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Review

Corneal stroma microfibrils

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ABSTRACT

Elastic tissue was first described well over a hundred years ago and has since been identified in nearly every part of the body. In this review, we examine elastic tissue in the corneal stroma with some mention of other ocular structures which have been more thoroughly described in the past. True elastic fibers consist of an elastin core surrounded by fibrillin microfibrils. However, the presence of elastin fibers is not a requirement and some elastic tissue is comprised of non-elastin-containing bundles of microfibrils. Fibers containing a higher relative amount of elastin are associated with greater elasticity and those without elastin, with structural support. Recently it has been shown that the microfibrils, not only serve mechanical roles, but are also involved in cell signaling through force transduction and the release of TGF-β. A well characterized example of elastin-free microfibril bundles (EFMBs) is found in the ciliary zonules which suspend the crystalline lens in the eye. Through contraction of the ciliary muscle they exert enough force to reshape the lens and thereby change its focal point. It is believed that the molecules comprising these fibers do not turn-over and yet retain their tensile strength for the life of the animal. The mechanical properties of the cornea (strength, elasticity, resiliency) would suggest that EFMBs are present there as well. However, many authors have reported that, although present during embryonic and early postnatal development, EFMBs are generally not present in adults. Serial-block-face imaging with a scanning electron microscope enabled 3D reconstruction of elements in murine corneas. Among these elements were found fibers that formed an extensive network throughout the cornea. In single sections these fibers appeared as electron dense patches. Transmission electron microscopy provided additional detail of these patches and showed them to be composed of fibrils (~10 nm diameter). Immunogold evidence clearly identified these fibrils as fibrillin EFMBs and EFMBs were also observed with TEM (without immunogold) in adult mammals of several species. Evidence of the presence of EFMBs in adult corneas will hopefully pique an interest in further studies that will ultimately improve our understanding of the cornea's biomechanical properties and its capacity to repair.

1. Introduction

The cornea is a complex and precisely structured tissue, resilient and uniquely transparent, endowed with optical properties making vision possible, yet deceivingly simplistic in its macroscopic appearance. It is composed of three cellular regions: stratified squamous anterior epithelium, stroma, and posterior endothelium. In humans, 90% of the corneal thickness is attributed to the stroma which is classified as regular, fibrocollagenous, dense connective tissue. Corneal transparency requires the stromal collagen fibrils, which are organized into tightly-spaced bands (lamellae) of parallel fibrils laid down in criss-crossed fashion, be uniformly sized and spaced (Goldman et al., 1968; Maurice, 1957). In addition to the major component, type I collagen, there are several other minor collagens and ground substance consisting of glycosaminoglycans, proteoglycans, and glycoproteins (Hassell and Birk, 2010).

As the main refractive component of the eye, the cornea must not only be transparent but must also maintain a precisely-shaped, regular surface while providing strength and resiliency to withstand significant mechanical forces from external sources and intraocular pressure variation (Coleman and Trokel, 1969; Hann and Fautsch, 2011). These biomechanical stresses require tissue deformation and recoil; a characteristic common to elastic tissue,

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yet definitive evidence of the presence of elastic tissue microfibrils in the corneal stroma is limited.

The purpose of this paper is to review what is known about elastic tissue microfibrils specifically in regards to the corneal stroma in hopes of stimulating new research efforts aimed at elucidating their role in the cornea. Corneal disease and trauma negatively impact vision, so there is a continuing need to better understand how the cornea responds to injury. A better understanding of corneal microfibrils is likely to lead to an improved understanding of corneal biomechanics, homeostasis and the cornea's response to insult.

2. Historical review of elastic tissue

A brief review of elastic tissue is important for understanding the possible role of elastic tissue microfibrils within the corneal stroma. The biomechanical properties of the cornea imply the presence of elastic tissue but what has been learned from microfibrils in structures other than the cornea reveals additional functions beyond mechanical. The corollary functions of microfibrils in the cornea are largely unknown.

A number of books and articles were published during the mid-19th century apparently as a result of a keen interest in understanding the characteristic of elastic tissue, which had only recently been described by Donders in 1846 (Kolliker, 1860; Lehmann, 1855). While many of the observations were quite accurate, conclusions drawn from these early studies have since been modified or outright rejected in light of new evidence. For example, one early author described a delicate system of nutrient channels in connective tissue that transforms into elastic tissue when required (Virchow, 1863). A century later Fullmer and Lillie (1958) (Fullmer and Lillie, 1958) used selective staining methods to reveal connective tissue fibers that could be distinguished from collagen, elastic, and reticular fibers (type III collagen secreted by reticular cells as a supporting mesh in soft tissues). These fibers were found to be resistant to acid hydrolysis and were named “oxytalan” (meaning acid resistant) fibers.

In the 1960's, the field of biological electron microscopy was developing rapidly and it was during this time that Low (1962) described the fine structure of microfibrillar elements of the extracellular matrix (Low, 1962). He reported microfibril ultrastructure as being variable in diameter (4–12 nm) with frequent branching. It was later shown that individual microfibrils are unbranching but form bundles which do branch, (Kielty and Shuttleworth, 1995; Sakai et al., 1986). According to Low's observations the fibrils seemed to be in all connective tissue but were most noticeable in formed elastic fibers and basement membranes with some microfibrils appearing to have a dense outer shell and lucid core. A few years later Haust, et al. (1965) (Haust, 1965; Haust et al., 1965) made similar observations and reported that elastic fibers had a homogenous core with a surrounding “elastic membrane” and fibrils around the outside. The elastic fibers were reported to be 50–150 nm in diameter and the microfibrils within the fibers had a diameter of 10 nm and a periodicity of 15 nm (microfibril periodicity was later shown to be consistently 50–56 nm when tension-free). They proposed connective tissue microfibrils and collagen fibrils share a common precursor (tropocollagen) and the microfibril of the extracellular matrix is a common denominator in collagenous and elastic tissues. They also subscribed to an earlier notion that degraded collagen fibers combine with mucopolysaccharide (glycosaminoglycan) to form microfibrils. The microfibrils they observed were 4–14 nm in diameter and therefore similar to those described by Low.

At that time (ca. 1965) the chemical composition of elastic fibers was not known but they could be identified by various stains such as aldehyde fuchsin and orcein. In 1966 Greenlee et al. (Greenlee et al., 1966) used an electron microscope to examine the fine structure of immature elastic fibers and concluded they were the precursor to mature elastic fibers. In 1969 Ross & Bornstein (Ross and Bornstein, 1969), proposed that microfibrils and the amorphous component of elastic fibers are two different proteins, or different forms of elastin (the amorphous substance being altered microfibrils). Using electron microscopy and selective degradation they showed the microfibrils to be a second but unknown protein and also described microfibrils as being in close apposition to cells, often found in cell in-foldings.

Fibers in the elastic system were grouped into 3 categories depending on the relative amount of elastin as compared to microfibrils (Gawlik, 1965). True “elastic” fibers are predominantly elastin with a coating layer of microfibrils on the outside of the fiber, “elaunan” fibers have proportionately more microfibrils, with some interspersed within the fiber core. “Oxytalan” fibers are composed of only microfibrils. Differential staining (aldehyde fuchsin, with and without oxidation, for example) identifies the different fibers (Alexander et al., 1981; Carrington et al., 1984; Fullmer and Lillie, 1958). The 3 types of fibers were confirmed with electron microscopy as having distinct ultrastructural morphologies (Cotta-Pereira et al., 1976). It has been proposed that microfibrils present a scaffold for elastin deposition during elastogenesis and many authors agree that the 3 fiber types are a continuum of maturity, where oxytalan represents immature elastic fibers. However, some authors raised the possibility that microfibrils of elastin-free fibers might be a somewhat different variant. Elastic tissue then includes fibers that are predominantly elastin, those that are exclusively microfibrils, and a continuum intermediate phase of varying proportional composition.

The term “microfibril” has been adopted by many authors to refer specifically to extracellular fibrillin-containing microfibrils. Hence, in this review we will simply use the term “microfibril” to refer to extracellular fibrillin-containing microfibrils and bundles of elastin-free microfibrils will be referred to as (EFMBs).

Elastic tissue is formed during early development with minimal if any turn over in mature tissues (Davis, 1993). Once damaged, elastic tissue may reactivate elastogenic cells but this typically results in abnormal tissue which may even impair normal function (Kuhn et al., 1976). Surprisingly this observation was also reported by Kolliker in 1858 (Kolliker, 1860). Little is known about the half-life of microfibrils but they apparently exist for exceedingly long periods of time possibly up to the life of the animal and it has been reported they are non-self-renewing (Garner and Alexander, 1986; Kielty and Shuttleworth, 1995; Sherratt, 2009; Yanagisawa and Davis, 2010). Degeneration of existing microfibrils results in loss of elasticity (increase in rigidity) with advancing age (senile elastosis) as seen for example in skin (Banfield and Brindley, 1963; Sherratt, 2009).

Elastic tissue is an integral/major component in most tissues and has been extensively studied in many tissues such as skin, aorta, and tendon. In tissues that are more elastic in nature, there are more elastic fibers; where strength and support are needed, there are more elastin-free fibers. These latter fibers have been extensively studied using the periodontal and nuchal ligaments.

Elastic tissue has been described in many ocular structures and generally consists of a range of fiber maturity from elastin-free microfibril bundles to mature elastic fibers with an elastin core and microfibril mantle. The human sclera is a good example of the continuum of elastic fiber maturity and like skin it shows signs of senile elastosis with advancing age (Kanai and Kaufman, 1972). Lens zonules have been one of the more thoroughly studied ocular structures and shown to consist of pure microfibrils (elastin-free) that never develop into elastic fibers, thereby providing a relatively