

Editorial

Keratocytes: more than a framework for the window

In this issue of *Clinical and Experimental Ophthalmology*, Poole, Brookes and Clover describe confocal microscope 3-D imaging and volume rendering reconstruction of the keratocyte network in the human corneal stroma.¹ They have used the cell tracker dye 5-chloromethylfluorescein diacetate (CMFDA) to show exquisite detail of the stromal network, following up earlier work they have completed on the porcine cornea² and fibroblast networks in connective tissue explants.³ They show previously unseen process ramifications, orientated in both lateral and anteroposterior directions. Taken in conjunction with increasing evidence for extensive gap junction connections within the corneal stroma,^{4–7} the stromal keratocyte network is clearly both very extensive and highly coupled.

Why is this important? In this avascular tissue the keratocyte network will provide a means by which nutrients and metabolites are passed into the central regions of the stroma, and waste products out. In this sense the corneal keratocyte has an analogous role to the astrocyte in the central nervous system. The astrocytes are also extensively coupled, vital for their role in maintaining the neuronal environment.⁸ These cells remove waste products (including neurotoxins such as potassium and glutamate released during neuronal depolarization) and pass back metabolites. In cases of injury, however, not only do the astrocytes become activated, but they increase their level of cell coupling.^{9,10} Raised interstitial potassium and glutamate from injured neurones then generate spreading cytosolic calcium waves that are thought to pass via gap junction coupled astrocytes.^{11–13} These calcium waves, in conjunction with intercellular astrocytic-neuronal signalling, encompass healthy neighbouring neurones into the injured area, eventually leading to cell death, a process also known as the 'gap junction mediated bystander effect'.^{14,15} In the 24–48 h following central nervous system trauma then, secondary propagation from the injury site increases the volume of damaged tissue.¹⁶ The bystander effect is not unique to the astrocyte: it is, for example, a significant factor following ionizing radiation¹⁷ and in cytomegalovirus retinitis contributes to the death of uninfected cells.¹⁸

As Poole *et al.* allude,¹ the bystander effect is almost certainly an important factor in the cornea following laser surgery. The side-effects of photorefractive keratectomy (PRK) include short-term pain, widespread keratocyte apoptosis in the anterior stroma and corneal haze, and laser *in situ* keratomileusis (LASIK) can have longer term adverse effects on corneal biomechanics.¹⁹ Although some have suggested that the emerging laser epithelial keratomileusis

(LASEK) procedure may overcome some of these complications, all of these corneal surgical procedures suffer from outcome variability or reduced precision, especially in eyes with high myopia.²⁰ This variability almost certainly arises from two sources: (i) the die back (or regression) resulting from the gap junction mediated bystander effect; and (ii) scarring (fibrosis) arising from the inflammatory response^{21,22} and the extent to which fibroblast and myofibroblasts move into the wound area to replace the injured keratocytes.²³ Following corneal damage, both the keratocytes and cells moving into the wound area remain coupled.⁴ Furthermore, in twin abstracts published in this journal last year, Laux-Fenton, Grupcheva and colleagues have indicated that down-regulation of this coupling appears to alleviate the inflammatory response, and hence should reduce scarring.^{24,25} They predict that down-regulation will also relieve regression, as has been reported following down-regulation of coupling between astrocytes in the central nervous system.²⁶

The highly coupled keratocyte network plays important roles during development, in maintaining the cornea, and in wound repair, but the coupling may not always be so helpful following extensive damage. Modulation of the wound healing process, which requires a better understanding of the corneal stroma, is a key factor to improving surgical outcomes. This is compounded by the existence of three distinct keratocyte subpopulations within the stroma, which the article by Poole *et al.* highlights in the human cornea.¹ The roles played by these layers, especially the denser, more variably sized keratocyte layer in the anterior stroma, will be critical following trauma to both the epithelium and the stroma, and in disease processes such as pseudophakic bullous keratopathy and keratoconus in which gap junction coupling may also be of significance.⁴

Until the last 10 years, within clinical ophthalmology, the role and functions of the keratocyte often seems to have only merited a cursory 'footnote' in comparison to the corneal endothelium and epithelium. However, advances in corneal and refractive surgery have highlighted the importance of understanding keratocyte function and laboratory science continues to demonstrate that the keratocyte network is much more dynamic than ever speculated.

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