turnover has special significance in lieu of the photochemical strengthening therapy known as CXL. This is of interest to clinicians because the rate at which collagen proteins are degraded and re-synthesized will impact upon the durability of a CXL treatment and will determine the future need for retreatments following CXL. A recent report from Mazzotta et al (2018) suggests that the CXL procedure in children may be more prone to needing retreatments, with a 10-year progression rate of 24% as compared to 5–6% in adults.[2] In the paper, the authors use references that estimate collagen turnover of 6–7 years. They include estimates based on these numbers to predict a time frame in which retreatments could be expected. Although the paper sheds light on an important clinically relevant question, a closer examination of the primary supporting literature should raise caution prior to making recommendations to patients. A review of the primary source literature indicates that the turnover time of collagen in the adult cornea is unknown and

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Conflicts of Interest: Patent pending through Columbia University. SLT and DCP are named inventors as employees of Columbia University. No other conflicts.

[*]Patent pending through Columbia University.

there are few studies that experimentally address the question of corneal collagen turnover. In the Mazzotta paper, the authors estimate a figure of 6–7 years based on the citation of a paper from Meek's group that quantitated advanced glycation end-products (AGEs), such as pentosidine, in the cornea.[3] Increased pentosidine accumulation has been used as an indication of slow protein turnover and the accumulation of age-related markers indicates that the proteins are present for long time periods.[4] Thus, although the Malik et al paper does provide an indication that corneal turnover rates will decrease with age, the authors admittedly are unable to assign a specific amount of time (or even an estimate) regarding collagen turnover rates. The Malik et al study quantitates the levels of cumulative age-related cross-linking (i.e. pentosidine) in corneal tissue rather than specifically estimating turnover times. Further, Malik et al do cite a book dedicated to protein turnover in mammalian tissue and in the whole body from 1978. This book does address turnover rates of proteins (and collagens) in various tissues and in the body as a whole. However, specific information regarding the cornea is not included.[5]

Other papers from the CXL literature also cite a figure of 2–7 years.[6–9] The two commonly cited source references to support these secondary reviews are from Smelser et al 1965 and a book chapter by Nishida in the textbook CORNEA from 1997 (1st edition). The Smelser et al study we discuss below. The Nishida chapter reports that "the turnover of collagen molecules in the cornea is slow, requiring 2–3 years" on page 13 of chapter 1.[10] This number is quoted in all subsequent editions and include the 2nd edition (2005), 3rd edition (2011), and 4th edition (2017).[11–13] In this most recent 4th edition, a review paper from Meek and Fullwood is cited (2001). This 2001 review paper is highly focused on corneal collagen microstructure, yet the review does not specifically address collagen turnover and is predominantly a review of light and electron microscopic findings.[14] The prior editions (1st, 2nd, and 3rd) of the textbook CORNEA (i.e. Nishida chapter) do not include references for the 2–3 years collagen turnover statement. There are four additional references found in the same Nishida paragraph and precede the "2–3 years collagen turnover" statement. None of these four references, however, address the specific turnover issue.[15–18] So we conclude that it is unclear as to how this number of 2–3 years was generated.

A traditional method to evaluate collagen turnover in a tissue is to follow the incorporation and elimination of radiolabeled [^{3}H]-proline into a tissue, since collagen is proline-rich (~10%). Three radiolabeling studies have been used with both transplanted rabbit tissues, newborn rabbits, and ex vivo calf. The first was reported by Smelser et al in 1965 and is cited by several recent papers (see above) including Wollensak et al (2003). In the Smelser work, the investigators first radiolabel excised rabbit corneas and then transplant them into living rabbits. After incorporation of the corneal tissue graft for 6–8 weeks, the tissue was re-excised and found to have intact radioactivity indicating very little turnover of proline.[19] However, longer term studies on the order of months to years were not available. Although an interesting and important study that provided meaningful information, the interpretation of the results as it could apply to CXL should be taken with caution. A corneal graft has severed nerves and collagen lamellae as well as various "healing zones" and may not accurately represent the metabolic turnover of intact non-transplanted native tissues. The second study was reported by Lee and Davison in 1981 in which newborn rabbit pup corneas were injected with ^{3}H-proline into the space between the closed eyelid and cornea.[20] Uptake

of label at this age was rapid although radiolabel incorporation declined rapidly after the 5th day post-partum. The half-life of collagen in this newborn rabbit was estimated to be 50 hours or less. A third study directly addressing collagen turnover was reported from France by Kern et al in 1991. In that study, the investigators used young growing calf corneas in organ culture to show turnover rates on the scale of hours supporting the newborn rabbit findings from Lee and Dawson from 1981 that indicates rapid turnover in actively growing tissues. The estimated half-lives for collagens were 36 hours for type I, 10 hours for type V, and 6 hours for type VI.[21, 22] Unfortunately, to the best of our knowledge, there are no reports in the literature that have carried out similar studies using adult corneal tissues.

A newer method using the quantitation of the racemization product of L- to D- aspartate has been used in several tissues to determine the protein residence time in a tissue. This gives an indication of how long the protein has been present in the tissue and can be used to estimate the turnover rates of proteins. Using this sophisticated analytical chemistry method, protein collagen half-lives were reported for human skin at 14.8 years, articular human cartilage at 117 and 174 years, and human dentin at >500 years.[23] A similar study was carried out by Gineyts et al (2010), who quantitated the isomerization and racemization of type I collagen C-teleopeptides. Many soft tissues were studied in this report and include heart, arteries, skeletal muscle, lung, intestine, kidney, liver, ligament, tendon, bone and dermis with collagen half-lives estimates between 40 and 190 years.[24] Unfortunately, corneal tissue was not included in this study. Thus, to our knowledge, such racemization techniques for estimating collagen turnover rates have yet to be applied to the study of corneal tissue.

In summary, based on the current content of literature on the subject, it is unclear as to the “turnover” time of corneal collagens in human children or human adults. What can be inferred from the literature however, is that the turnover time is significantly faster in young actively growing children. This is based upon radiolabeled ^{3}H-proline incorporation studies in young calf and newborn rabbit tissues. Future studies are needed using isomerization and racemization detection techniques to more accurately address this important gap in our knowledge. This is a fundamental and clinically relevant question since keratoconus treatments in pediatric populations suggest that retreatments are more likely to be needed. In conclusion, what we know about corneal collagen turnover indicates that a CXL treatment in a younger patient will likely need retreatments. Because of the rapid turnover in children, alternative approaches to cross-link the cornea using topical solutions (such as an eyedrop) could provide an attractive new “re-treatment” option to patients.[25]

Acknowledgments

Supported in part by Research to Prevent Blindness and by National Institutes of Health Grants NCRR UL1RR024156, NEI P30 EY019007, and NEI R01EY020495 (dcp).

Disclosure: D.C. Paik, None; S.L. Trokel, None; L. Suh, None.

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