Review

Corneal neuromediator profiles following laser refractive surgery

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Abstract

Laser refractive surgery is one of the most commonly performed procedures worldwide. In laser refractive surgery, Femtosecond Laser *in Situ* Keratomileusis and Refractive Lenticule Extraction have emerged as promising alternatives to microkeratome Laser *in Situ* Keratomileusis and Photorefractive Keratectomy. Following laser refractive surgery, the corneal nerves, epithelial and stromal cells release neuromediators, including neurotrophins, neuropeptides and neurotransmitters. Notably, nerve growth factor, substance P, calcitonin gene-related peptide and various cytokines are important mediators of neurogenic inflammation and corneal nerve regeneration. Alterations in neuromediator profiles and ocular surface parameters following laser refractive surgery are attributed to the surgical techniques and the severity of tissue insult induced. In this review, we will discuss the (1) Functions of neuromediators and their physiological and clinical significance; (2) Changes in the neuromediators following various laser refractive surgeries; (3) Correlation between neuromediators, ocular surface health and corneal nerve status; and (4) Future directions, including the use of neuromediators as potential biomarkers for ocular surface health following laser refractive surgery, and as adjuncts to aid in corneal regeneration after laser refractive surgery.

Key Words: cornea; corneal nerves; dry eye; femtosecond laser; laser *in situ* keratomileusis; neuromediator; refractive surgery; small incision lenticule extraction

Laser Refractive Surgery

Refractive error is a leading cause of reversible visual impairment worldwide (Lou et al., 2016). For the correction of refractive errors, laser refractive surgery remains a mainstay treatment in achieving spectacle independence, and is one of the most commonly performed ophthalmic surgeries globally (Kim et al., 2019). Laser refractive surgery has been established as a safe and effective procedure associated with excellent visual outcomes, improvement in the quality of life, and high patient satisfaction (Sandoval et al., 2016). The number of laser refractive surgeries has seen a burgeoning increase since its introduction in ophthalmology, with more than 16 million refractive surgeries performed worldwide (Bandeira et al., 2019).

The femtosecond laser (FSL) represents a significant milestone in ophthalmic surgery, including refractive surgery (Liu et al., 2018; Han et al., 2020), keratoplasty (Liu et al., 2019), conjunctival and cataract surgery (Fuest et al., 2017; Liu et al., 2017). The FSL utilizes ultrashort pulses of near-infrared wavelength light to make tissue incisions (Liu et al., 2015). Reducing the pulse duration to a femtosecond (10^{-15}) level produces smaller microcavitation bubbles (**Figure 1A**) and shock waves, thereby reducing the degree of collateral damage (**Figure 1B**), which is often associated with conventional lasers such as the argon fluoride excimer laser for photoablation (Liu et al., 2015; Fuest et al., 2017). With an accuracy of 5 μm, the FSL's ability to photodisrupt tissue with high precision is ideal for surgeries wherein precision is crucial to achieving good outcomes (Liu et al., 2016b).

Femtosecond Laser *in Situ* Keratomileusis (FSL-LASIK) has emerged as an alternative to conventional mechanical microkeratomes for flap creation. It has been increasingly preferred due to its better precision, greater surgeon flexibility in flap characteristics, ability to produce thinner flaps, and reduced flap-related complications, attributable to the uniform flap morphology that enhances adhesion strength (Salomão and Wilson, 2010). Whilst FSL-LASIK involves two lasers, that is, the FSL for flap creation and excimer laser for stromal ablation, Refractive Lenticule Extraction (ReLEx) requires only a single FSL. Since the first study of Femtosecond Lenticule Extraction (FLEx) published in 2008 by Sekundo et al., ReLEx procedures have gained increasing popularity as a comparable, if not better, alternative to FSL-LASIK. A variant of ReLEx, Small Incision Lenticule Extraction (SMILE), is the most advanced form of ReLEx. Even though FLEx is performed without the need for an excimer laser, a corneal flap similar to the LASIK flap is created prior to lenticule extraction. On the other hand, SMILE involves a small corneal incision created by the FSL, through which the lenticule is dissected and extracted (Liu, 2016). While SMILE has been shown to have similar safety, efficacy and predictability profiles to FSL-LASIK, it provides better outcomes regarding the impact on the ocular surface, as well as with respect to corneal wound healing and inflammatory responses postoperatively (Liu et al., 2016b).

Following laser refractive surgery, the corneal nerves, epithelial and stromal cells release neuromediators that play an important role in postoperative neurogenic inflammation, wound healing and corneal nerve regeneration. In this review, we will discuss the functions of neuromediators, the changes

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in neuromediators following various laser refractive surgeries, and their clinical implications. The authors searched the electronic database PudMed for relevant articles relating to corneal neuromediators following laser refractive surgery. Keywords including "nerve regeneration", "corneal healing", "neurogenic inflammation", "neurotrophic factors", "nerve growth factor", "substance P", "calcitonin gene-related peptide", "femtosecond laser", "neurotrophic keratitis", "neurotrophic keratopathy", "ocular surface", "corneal nerve" and "corneal biomarker" were used. Only articles published in English were used. The date of publication was restricted to the last five years as best as possible. Additional relevant articles were identified from the references of these included articles. The latest search date was September 14, 2020. After removing duplicates, we independently screened the articles to ensure fulfilment of the inclusion criteria, and subsequently assessed the full-text version of all included articles. Of 401 articles identified through database searching, 329 articles were screened, and 85 articles were eventually included in the final manuscript.

Corneal Neuromediators

Corneal neuromediators refer to the chemical substances released by corneal nerves and they include neurotrophins, neuropeptides and neurotransmitters (Al-Aqaba et al., 2019). They play a significant role not only in physiological homeostatic processes, but also in corneal wound healing following injurious stimuli (Chao et al., 2016). The release of neuromediators is vital in corneal nerve regeneration and return of normal neuronal function after refractive surgery. Moreover, the presence and levels of specific neuromediators have been shown to influence the occurrence of adverse effects following laser refractive surgery, such as postoperative dry eye, as will be discussed subsequently.

The cornea is densely innervated with an average of 351 ± 53.5 bundles per human cornea (Mansoor et al., 2020). Corneal innervation is maintained by a homeostatic and neurochemical milieu of neurotrophins, neuropeptides and neurotransmitters, a group of biologically active chemicals collectively known as neuromediators. These neuromodulating chemicals are produced by, and exert effects on a myriad of cells in the cornea, in a complex neurobiological interplay.

The cornea is innervated predominantly by sensory nerves, which confer both afferent and efferent functions, receiving touch and pain sensation, as well as producing neuromediators. Most sensory nerves produce neuropeptides and neurotrophins. Autonomic innervation of the cornea arises from sympathetic and parasympathetic fibers. The corneal sympathetic innervation, although scarce, is a source of important neurotransmitters such as catecholamines (Müller et al., 2003). On the other hand, parasympathetic nerves of the cornea produce acetylcholine. Other corneal cells apart from neurons contribute to the diversity of neuromediators in the cornea. Corneal epithelial cells are a source of acetylcholine, cholinergic synthetic and degradative enzymes, as well as neurotrophins. Neurotrophins are also derived from corneal endothelial cells and stromal cells (Lambiase et al., 2000).

Neurotrophins belong to a class of growth factors that regulate neuronal development, survival, death and plasticity (Al-Aqaba et al., 2019). They are synthesized as inactive precursors, then cleaved by extracellular proteinases to mature neurotrophins, which then activate the p75 neurotrophin receptor and tropomyosin-related tyrosine kinase receptor to affect downstream biological functions (Müller et al., 2003). Nerve growth factor (NGF) is the best-characterized neurotrophin, which is important in sustaining normal corneal nerve density and corneal sensation (Liu, 2010). NGF has garnered considerable attention for its potential in corneal nerve regeneration. Its recombinant form, cenegermin, has recently been used in patients with non-healing corneal epithelial defects in neurotrophic keratopathy (NK) to enhance the rate of corneal healing (Bonini et al., 2018). It has been postulated that NGF serves as a pleiotropic factor for an injured cornea via several mechanisms. Through *in vitro* studies, Aloe et al. (2015) demonstrated the role of NGF in the stimulation of corneal nerve regeneration, modulation of corneal stem cells through the induction of fibroblastic differentiation into myofibroblasts, and migration of wounded fibroblasts. NGF is also thought to play an instrumental role in the migration, colony formation and proliferation of epithelial cells, through the activation of tropomyosin receptor kinase A, a high-affinity receptor for NGF located on corneal epithelial cells (Al-Aqaba et al., 2019). Other neurotrophins, including glial-derived neurotrophic factor, brain-derived neurotrophic factor, ciliary neurotrophic factor (CNTF) and neurotrophin-3 (NT-3) have also been described in the cornea. *In vitro* studies of the corneal epithelium have demonstrated that glial-derived neurotrophic factor parallels the functions of NGF in cell migration, colony formation and proliferation (You et al., 2001), whereas brain-derived neurotrophic factor promotes colony formation (You et al., 2000). CNTF exerts trophic effects through the activation of corneal epithelial progenitor cells. Exogenous CNTF has been shown to accelerate healing and nerve regeneration of the wounded corneal epithelium in a mouse model (Zhou et al., 2015). Lastly, mouse corneal studies have demonstrated NT-3 as a survival factor of both sensory and sympathetic nerves, as well as a modulator of neuronal branching (Bennett et al., 2002).

Neuropeptides and neurotransmitters function as important messengers, transmitting nervous impulses from the presynaptic to the postsynaptic neuron, through the synaptic cleft. Neuropeptides are released slowly, act over an extended duration on many receptors, and exert paracrine effects aside from their neurotransmission function. Substance P (SP) and calcitonin gene-related peptide (CGRP) are the most common neuropeptides in mouse cornea (He and Bazan, 2016). SP, much like NGF, exerts a trophic effect on the rabbit corneal epithelium by modulating cell proliferation, migration and adhesion (Garcia-Hirschfeld et al., 1994). SP is constitutively expressed in tears. Its effects are important in both the maintenance of normal corneal epithelium, and healing of cornea after injurious stimuli (Al-Aqaba et al., 2019). SP exerts physiological roles in rabbit corneal homeostasis (Yamada et al., 2003), serving as an important mediator of reflex tear production, protector of corneal epithelial barrier function, and inhibitor of epithelial cell apoptosis. Interestingly, in diseased rabbit corneas, Nishida et al. (1996) established the synergistic interaction of SP with other trophic factors, such as insulin-like growth factor-1 (IGF-1), in stimulating corneal epithelial healing. Nishida et al. (2007) subsequently demonstrated the use of eye drops comprising an SP-derived peptide and IGF-1, to successfully treat epithelial defects in NK. The SP/IGF-1 complex upregulates integrin alpha-5, a fibronectin receptor, hence promoting cell adhesion to a fibronectin complex which plays a key role in epithelial cell migration and regeneration in a rabbit model (Nakamura et al., 1998). In clinical cross-sectional studies (Chao et al., 2016), tear SP levels have been shown to be positively correlated with the severity of dry eye symptoms, and negatively correlated with corneal sensitivity following LASIK. SP is also associated with corneal nerve degeneration in diabetes mellitus. Tummanapalli et al. (2019) demonstrated a significant correlation between the tear SP concentrations and corneal nerve fiber density (CNFD), as well as the Total Neuropathy Score of the severity of diabetic peripheral neuropathy. As a result, SP may serve as a useful biomarker for assessing post-laser refractive surgery dry eye and diabetic peripheral neuropathy.

CGRP is another neuropeptide expressed constitutively in tears for corneal epithelial maintenance, whose secretion increases after corneal wounding (Al-Aqaba et al., 2019). An earlier study has postulated that CGRP exerts vasoactive effects and increases blood flow in the eye after corneal epithelial injury (Uusitalo et al., 1989). CGRP has further been shown to enhance corneal re-epithelialization via the facilitation of epithelial cell migration in dog cornea, as well as cell differentiation in rabbit cornea (Garcia-Hirschfeld et al., 1994). CGRP also modulates innate immunity by upregulating cyclic adenosine monophosphate and interleukin (IL)-8 expression, thereby promoting neutrophil chemotaxis to areas of acute inflammation in the cornea (Tran et al., 2000). Diminished CGRP levels have been correlated with dry eye severity (Lambiase et al., 2001), and as such, have been suggested by many researchers (Di Zazzo et al., 2019; Tamhane et al., 2019) as useful biomarkers of dry eye disease in tandem with SP. Moreover, CGRP serves vital functions in nociception of the central and peripheral nervous system (Schou et al., 2017), with the cornea being no exception. CGRP stimulates the release of nitric oxide from trigeminal ganglia in rat cornea (Vause and Durham, 2009) and algogenic factors such as bradykinin from satellite glial cells in mouse cornea (Ceruti et al., 2011). These effects help produce a favorable neurochemical environment that enhances neural activity. In addition, increased CGRP levels have been associated with increased corneal hyperalgesia following corneal injury in a rat model (Hegarty et al., 2018).

Another neuropeptide, vasoactive intestinal peptide (VIP), is expressed in limited quantities in the corneal nerves (Al-Aqaba et al., 2019), but nonetheless plays important roles in wound healing in the corneal epithelium. Zhang et al. (2020) demonstrated the role of VIP in modulating corneal epithelial healing and nerve regeneration, as well as exerting anti-inflammatory effects in a signaling pathway-dependent manner. Moreover, in their animal diabetic corneal model, exogenous VIP improved the epithelial healing, upregulated the wound-induced production of neurotrophic factors, and dampened the inflammatory response (Zhang et al., 2020). As an autocrine trophic factor in the corneal endothelium, VIP has also been shown to promote the survival of corneal endothelial cells under oxidative stress (Koh and Waschek, 2000), postulated to be via the upregulation of the anti-apoptotic factor Bcl-2 and differentiation marker N-cadherin in a kinase A inhibitor-dependent mechanism (Koh et al., 2009). Like VIP, neuropeptide Y (NPY) confers anti-inflammatory properties as well. It is the most abundant peptide in the central and peripheral nervous systems (Medeiros and Turner, 1996). It has been shown to serve bimodal functions, as both a strong negative regulator of T cells, and an activator of antigen-presenting cells in a mouse model (Wheway et al., 2005). The distribution of NPY in the human cornea was shown to be closely related to vascular distribution (Stone, 1986), and subsequently in a mouse model, its role as a stimulator of angiogenesis and angiogenesis-dependent wound healing was discovered (Ekstrand et al., 2003).

Neurotransmitters, in contrast to neuropeptides, are released rapidly at the synaptic junction, exerting short-term effects on a limited number of receptors. The catecholamines, norepinephrine and epinephrine, have been found to exert neurotrophic functions, aiding corneal wound healing through epithelial cell proliferation, migration and transcellular transport processes (Al-Aqaba et al., 2019). The neurotransmitter acetylcholine is present in high concentrations in the cornea, helping to maintain the ionic gradient during propagation of nerve impulses along an axon (Al-Aqaba et al., 2019). Acetylcholine stimulates corneal epithelial cell DNA synthesis, epithelial cell migration and keratocyte proliferation (Słoniecka et al., 2015). Acetylcholine also reduces apoptotic activity and corneal fibrosis by inhibiting the formation of myofibroblasts and dampening the excessive production of extracellular matrix (Słoniecka and Danielson, 2020). Corneal trigeminal axons also contain the enzyme acetylcholinesterase (AChE) (Al-Aqaba et al., 2019), which has been used as a technique for corneal nerve staining (Liu et al., 2021). This enzyme has been postulated to confer neurotrophic effects on the cornea, evidenced by the loss of corneal sensation when AChE production is repressed, and the absence of AChE and acetylcholine in denervated corneas (Al-Aqaba et al., 2019).

These neuromediators serve vital physiological roles in homeostatic processes of the cornea (**Table 1**). In corneas with normal physiological conditions, they help maintain essential cellular processes, including normal proliferation, apoptosis and neurotransmission. In corneas that have pathological changes or surgical insults including laser refractive surgery, they provide additional stimuli for healing and nerve regeneration, as well as the modulation of inflammatory processes (Gao et al., 2014; Zhang et al., 2016).

Changes in Neuromediators Following Laser Refractive Surgery

Corneal wound healing is associated with the postoperative refractive stability, predictability, visual outcomes and resultant patient satisfaction following refractive surgery (Liu et al., 2015). The healing process is complex, involving various interactions between cells, neuromediators, cytokines and chemokines on the cornea and ocular surface (Lim et al., 2016; Yawata et al. 2019). In response to stimuli such as FSL ablation or surgical incision, corneal sensory nerves of rats are stimulated to release the neuropeptides SP and CGRP, the principle mediators of neurogenic inflammation (Liu et al., 2020a). In rabbit cornea, SP interacts synergistically with IGF-1 to promote the migration of epithelial cells to the site of tissue injury (Al-Aqaba et al., 2019). Furthermore, through the activation of calmodulin-dependent protein kinase II in rabbit corneal epithelial cells, SP enhances cell migration via the induction of fibronectin and interleukin (IL)-6 (Yamada et al., 2005). Another neuropeptide, CGRP, modulates the innate immune response through the activation of cyclic adenosine monophosphate and IL-8, hence promoting the migration of inflammatory cells such as neutrophils to the wounded site. The epithelium also produces cytokines and growth factors, including IL-1, tumor necrosis factor-α, epidermal growth factor, and platelet-derived growth factor (Mohan et al., 2000). These inflammatory and trophic factors function to regulate apoptosis, proliferation and the migration of keratocytes after laser refractive surgery (**Figure 2**).

Changes in various neuromediators have been observed following LASIK, photorefractive keratectomy, FLEx or SMILE. Mertaniemi et al. (1995) examined the effects of PRK on CGRP in a prospective study of 14 patients. Compared to the preoperative values, the release of CGRP in tears increased and peaked on postoperative day 2, and thereafter declined on day 7. It was postulated that the significant elevation of CGRP release on days 1–2 may be attributed to the secretion from damaged stromal nerves. Notably, despite the hypersecretion of tears postoperatively, there was no significant decrease in tear CGRP concentrations, signifying a simultaneous increased production of CGRP in tears possibly by the corneal sensory nerves. Similarly, in a longitudinal study comparing PRK and microkeratome LASIK, Lee et al. (2005) demonstrated that tear NGF/total tear protein (NGF/tP) ratio had the greatest increase in the immediate postoperative period following PRK, peaking at day 1, before decreasing from week 1 to month 6. LASIK resulted in a lower NGF/tP ratio as compared to the PRK procedure up to 1 month postoperatively, but there were no significant differences observed between both groups thereafter. At 6 months, the
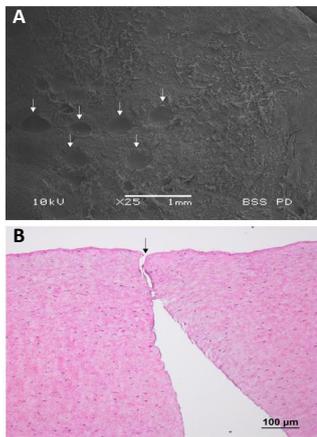
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Figure 1 | Scanning electron microscopy picture and corneal histologic section with hematoxylin and eosin staining showing the photodisruption process of femtosecond laser.
Bubbles cavities left after bubble expansion (arrows) are generated during the laser firing process, cleaving the tissue (A). The histological section shows no thermal damage, coagulative necrosis or obvious inflammatory response observed along the laser path (B; arrow). Sourced from unpublished data of the authors' laboratory.
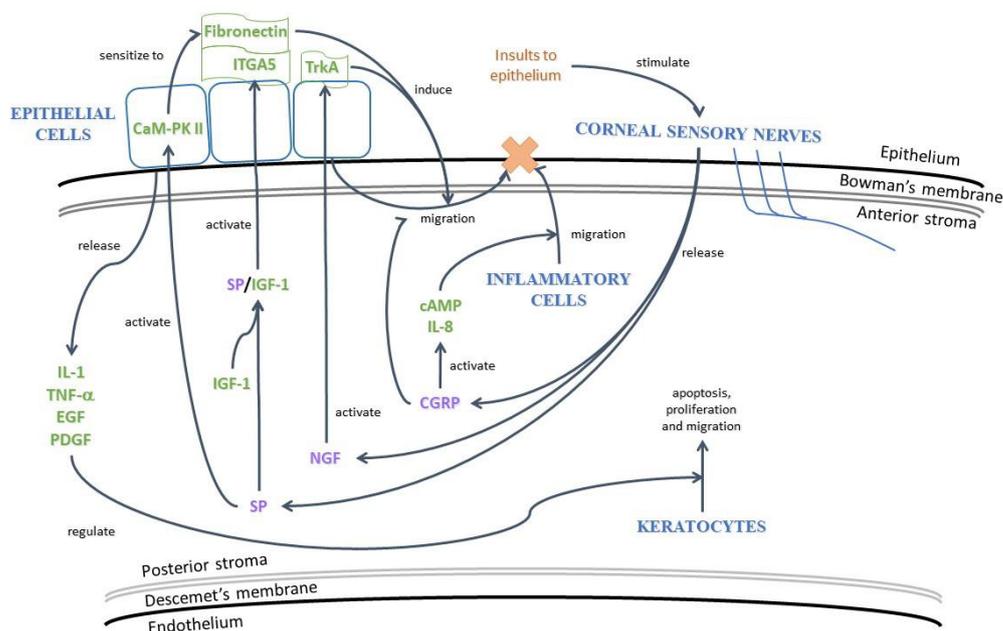

Figure 2 | The neurogenic inflammation process following injury to the cornea.
The neurogenic inflammation process following injury to the cornea. Damage of corneal nerves triggers inflammatory, neuroinflammatory and wound healing cascades in the cornea. cAMP: Cyclic adenosine monophosphate; CaM-PK II: calmodulin-dependent protein kinase II; CGRP: calcitonin gene-related peptide; EGF: epidermal growth factor; IGF-1: insulin-like growth factor-1; IL: interleukin; ITGA: integrin alpha; NGF: nerve growth factor; PDGF: platelet-derived growth factor; SP: substance P; TNF-α: tumor necrosis factor-α; TrkA: tropomyosin receptor kinase A.

Table 1 | Summary of the physiological roles and translational applications of neuromediators in corneas

	Neuromediator	Functions	Translational applications
Neurotrophin	Nerve growth factor	Epithelial cell migration, colony formation and proliferation *in vivo* Corneal nociception *in vitro* Maintenance of corneal nerve density *in vivo* Induction of fibroblastic differentiation into myofibroblasts *in vitro*	Nerve growth factor eye drops for neurotrophic keratopathy and post-refractive surgery regeneration (Nishida et al., 2007; Joo, 2014; Ma et al., 2014) Biomarker for dry eye disease (Tamhane et al., 2019)
	Glial-derived neurotrophic factor	Epithelial cell migration, colony formation and proliferation *in vitro*	
	Brain-derived neurotrophic factor	Epithelial cell colony formation *in vitro*	
	Ciliary neurotrophic factor	Epithelial progenitor cells activation *in vitro*	
	Neurotrophin-3	Corneal sensory and sympathetic nerve survival *in vivo* Modulator of corneal nerve branching *in vivo*	
Neuropeptide	Substance P	Epithelial cell migration, proliferation and adhesion *in vivo* Mediator of reflex tear production *in vivo* Protection of corneal epithelial barrier *in vitro* Inhibition of epithelial cell apoptosis *in vitro*	Substance P and insulin-like growth factor-1 eye drops for neurotrophic keratopathy (Bonini et al., 2018) Biomarker for dry eye disease and diabetic peripheral neuropathy (Chao et al., 2016; Tummanapalli et al., 2019)
	Calcitonin gene-related peptide	Epithelial cell migration and differentiation *in vivo* Innate immunity and neutrophil chemotaxis *in vitro* Vasoactive effects *in vivo* Corneal nociception *in vitro*	Biomarker for dry eye disease (Di Zazzo et al., 2019; Tamhane et al., 2019)
	Vasoactive intestinal peptide	Corneal nerve regeneration *in vivo* Upregulation of neurotrophin production *in vivo* Anti-inflammatory effects *in vitro* Corneal endothelial survival *in vitro*	
	Neuropeptide Y	Anti-inflammatory effects *in vivo* Angiogenesis and angiogenesis-dependent wound healing *in vivo*	
Neurotransmitter	Epinephrine and norepinephrine	Epithelial cell migration, proliferation and transcellular transport *in vitro*	
	Acetylcholine	Epithelial cell DNA synthesis and migration Keratocyte proliferation *in vitro* Reduces corneal apoptosis and fibrosis *in vitro* Maintains ionic gradient during propagation of nerve impulse *in vitro*	Corneal nerve staining to visualize corneal nerves distribution (Liu, 2020)

NGF/tP ratio returned to preoperative levels for both PRK and LASIK. In a similar study, Pérez-Santonja et al. (1999) showed that LASIK-treated eyes had significantly lower tear NGF levels compared to PRK during the first 3 months, and these differences became insignificant only after 6 months.

It has been hypothesized that the early postoperative differences in the tear NGF levels between PRK and LASIK are attributed to differences in the corneal wound healing processes. In PRK, the epithelium is removed, and the anterior stroma is ablated. On the other hand, LASIK relatively spares the epithelium, superficial stroma and Bowman's layer, and only the anterior stroma is ablated fully. As a result, PRK stimulates a more enhanced inflammatory response, with the presence of cytokines such as IL-1β acting to upregulate NGF and tropomyosin receptor kinase A production (You et al., 2000). Similarly, the relative lack of an NGF surge in the immediate postoperative period in LASIK, as compared to epi-LASIK, which has greater damage to the corneal epithelium, has been reported in a rabbit model. This low NGF level might partially explain the slower corneal sensory recovery in LASIK than epi-LASIK (Wu et al., 2009). In a clinical study by Erie et al. (2005) on the evaluation of corneal nerve plexus, the subbasal nerve density did not recover to preoperative levels until 5 years after LASIK as compared with 2 years after PRK. As a consequence, exogenous NGF has been proposed as a potential treatment to improve corneal neural healing post-LASIK (Joo, 2004; Ma et al., 2014). Besides the neurotrophic effects reported in clinical trials of patients with NK, topical NGF has been demonstrated to enhance the regeneration of the subbasal nerve plexus, as well as corneal sensitivity after LASIK in rabbits (Joo, 2004; Ma et al., 2014). Moreover, NGF is involved in nociception in the cornea, with topical NGF shown to stimulate thermal and mechanical hyperalgesia in rats (Lewin et al., 1993). As a result, the lower NGF secretion after LASIK might therefore account for a diminished corneal sensitivity, compared to after PRK.

Several studies have also demonstrated increased tear IL-6 and SP levels in the early post-LASIK period of up to 3 months (Gao et al., 2014; Chao et al., 2015), and Gao et al. (2014) found the SP tear concentrations inversely associated with CNFD. Studies on the changes of CGRP level following LASIK have shown inconsistent results; Chao et al. (2015) reported that there were no significant elevations of CGRP in the postoperative period of up to 3 months, while another cross-sectional study by Chao et al., (2016) showed that the tear CGRP concentrations at postoperative 12 months were significantly higher than those of normal subjects.

Following SMILE, there are different tear neuromediator profiles, as well as ocular surface changes compared to LASIK. NGF and IL-6 concentrations in tears are increased in both SMILE and FSL-LASIK, and the levels return to baseline more rapidly in SMILE at 3 months (Gao et al., 2014). Several reasons underlie the differences in the neuromediator profiles in these two procedures. In LASIK, the stromal nerve fibers that run across the circumferential flap cut are truncated and resected, and the excimer laser ablation on the stromal bed further vaporizes deeper stromal nerves. On the contrary, in SMILE, only the nerves near the small incision and inside the refractive lenticule are interrupted, but the nerve bundles located outside the cap/lenticule area remained untouched (Mastropasqua, 2015). Our group has previously demonstrated that at 4 years postoperatively, SMILE patients had significant higher corneal nerve fiber length and fiber density as well as total corneal nerve branch density compared to LASIK patients, indicating better preservation and faster recovery of corneal nerves following SMILE (Liu et al., 2020a). Furthermore, LASIK has been proven to invoke a greater inflammatory response, extracellular matrix deposition and stromal interface reaction as compared to SMILE, hence resulting in a greater trigger of NGF release through neurogenic inflammation (Liu et al., 2016a).

As for FLEx and SMILE, the tear NGF levels are significantly higher at 1 day, 1 week and 1 month following FLEx, in comparison to SMILE. Transforming growth factor-β1 (TGF-β1) levels are also significantly higher in FLEx compared to SMILE, at 1 day and 1 week postoperatively. Zhang et al. (2016) postulated that the flap creation following FLEx stimulates a more extensive inflammatory reaction, and therefore a greater resultant secretion of NGF and cytokines. FLEx involves a 330° flap creation, while SMILE requires just a 30° incision. Furthermore, the authors also showed that the tear NGF, TGF-β1 and IL-1α levels were moderately and significantly correlated with the ocular surface disease index scores, corneal fluorescein staining and non-invasive TBUT values in both FLEx and SMILE groups. **Table 2** summarizes the changes of tear neuromediators following laser refractive surgery.

Correlation of Neuromediators with Clinical Dry Eye Parameters

The consequences of corneal denervation and neuroinflammation following refractive surgery are seen clinically on the ocular surface (Liu et al., 2020b). Refractive surgery results in a decrease in tear production, tear film quality, and blinking reflex, which are involved in the pathogenesis of dry eye disease. Tear inflammatory cytokines and neuromediators have been shown to be associated with the clinical evaluation of dry eye such as ocular surface or corneal staining, Schirmer test, TBUT, tear osmolarity, corneal sensitivity and ocular surface disease index scores, not only in post-laser refractive surgery patients but also in other ocular surface conditions. Studies in contact lens wearers have found that tear NGF correlated significantly and moderately with clinical grading of dry eye severity, ocular surface fluorescein staining and conjunctival hyperemia. Results on the CGRP levels showed opposite changes compared to those of NGF, whereby the CGRP concentration was correlated inversely with the severity of clinical dry eye (Lambiase et al., 2011). The decreased tear CGRP levels resulted from decreased mucin production by goblet cells, which is itself a feature of dry eye disease (Mantelli and Argüeso, 2008). Another study by Golebiowski et al. (2017) on contact lens wearers showed that the tear CGRP level was correlated with CNFD.

Increased tear interferon-γ concentrations are also correlated with ocular surface staining, Schirmer test scores and tear hyperosmolarity (Jackson et al., 2016), whereas elevated IL-6 levels are related to TBUT and disease severity in patients with dry eye (Yoon et al., 2007).

Given the correlation established between these neuromediators as well as inflammatory mediators and clinical parameters, these mediators may serve as useful biomarkers for ocular surface and corneal nerves status following laser refractive surgery. Moreover, by targeting these neuromediators, it may open a new avenue to enhance the corneal wound healing and nerve regeneration processes, as well as to alleviate complications such as dry eye following laser refractive surgery.

Conclusion

Neuromediators represent the complex interplay between corneal nerves, epithelial, stromal and endothelial cells, whose mutual release of neurotrophins, neuropeptides and neurotransmitters are vital to corneal homeostasis. Alterations in neuromediator profiles following laser refractive surgery are closely related to the surgical techniques and the severity of the corneal tissue insult induced. The growing understanding of neuromediators, as well as advancements in corneal nerve assessment, have inspired the use of tear neuromediators as potential biomarkers for ocular surface health and corneal

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Author	Study design	Surgery type and patient number	Findings on neuromediators
Mertaniemi et al. (1995)	Prospective	PRK (n = 14 eyes)	Tear CGRP increased at d 1, peaked at d 2, and decreased at d 7 after PRK.
Lee et al. (2005)	Prospective	PRK (n = 70 eyes) and microkeratome LASIK (n = 76 eyes)	Up to 1 mon postoperatively, LASIK resulted in significantly lower tear NGF/total protein ratio compared to PRK, but differences were not significant at 3 and 6 mon. The mean NGF/total protein values at 1 d and 1 wk postoperatively correlated with corneal sensation, TBUT and Schirmer score at 6 months postoperatively for both groups.
Wu et al. (2009)	Rabbit model	Microkeratome LASIK (n = 24 eyes) and epi-LASIK (n = 24 eyes)	Tear NGF level was significantly lower in LASIK as compared to epi-LASIK on 1 d and 3 d, but differences were not significant at 7 d and 1 mon.
Chen et al. (2014)	Monkey model	Microkeratome LASIK (n=20 eyes)	NGF mRNA increased to 5.4-fold at day 3 and 2-fold above preoperative levels at d 7, and then returned to preoperative levels at 3 mon. Increased NGF mRNA level correlated with CNFD at 3 and 7 d postoperatively.
Pérez-Santonja et al. (1999)	Prospective	PRK (n = 18 eyes) and microkeratome LASIK (n = 17 eyes)	Tear NGF level was significantly lower in LASIK than PRK during the first 3 mon, with no significant differences after 6 mon.
Chao et al. (2015)	Prospective	Microkeratome LASIK (n = 40 eyes)	Tear SP level increased during the early post-LASIK period, and did not return to the preoperative ranges until after 3 mon. CNFD following LASIK was inversely correlated with dry eye symptoms and tear SP.
Chao et al. (2016)	Cross-sectional	Microkeratome LASIK (n = 40 eyes)	Tear SP level was positively and significantly associated with decreased CNFD and increased dry eye symptoms. Tear CGRP level was significantly higher than that of controls at 12 mon.
Gao et al. (2014)	Prospective	FSL-LASIK (n = 64 eyes) and SMILE (n = 30 eyes)	Tear NGF peaked on the first day, recovered to preoperative values at 3 mon after SMILE, but remained slightly elevated after 3 mon following FSL-LASIK.
Zhang et al. (2016)	Prospective	FLEx (n = 18 eyes) and SMILE (n = 23 eyes)	FLEx resulted in significantly higher tear NGF level for the first 1 mon, and significantly higher TGF-β1 for the first 1 wk postoperatively, compared to SMILE. NGF was significantly correlated with OSDI in both the FLEx and SMILE groups. NGF also had a significant association with NI-BUT in the FLEx and SMILE groups.

CGRP: Calcitonin gene-related peptide; CNFD: corneal nerve fiber density; epi-LASIK: epi-laser *in situ* keratomileusis; FLEx: Femtosecond Lenticule Extraction; FSL-LASIK: Femtosecond Laser *in Situ* Keratomileusis; LASIK: Laser *in Situ* Keratomileusis; NGF: nerve growth factor; NI-BUT: non-invasive TBUT; OSDI: ocular surface disease index; PRK: photorefractive keratectomy; SMILE: Small Incision Lenticule Extraction; SP: substance P; TGF: transforming growth factor.

nerve status following laser refractive surgery. Future directions include the validation of neuromediators as potential biomarkers, and the exploration of neuromediators as adjuncts to aid corneal regeneration after laser refractive surgery, or other ocular surface diseases such as neurotrophic keratopathy and dry eye.

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