# Trefoil Factor Family Peptide 3 (TFF3) is Upregulated Under Experimental Conditions Similar to Dry Eye Disease and Supports Corneal Wound Healing Effects In Vitro

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**Purpose.** To elucidate the role of trefoil family peptide (TFF) 3 at the ocular surface under conditions similar to dry eye disease (DED) and in tears of patients suffering from DED.

**Methods.** Trefoil family peptide 3 levels in tear samples from non-Sjögren's DED patients with moderate dry eye were analyzed by ELISA and compared with tears from healthy volunteers. A human corneal epithelial (HCE) cell line was treated with proinflammatory cytokines IL-1β and TNF-α, hyperosmolar medium, or scratching for up to 24 hours. *Trefoil family peptide 3* gene expression and protein biosynthesis were analyzed by RT-PCR, immunofluorescence, and ELISA. Migration and proliferation of HCE cells under recombinant (r) human (h) trefoil factor family peptide 3 (TFF3) stimulation were investigated by scratching and bromodeoxyuridine (BrdU) proliferation assays.

**Results.** Tears of patients suffering from DED contained significantly higher TFF3 levels than tears from healthy volunteers. Stimulation of HCE cells with proinflammatory cytokines, culture under hyperosmolar conditions, or scratching resulted, with the exception of hyperosmolar conditions, in an increase in TFF3 expression and elevated secretion level of TFF3. Cell proliferation decreased and cell migration increased after 24-hours stimulation with rhTFF3.

**Conclusions.** These results suggest that inflammatory factors or ocular surface damage as they occur in DED, lead to an increase of TFF3 tear film concentration, whereas hyperosmolarity does not. Our data underline a potential role for TFF3 as a candidate therapeutic for the ocular surface damage observed in DED.

Keywords: TFF3, ITF, ocular surface, dry eye syndrome

The tear film coats the ocular surface, serves as a barrier from environmental and microbial impact, lubricates the underlying epithelial cell layer, provides nutrients, and enables a smooth refractive surface.[1–3]

Trefoil factor family peptide 3 (TFF3), which is a member of the trefoil factor family (TFF) that can interact with mucins, and thereby influence mucus viscosity. Moreover, they have been shown to promote migration of epithelial cells in vitro, among other functions.[4] At the ocular surface, TFF3 was first described by Langer et al.[5] as a product of conjunctival goblet cells. It is absent in healthy cornea but is induced under certain pathological conditions such as herpetic infection.[6] So far, a TFF receptor has not yet been identified although the chemokine receptor CXCR4 has been shown to interact with TFF2,[4,7] promoting cell proliferation in pancreatic β-cells[8] and a CXCR4 receptor antagonist inhibited the action of topical rat TFF3 to accelerate epithelial restitution in TFF2(–/–) mice.[9] The dimeric form of TFF3 has been shown to increase viscosity of a mucin solution resulting in a spider web–like structure, whereas the monomer form of TFF3 has very little effect on viscosity and elasticity.[10] These rheological properties of TFF3 may have a functional role in dacryolith formation, where TFF3 is increased.[11]

In western industrial nations around 10% to 30% of the population suffer from dry eye disease (DED), a condition characterized by drastic changes in tear film composition and stability caused by several intrinsic or extrinsic factors.[3] It can result in a feeling of discomfort accompanied by inflammation of the corneal or conjunctival epithelial cells and hyperosmolarity of the tear film initiating corneal wound healing mechanisms.[3] Consistent research has so far provided insights into changes in tear film composition as well as changes in the ocular epithelial cells. Growth factors are decreased, proinflammatory cytokines and matrix metalloproteinases are increased, and salt concentrations are changed.[3]

Since TFF3 has been demonstrated to have positive effects on the corneal wound healing mechanism, has growth factor–like functions, and expression that are influenced by inflammatory conditions, we expected to gain further insight into TFF3 occurrence and action in the course of DED. To

investigate the role of TFF3 under conditions similar to DED we simulated these conditions in vitro by studying distinct inflammatory cytokines, hyperosmolar conditions, and epithelial damage in cultured corneal epithelial cells.

## Materials and Methods

### Tear Fluid

The study was approved by the local ethics committee of Martin Luther University Halle-Wittenberg, Germany and was conducted in accordance to the tenets of the Declaration of Helsinki, in compliance with good clinical practice and with informed consent. All subjects completed an institutional review board–approved questionnaire and underwent an ophthalmological examination in accordance with the valid Berufsverband der Augenärzte Deutschlands (BVA [Professional Association of German Ophthalmologists]) and Deutsche Ophthalmologische Gesellschaft (DOG [German Society of Ophthalmology]) guidelines for Germany. Healthy subjects had no dry eye symptoms, no ocular discomfort, no use of artificial tears, lubricants, or rewetting drops, no autoimmune disorders, no other eye diseases, and no history of eye surgery or contact lens wear. Subjects were considered to have a history of moderate (non-Sjögren's) DED if they had a documented diagnosis for more than 6 months prior to the study visit and a longer than 3-months history of ocular discomfort consistent with DED (burning, stinging, blurring, gritty sensation, or other); in addition, subjects currently used or wished to use artificial tears, ocular lubricants, or rewetting eye drops. Exclusion criteria were symptomatic xerostomia and an underlying autoimmune disease suggestive of Sjögren's syndrome as well as menopause. Human tear fluid was obtained from 10 patients suffering from moderate (non-Sjögren's) DED (mean age, 45.6 ± 3.8 years; seven women, three men) and 10 healthy controls (mean age, 35.2 ± 3.8 years; seven women, three men). Tears were obtained from both eyes of each subject using Schirmer strips (Bausch & Lomb, Berlin, Germany) as described recently.[12] Following the tear fluid sampling procedure, the tear fluid–soaked Schirmer strips were transferred to an Eppendorf reaction tube (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany), and stored at −20°C until testing.

### Isolation of the Tear Fluid From Schirmer Strips

For extraction of the tear fluid, the Schirmer strips were transferred to a 0.5-mL tube punctured at the bottom with a cannula. The tube was placed in a larger (1.5 mL) tube and centrifuged at maximum rpm (17.900 rct) for 5 minutes. The centrifugal force pulled the tear fluid out of the Schirmer strip, through the central "pore" in the bottom of the smaller tube and into the outer 1.5-mL tube.[12]

### Corneal Tissue

Four corneas were obtained from cadavers (three female, one male, aged 53–92) donated to the Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Germany. All corneas used were dissected from the cadavers between 4 and 12 hours postmortem. The donors were free of recent trauma, eye and nasal infections, or diseases affecting lacrimal functions.

### Cell Culture

SV40-transformed human corneal epithelial cells (HCE cells, passage number 18–27, obtained from Kaoru Araki-Sasaki; Tane Memorial Eye Hospital, Osaka, Japan)[13] were cultured as monolayer and used for stimulation experiments. Human corneal epithelial cells were cultured with Dulbecco's modified Eagle medium (DMEM)/HAM's F12 (1:1; Biochrom AG, Berlin, Germany) supplemented with 10% fetal calf serum (FCS) in a 5% $CO_2$ incubator at 37°C. For stimulation experiments, cells ($3 \times 10^6$) were seeded in Petri dishes and cultured until confluence was reached. Cells were washed with PBS and changed to serum-free mediums for 3 hours. Afterwards, cells were treated with proinflammatory cytokine IL-1β, (10 ng/mL; ImmunoTools, Friesoythe, Germany), TNF-α (20 ng/mL; ImmunoTools), or hyperosmolar media (containing 120 mM sodium chloride resulting in media with 550 milliosmolar [mOsM]) for 6 or 24 hours.[14] For in vitro simulation of wound healing, scratch experiments were conducted using confluent cells. The confluent monolayer was scratched three times with a 100-µL pipette tip, washed three times with PBS and incubated with serum-free cell culture media for different time periods (5 minutes, 1, 6, 12, and 24 hours). On completion of each experiment, conditioned media were collected for ELISA TFF3 secretion analysis. For immunofluorescence experiments, treated cells were fixed with ice-cold methanol for 7 minutes at −20°C.

### RNA Preparation and Complementary DNA (cDNA) Synthesis

Total RNA from cultured HCE was extracted using TRIZOL reagent (Invitrogen, Karlsruhe, Germany) according to manufacturer's protocol. Crude RNA was purified with isopropanol and repeated ethanol precipitation and contaminating DNA was destroyed by digestion with RNase-free DNase I (30 minutes, 37°C; Boehringer, Mannheim, Germany). The enzyme DNase was heat-inactivated for 10 minutes at 65°C. Reverse transcription of all RNA samples to first-strand cDNA was performed by RevertAidTM H Minus M-MuL V Reverse Transcriptase Kit (Fermentas, St. Leon-Rot, Germany) according to manufacturer's protocol. For each reaction, 2 µg total RNA and 10 pmol oligo (dT) 18 primer (Fermentas) were used. The ubiquitously expressed β-actin served as the internal control for the integrity of the translated cDNA.

### Polymerase Chain Reaction (PCR)

For TFF3 gene expression analysis, conventional RT-PCR amplification was carried out in a final volume of 20-µL containing 2 µL cDNA, 0.15 µL Taq polymerase, 1 µL 50 mM $MgCl_2$, 2 µL PCR buffer, 0.5 µL desoxynucleosidtriphosphate, and 0.5 µL forward/reverse primer mix. Polymerase chain reaction cycles were performed as follows: 94°C for 3 minutes, 40 cycles of 94°C for 40 seconds, 56°C (TFF3)/57°C (β-actin) for 40 seconds, 72°C for 40 seconds, and a final elongation step at 72°C for 10 minutes. Ten microliters of PCR reaction product were loaded onto 1% agarose gel to confirm the expected amplicons at 303 bp (TFF3) and 275 bp (β-actin). BigDye sequencing (Applied Biosystems, Foster City, CA, USA) was performed to verify PCR products. Beta-actin transcript amplification served as a positive control for RNA integrity and PCR success. The primer pairs used were as follows[15]: TFF3 sense (5′-GTG CCA GCC AAG GAC AG-3′), antisense (5′-CGT TAA GAC ATC AGG CTC CAG-3′), 303 bp, 65°C; β-actin sense (5′-CAA GAG ATG GCC ACG GCT GCT-3′), and antisense (5′-TCC TTC TGC ATC CTG TCG GCA-3′), 275 bp, 57°C.

### Enzyme-Linked Immunosorbent Assay

Trefoil factor family peptide 3 amounts in human tear fluid were evaluated by sandwich ELISA[15]: ELISA plates were coated

with capture antibody anti-TFF3 (mouse monoclonal antibody M01 clone 3D9, concentration of 0.6 μg/mL in 0.05 M carbonate buffer; Abnova, Taipei, Taiwan) overnight at 4°C, followed by four washing steps, 5 minutes each with washing buffer solution (0.5 M Tris-buffered saline [TBS; containing 50 mM Tris-Cl, pH 7.6, 150 mM NaCl]; 0.05% Tween 20). To prevent unspecific binding of the antibodies a blocking step was performed using 3% BSA in TBS/0.05% Tween 20 solution for 1 hour at room temperature (RT) and washed for 5 minutes once afterwards. Tear fluid samples and cell culture supernatants (diluted 1:20–1:50 in washing buffer) as well as recombinant human (rh) TFF3 peptide (nonglycosylated homodimer; ProSpec, Rehovot, Israel) for standard curve evaluation (at the concentrations of 0, 1.6, 3.1, 6.3, 12.5, 25, 50, and 200 ng/mL) were incubated for 2 hours at RT, and the plate was washed four times for 5 minutes afterwards. For detection biotinylated anti-human TFF3 (biotinylated polyclonal human TFF3, Sheep immunoglobulin G [IgG], BAF4407, 1:200 dilution in washing buffer [1 μg/ml]; R&D Systems, Abingdon, UK) was incubated for 2 hours at RT. Afterwards, the plate was washed four times, for 5 minutes each, and treated with HRP-streptavidin (dilution 1:8000 [0.25 μg/ml] in 0.1% BSA in TBS/0.05% Tween 20; DakoCytomation, Glostrup, Denmark). After five final washing steps of 5 minutes each, color reaction was enabled by adding 3, 3′, 5, 5′-tetramethylbenzidine (TMB) for 30 minutes (Sigma, Hamburg, Germany) and stopped by 1 N sulfuric acid and measured at 405 nm. For in vitro analysis, experiments were performed in triplicate and repeated at least three times. Concentrations were calculated in relation to total protein concentration. The assay was validated for the use of tears and cell culture medium samples by spiking a tear sample with known concentrations of rhTFF3. Recovery rates of 82.5% to 85.9% were obtained.

### Immunofluorescence

Corneas were cut into two pieces and one half was fixed in 4% paraformaldehyde (Roth, Karlsruhe, Germany) for 4 hours and washed in PBS. Corneas were then prepared for 10-μm sagittal cryostat sections. Sections were incubated in BLOTTO's Blocking Buffer (Thermo Scientific, Erlangen, Germany) at RT for 1 hour to reduce nonspecific background staining. Following three washes in PBS, sections were incubated with the primary antibody (1:100 dilution, anti-rTFF3-1, a gift from Werner Hoffmann; Institute of Molecular Biology and Medical Chemistry, Otto von Guericke University, Magdeburg, Germany)[16] at 4°C overnight, washed three times with PBS, and incubated with a secondary goat anti-rabbit antibody (1:2000 dilution [1 μg/ml], Alexa 488, A11034; MoBiTec, Göttingen, Germany) for 2 hours. Two negative control sections were used in each case: one was incubated with the secondary antibody only, the other with the primary antiserum only. Sections of human conjunctiva were used for positive control. The slides were examined with a Keyence Biorevo BZ9000 microscope (Keyence, Neu-Isenburg, Germany).

Control and treated cells were fixed with ice-cold methanol for 5 minutes at −20°C. For TFF3 immunoreactivity fixed cells were pretreated with dry milk solution (Blotto; Santa Cruz Biotechnologies, Heidelberg, Germany) for 1 hour at RT to prevent nonspecific binding. Primary antibody (monoclonal anti-TFF3, donor species: mouse; Nanotools, Teningen, Germany; 1:100 in PBS buffer [PBS with 2% (wt/vol) bovine serum albumin; Merck, Darmstadt, Germany; and 0.2% (vol/vol) Triton X-100]) was incubated overnight at 4°C, rinsed in PBS three times for 10 minutes each time, followed by secondary antibody treatment (Alexa488 conjugated goat-anti-mouse antibody, 1:500 in PBS [4 μg/ml]; MoBiTec GmbH, Göttingen, Germany). Nuclei counterstaining was done with 4′,6-Diamin-2-phenylindol (DAPI). Control sections were incubated with nonimmune IgG to determine possible nonspecific binding of mouse IgG. Positive control sections included pylorus or antrum (not shown).[15] Negative control sections, two per experiment, were obtained by incubating slides: one with primary antibody only and the other with secondary antibody only.

### Scratch Assay

For migration assay cells were stimulated with different TFF3 concentrations (0.1–300 μg/mL) as well as epidermal growth factor (EGF; 20 ng/mL) after scratching and washing steps. Cells were fixed with a 4% paraformaldehyde solution and stained with haemotoxylin. To ensure motogenic effects are visible instead of proliferation, cells were treated with 5 μM (2.4 μg/mL) cytochalasin B (Sigma-Aldrich, Taufkirchen, Germany) and 5 μg/mL mitomycin D (Sigma-Aldrich), inhibitors of migration (cytochalasin B), and proliferation (mitomycin D).[17] Dimethylsulfoxide (DMSO) served as a solvent for cytochalasin B. For obtaining three linear gaps cells were wounded with a pipette tip by scraping across the cell monolayer. Gap width was measured at 0 hour after 24 hours at 10 different positions per gap per well and at least four different wells per time point/concentration/stimulant were evaluated.

### Statistical Analysis

Data are represented as mean ± SEM of all studied samples. Statistical significance was evaluated by one-way ANOVA using InStat statistical software after confirming normal distribution and homogeneity of variance (Graph-pad Software, San Diego, CA, USA) or Kruskal-Wallis test, respectively. $P$ values below 0.05 were considered statistically significant.

## Results

### Tear Fluid From Patients Suffering From Non-Sjögren's Moderate DED Contains a Higher TFF3 Concentration Than Tears From Healthy Controls

Tear fluid samples obtained from 10 patients with diagnosed DED as well as from 10 healthy controls collected by Schirmer strips were tested for TFF3 concentrations. Enzyme-linked immunosorbent assay analysis revealed significantly increased levels of TFF3 in tear fluid samples from DED patients (5549 ± 1529 pg/mg total protein) compared with healthy control samples (1872 ± 259 pg/mg total protein; $P = 0.0019$).

### Human Corneal Epithelial Cells Express TFF3

Sections from four corneas of cadavers were analyzed by immunohistochemistry for the expression of TFF3. All sections analyzed revealed cytoplasmic immunoreactivity for TFF3 in epithelial cells, whereas the corneal stroma and the endothelium did not react with the antibody. Of interest, especially superficial epithelial cells revealed strong reactivity with the antibody but also basal cells of the epithelial cell layer were positive (Fig. 1).

### Proinflammatory Cytokines, but Not Hyperosmolarity, Increase TFF3 Expression, and Secretion In Vitro

Conventional RT-PCR showed basal mRNA expression levels of TFF3 in untreated cells after 6 and 24 hours as well as under

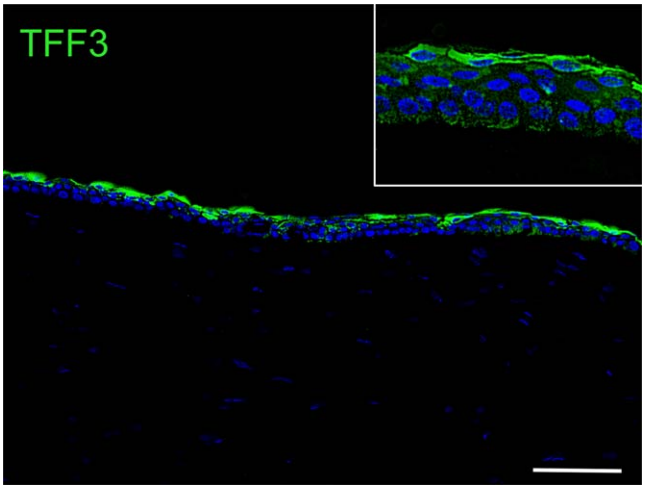


**Figure 1.** Trefoil factor family peptide 3 expression in human cornea. Trefoil factor family peptide 3 immunoreactivity occurs cytoplasmatically in corneal epithelial cells but not corneal stroma cells. Superficial epithelial cells reveal strongest immunoreactivity. The insert shows an epithelial area in higher magnification. *Scale bar*: 165 μm.

hyperosmolar conditions. Increased mRNA was measured after exposure to proinflammatory cytokines (IL-1β, TNF-α; Fig. 2A).[18] Trefoil factor family peptide 3 peptide in cultured HCE cells detected by immunofluorescence indicated increased intracytoplasmic reactivity of TFF3 after proinflammatory cytokine stimulation after 6 and 24 hours compared with nontreated control cells, whereas the cells did not show obvious changes in TFF3 reactivity when cultured under hyperosmolar conditions (550 mOsM; Fig. 2B). Proinflammatory cytokine challenge of HCE cells led to significantly increased TFF3 secretion into culture medium measured by ELISA. A 6- and 24-hour IL-1β treatment led to a 3.6- and 8.9-fold increase in secretion, respectively. Treatment with TNF-α after 6 and 24 hours resulted in a 2.7- and 7.2-fold increase in

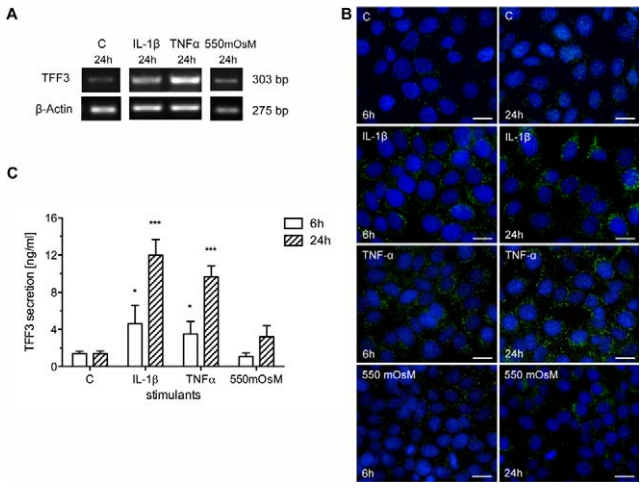


**Figure 2.** Trefoil factor family peptide 3 expression under inflammatory and hyperosmolar conditions. Semiquantitative RT-PCR (**A**) and immunofluorescence (**B**) reveal increased expression of *TFF3* gene after treatment with proinflammatory cytokines. Hyperosmolar conditions have no effect. *Scale bars*: 20 μm. (**C**) Control, *blue*: nuclear staining with DAPI. (**C**) Proinflammatory cytokines significantly increase the secretion of TFF3 (ng/mL), whereas in hyperosmolar conditions TFF3 secretion is increased 2.4-fold after 24 hours (ANOVA with Dunnett's test, $^{*}P < 0.05$; $^{***}P < 0.001$).

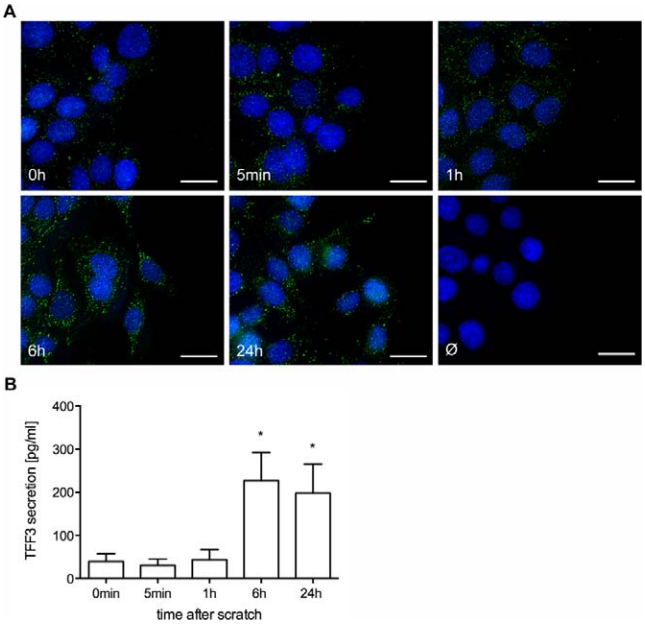


**Figure 3.** Corneal epithelial damage in vitro leads to a time-dependent increase in TFF3 expression and secretion. (**A**) Immunofluorescence after 6 and 24 hours. (**B**) Enzyme-linked immunosorbent assay: increased TFF3 secretion after 6 and 24 hours, but not at 1 hour. One-way ANOVA with Dunnett's test, $^{*}P < 0.05$.

the TFF3 secretion rate, respectively. A 24-hour treatment with hyperosmolar medium increased the TFF3 secretion by 2.4-fold compared with untreated controls, which, however, was not statistically significant, whereas a 6-hour treatment had no effect (Fig. 2C).

## Epithelial Cell Damage Increases the TFF3 Secretion In Vitro

Epithelial cell damage was induced by scratching confluent HCE cell monolayers (Fig. 3). Trefoil factor family peptide 3 expression and secretion analyzed by immunofluorescence (Fig. 3A) revealed a clear increase in the intracytoplasmic TFF3. Enzyme-linked immunosorbent assay showed that the basal secretion was 39.6 pg/mL ± 16.5 pg/mL immediately after wounding and remained constant for at least the first hour (Fig. 3B). After 6 hours the TFF secretion increased significantly to 227 pg/mL ± 60.2 pg/mL (6-fold) and after 24 hours it was still high, at 201 pg/mL ± 63.4 pg/mL (5-fold) although slightly decreased compared with the 6-hour measurement.

## rhTFF3 Augments Migration Alone and Together With EGF

Treatment of HCE cells with different concentrations of TFF3 showed an increased migration rate at 10 and 300 μg/mL, whereas lower concentrations had no effect (Fig. 4A). Combined treatment of migration responsive concentrations of 10 and 300 μg/mL rhTFF3 and EGF led to even higher migration rates compared with single treatment with either stimulant (Fig. 4B). Treatment with a combination of rhTFF3 and cytochalasin B, an inhibitor of migration, resulted in significant inhibition of wound closure at both concentrations (10 and 300 μg/mL TFF3). Also treatment with a combination of rhTFF3 and mitomycin C, an inhibitor of proliferation, significantly inhibited wound closure at both rhTFF3 concentrations tested. However, cytochalasin B inhibited wound

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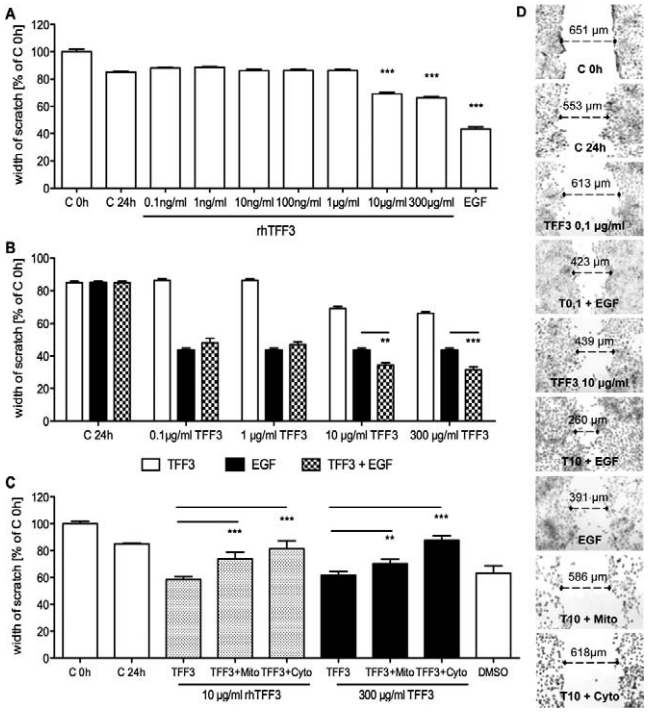


FIGURE 4. Effect of rhTFF3 on migration after 24 hours. (**A**) Trefoil factor family peptide 3 peptide affects migration of HCE cells only at higher concentrations (10 and 300 µg/mL). Combined stimulation of TFF3 together with EFG (20 ng/mL) resulted in elevated migration rates compared with single peptide. (**B**). Migration and proliferation inhibitors indicate motogenic rather than proliferative effects of TFF3 (**C**). Mito, mitomycin D (5 µM); Cyto, cytochalasin B (5 µg/mL); DMSO. (**D**) Shows examples of scratch gaps left.

closure more than mitomycin C (Fig. 4C). Thus, the main effect of rhTFF3 in scratch closure pertained to increased cell migration. Figure 4D shows example photographs of scratch widths of HCE cell monolayers under different time and stimulation conditions.

## DISCUSSION

Cultured HCE cells and sections of human cornea from cadavers express and secrete TFF3 in low concentrations that were not detectable in formalin-fixed tissue.[6] We speculate that the TFF3 concentration in corneal epithelial cells of formalin fixed corneal specimens was below the detection level of the antiserum we used in this earlier study.[6] Another explanation might be one of antibody specificity.

Recent studies have shown that proinflammatory cytokines, such as those used in the present study (e.g., IL-1β and TNF-α) are elevated in dry eye patients,[3,19–24] occur in all forms of DED[3] and regulate, for example, mucin expression. In the present study, such ocular stressors resulted in increased TFF3 secretion in vitro as in inflammatory conditions in other mucosae, but not all in vitro studies.[25,26] Rösler et al.[18] found an increase in the TFF3 protein level in primary osteoarthritis chondrocytes treated with IL-1β, TNF-α, or a combination of both. In contrast, hyperosmolar conditions that are also a common feature in patients suffering from DED[27] had no significant effect on TFF3 secretion rate in the cultured HCE cells.

Tear fluid of symptomatic DED patients showed significantly higher TFF3 concentrations than tears of healthy volunteers. In DED tear film instability causes damage of the ocular surface cells by desiccation. Trefoil factor family peptide 3 is known to act as an early-phase initiator of the wound healing cascade by activating a process called restitution, thereby promoting migration in the gastrointestinal mucosa.[28–30] This has also been shown in vitro and in vivo in mice for the ocular surface.[31,32] We hypothesize that also in DED, TFF3 is secreted to support this process. Irritation and epithelial damage caused (e.g., by inflammatory conditions), accompanied by disturbance of surface integrity, lead to TFF3 release by corneal epithelial cells, which can in turn be detected and measured in the tear samples of non-Sjögren's patients suffering from moderate DED.

Trefoil factor family peptide 3 levels in tear film are lower than in inflammatory conditions or in wounded epithelium. Ten to 300 µg/mL rhTFF3 is six orders of magnitude larger than the measured secretion from the cultured cells (Fig. 3) supporting the argument that increases in TFF3 are not sufficient to restore the status quo ante. Combined treatment of rhTFF3 and EGF, which is a very potent motogen showed a synergistic effect. These findings are in line with previous observations in other tissues.[33–36]

In conclusion, dimeric TFF3 in the tear film and at the ocular surface remains very promising as a potential therapeutic[37] and suggests broad implications for development of novel strategies for treating DED. First data from a phase II multicenter, randomized placebo-controlled trial of the prophylactic effects of rhTFF3 in treatment of chemotherapy-induced oral mucositis indicated that rhTFF3 in oral spray formulation is a safe and well-tolerated drug when given concurrently with chemotherapy. Prophylactic use of rhTFF3 at either high or low dosage was associated with a significant reduction (≈80%) in the occurrence of mucositis in patients at high risk for development of the lesion.[38] As DED, like mucositis, has an inflammatory component, these results prompt us to further elucidate the function and effects of rhTFF3 at the ocular surface with regard to the development of novel treatment strategies.

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