Doxycycline Prevents Matrix Remodeling and Contraction by Trichiasis-Derived Conjunctival Fibroblasts

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PURPOSE. Trachoma is a conjunctival scarring disease, which is the leading infectious cause of blindness worldwide. Elimination of blinding trachoma is being held back by the high rate of trichiasis recurrence following surgery. There is currently no treatment available to suppress the profibrotic state and reduce recurrence. Although the mechanisms underlying trichiasis development are unknown, the profibrotic phenotype has been linked to matrix metalloproteinase (MMP) expression. Doxycycline, a well-known tetracycline antibiotic, can act as a broad MMP inhibitor and has showed some success in preventing fibrosis in various clinical contexts. The purpose of this work was to assess the antiscarring properties of doxycycline in an in vitro model of trichiasis fibroblast–mediated tissue contraction.

METHODS. Primary cultures of fibroblasts were established from conjunctival samples obtained from normal donors or during surgery for trachomatous trichiasis. The effect of doxycycline on matrix contraction was investigated in our standard collagen gel contraction model. Cell morphology and matrix integrity were assessed using confocal reflection microscopy. Quantitative real time polymerase chain reaction and a FRET-based assay were used to measure MMP expression and activity, respectively.

RESULTS. Doxycycline treatment successfully suppressed the contractile phenotype of trichiasis fibroblasts, matrix degradation, and significantly altered the expression of MMP1, MMP9, and MMP12 associated with the profibrotic phenotype.

CONCLUSIONS. In view of the results presented here and the wider use of doxycycline in clinical settings, we propose that doxycycline might be useful as a treatment to prevent recurrence following trichiasis surgery.

Keywords: doxycycline, trichiasis, contraction, MMPs

Trachoma is the leading infectious cause of blindness worldwide.[1] The disease begins with recurrent infection by the bacterium *Chlamydia trachomatis* in early childhood, promoting chronic inflammation of the upper tarsal conjunctiva, which leads to progressive scarring and distortion of the eyelid. The edge of the eyelid turns in (entropion), so that the lashes scratch the surface of the eye (trichiasis). This can result in corneal opacity and irreversible sight loss.[2] Trachoma is a public health problem in over 50 countries, predominantly in Sub-Saharan Africa, the Middle East, the Indian subcontinent, Southeast Asia, and South America.[3] The most recent global estimation from the World Health Organization (WHO) suggests that 40 million people currently have active trachoma, a further 8.2 million have trichiasis, and 1.3 million are estimated to be blind as a result.[1] The WHO is leading a global alliance to eliminate blinding trachoma by 2020. This focuses on the implementation of the SAFE strategy: surgery for trichiasis, antibiotics for infection, facial cleanliness, and environmental improvements to reduce transmission of infection. However, there is growing evidence that the scarring complications can progress even in the absence of detectable chlamydial infection,[4] and following trichiasis surgery, the anatomical abnormality can redevelop (from 10% at 1 year to 60% at 3 years), in part through an ongoing immunofibrogenic process.[5,6] There is currently no adjuvant treatment available to suppress the profibrotic state and reduce recurrence.

The mechanisms underlying postsurgical recurrence of trichiasis are not fully understood. However, the dysregulated extra cellular matrix (ECM) proteolysis observed following infection and inflammation is suggested to play a key role in the development of fibrotic sequelae.[6] MMPs are a tightly regulated family of zinc-dependent enzymes responsible for degrading structural proteins of the ECM, and are produced by a variety of cell types after injury.[7] A number of MMPs have been found to associate with conjunctival scarring in in vitro models,[8] as well as in vivo.[9] MMP9 expression increases when conjunctival inflammation is associated with non-chlamydial bacterial infection in recurrent trichiasis,[10] and an increased level of MMP7 gene expression was also identified in trichiasis conjunctival samples.[11] Moreover, microarray analysis has confirmed the increased expression of MMP7, MMP9, and MMP12 in conjunctival samples from trichiasis subjects.[12] Overall, this suggests that the accumulation of fibrotic tissue in trichiasis might be due at least in part to altered MMP expression.

Doxycycline, a well-known tetracycline antibiotic, is widely used to prevent and treat bacterial and parasite infection, including *Chlamydia trachomatis*. More recently, its role as MMP inhibitor and in apoptosis has gathered more attention in the context of vascular disease,[13,14] pulmonary fibrosis,[15] periodontitis,[16] as well as ocular pathology.[17–19] Doxycycline inhibits MMPs, and particularly MMP9, at subantimicrobial doses in patients.[13,14,19,20] In addition, recent work suggests that doxycycline treatment can dampen local[21] as well as systemic[22] inflammation, thus making it a good candidate to prevent tissue remodeling and fibrosis in trachoma. Using for the first time conjunctival cells directly isolated from trachomatous trichiasis-affected individuals, we demonstrate that doxycycline significantly reduced collagen matrix remodeling and contraction, and specifically inhibited the mRNA expression of MMP1, MMP7, MMP9, and MMP12 during contraction, suggesting that it could be a potential adjuvant treatment following trichiasis surgery.

MATERIALS AND METHODS

Ethics Statement

This study adhered to the tenets of the Declaration of Helsinki. It was approved by the Tanzanian National Institute of Medical Research, the Kilimanjaro Christian Medical Centre, and the London School of Hygiene and Tropical Medicine Ethics committees. The study was explained to potential study participants and written informed consent was obtained before enrollment.

Clinical Samples

Conjunctival biopsies were obtained from the upper tarsal conjunctiva from Tanzanian patients undergoing trichiasis surgery. All cases had tarsal conjunctival scarring with entropion trichiasis. The eyelid was anesthetized with an injection of 2% lignocaine and the eye cleaned with 5% povidone iodine. A biopsy sample was taken using a 3-mm trephine from the tarsal conjunctiva, 2 mm from the lid margin, at the junction of the medial two-thirds and lateral one-third of the everted lid. The biopsies were wrapped in sterile gauze, moistened with normal saline, and transported to the laboratory at $+8°C$.

Cell Culture and Reagents

The biopsies were mechanically dispersed and the tissue fragments were placed in tissue culture dishes in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L L-glutamine (PAA Laboratories, Piscataway, NJ), supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO), 100 IU/mL penicillin, 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA) at 37°C with 5% CO_2. Following growth from the explant, the fibroblast populations (F07, F09, F10, and F11) were trypsinized and maintained routinely in the above medium. All four cell lines were tested for *C. trachomatis* infection using the Amplicor CT/NG Kit (Roche Molecular Systems, Branchburg, NJ) and were found to be negative. The cells were used between passage 4 and 9 for all experiments. For doxycyline treatment, a stock solution of 48.7 mM doxycycline hyclate (Sigma-Aldrich) was made in sterile ultrapure water (Biocel; Millipore Corp., Billerica, MA) and added to the cell culture medium at final concentrations of 104 and 416 μM.

Collagen Contraction Assay

The collagen contraction assays were performed as previously described.[8] Trachoma cells were seeded in a 1.5 mg/mL collagen type-I matrix (First Link [UK], Ltd, Wolverhampton, UK) at a concentration of 7×10^4 cells/mL. The gels were detached from the edge of the well, and 2 mL of DMEM with/without doxycycline was added. Gel contraction was monitored daily for 7 days by digital photography. Gel areas were measured using Java-based imaging software (ImageJ; provided in the public domain by the National Institutes of Health [NIH], Bethesda, MD; http://rsb.info.nih.gov/ij/), and the contraction was plotted as a percentage of gel area normalized to original area (day 0 measurement).

Cytotoxicity Assay

Cytotoxicity was determined using a cytotoxicity detection kit (Cytotoxicity Detection Kit [LDH]; Roche Applied Science, Penzberg, Upper Bavaria, Germany), on media collected at the termination of the gel contraction experiment (in phenol red-free DMEM) to measure the percentage of lactate dehydrogenase activity (LDH) present in the samples. The gels were lysed in 2% Triton X-100 (Sigma-Aldrich) in phenol red free, serum free DMEM for 10 minutes to achieve the maximum LDH release. Absorbances were measured at 490 nm (FLUOstar Optima; BMG LABTECH, Ortenberg, Germany) and the percentage of cytotoxicity was calculated according to the manufacturer's protocol.

Cell and Matrix Imaging

Following contraction for 7 days with/without 416 μM doxycycline, gels were fixed in 3.7% paraformaldehyde (Sigma-Aldrich) at room temperature for 30 minutes, followed by permeabilization with 0.5% Triton X-100 (Sigma-Aldrich) for 30 minutes, and staining with rhodamine-phalloidin (Invitrogen) for 1 hour.[8,23] Imaging was carried out on a confocal laser scanning microscope (Zeiss Axiovert S100; Carl Zeiss Microscopy, LLC, Thornwood, NY; Radiance 2000; Bio-Rad Laboratories, Inc., Hercules, CA) using simultaneous reflection microscopy and fluorescence imaging.[23] Representative images were acquired as *z*-stacks using a long working distance objective (Carl Zeiss Microscopy, LLC; 63×/0.75 Plan-Neofluar with correction collar). The resulting volumes were imported into the ImageJ software (NIH) where the fluorescence channel (F-actin staining) was compressed to a single projection and merged with a representative section of the matrix.

Quantitative Real-Time PCR

Collagen gel contraction assays were ended at days 0, 3, and 7 by placing the gels straight into TRIzol (Invitrogen) at 4°C for 1 hour. Control mRNA at day 0 were obtained after 1 hour of initial gel polymerization. Homogenization and phase separation were carried out according to the manufacturer's instructions. The aqueous phase was harvested and used for RNA isolation using an RNA purification kit (RNeasy Mini Kit; Qiagen, Hilden, Germany) according to the standard protocol. Reverse transcription was carried out using a reverse transcription kit (QuantiTect Reverse Transcription Kit; Qiagen) according to manufacturer's instructions. MMP gene expression was measured by QRT-PCR using validated primers and probes (Assay-on-Demand; Applied Biosystems, Foster City, CA). Assay identification numbers are MMP1 (Hs00899658_m1); MMP2 (Hs01548727_m1); MMP7 (Hs01042796_m1); MMP9 (Hs00234579_m1); and MMP12 (Hs00899662_m1). The hypoxanthine phosphoribosyl transferase-1 gene was used as an endogenous control to normalize the sample concentration. RT-PCR reactions were performed on a real-time PCR system (HT7900 Fast Real-Time PCR; Applied Biosystems), and the 2(-Delta Delta C(T)) method was used for quantification of mRNA levels.
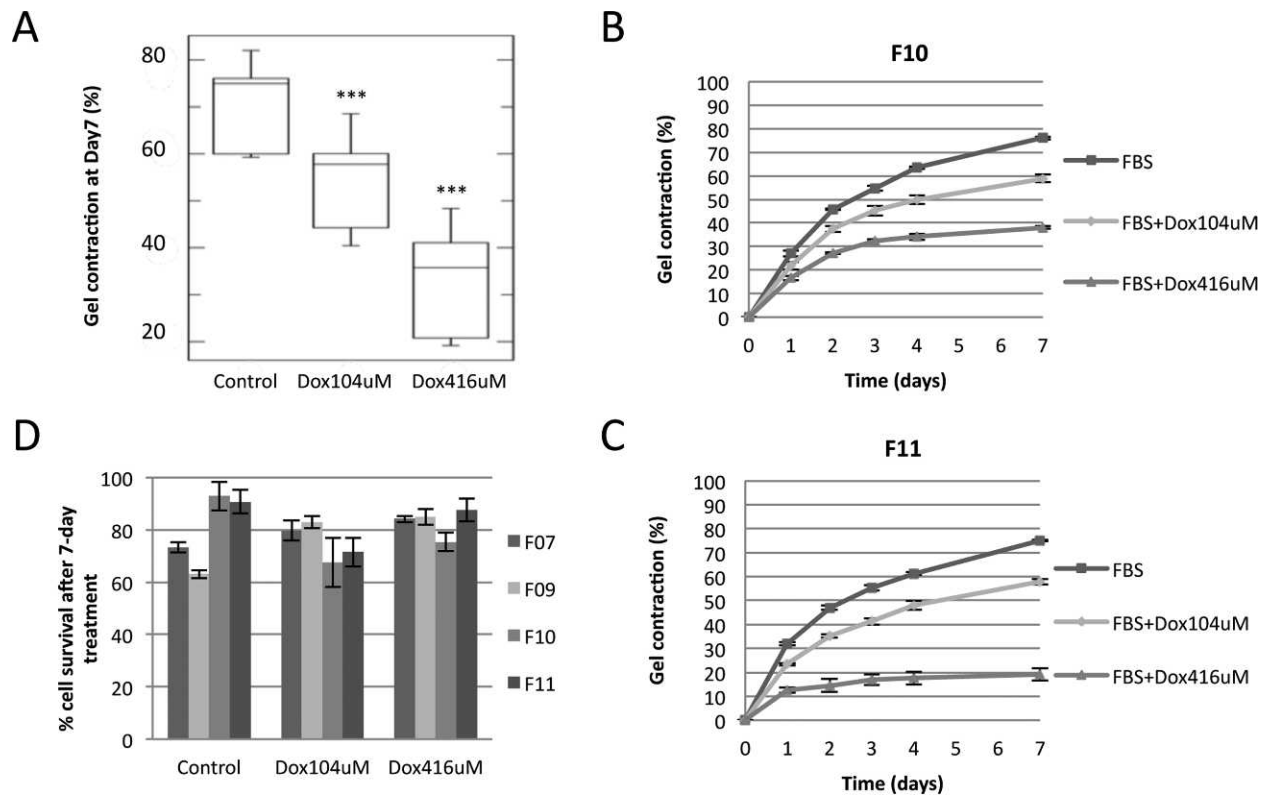
FIGURE 1. Doxycycline treatment prevents collagen matrix contraction by trichiasis fibroblasts. (**A**) Effect of doxycycline on trichiasis fibroblasts (pooled data for F07, F09, F10, and F11) gel contraction at day 7. Each data point was averaged from triplicate gels, $n = 3$. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. (**B**, **C**) Representative collagen gel contraction profile for F10 and F11 (mean ± SEM, three gels each). (**D**) Cytotoxicity as measured by LDH activity release into the medium during contraction after 7 days. The data is shown as percentage cell survival (mean ± SEM, for $n =$ three gels each).

MMP Activity Assay

Total MMP activity was determined using a FRET-based MMP activity assay kit according to the manufacturer's protocol (ab112147; Abcam, Cambridge, England). In brief, 25 μL of medium from control and doxycycline-treated collagen gel contraction cultures at day 0, 3, and 7 were added to 25 μL of 2 mM p-aminophenylmercuric acetate solution and incubated at 37°C for 3 hours. MMP red substrate (50 μL) was then added and the mix was incubated at room temperature for 1 hour. Fluorescence was measured at Ex/Em = 540/590 nM (FLUOstar Optima; BMG LABTECH).

Statistical Analysis

All graphs display mean and standard error. Statistical analysis was performed using the Students t-test to establish significant differences and individual P values displayed.

RESULTS

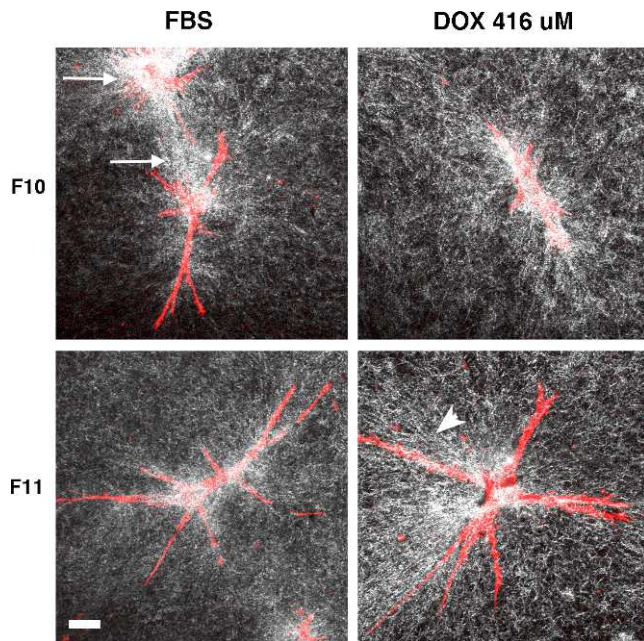### Doxycycline Prevents Collagen Matrix Remodeling and Contraction by Trichiasis Fibroblasts

We used our well-characterized in vitro model of cell-mediated matrix contraction[8,23–26] to assess the contractile potential of primary fibroblasts isolated from the conjunctiva of patients with trachomatous trichiasis and evaluate the potential of doxycycline as a modulator of contraction. As expected from their conjunctival and fibrotic origin,[23,25] trichiasis fibroblasts (F07, F09, F10, and F11) contracted collagen matrices strongly, down to 20% to 30% of their original size over 7 days in the

presence of 10% serum. The application of 104 μM doxycycline for 7 days was sufficient to reduce matrix contraction by 25% and more significantly, a 7-day treatment with 416 μM doxycycline prevented the contraction by up to 75% (Fig. 1A). Figures 1B and 1C show two representative contraction kinetics from F10 and F11 fibroblast lines, illustrating that doxycycline treatment reduced gel contraction as early as at day 1, with the effect of the drug increasing with incubation time for the higher concentration. To confirm that this effect was not due to drug toxicity, an LDH assay was performed on the cells within collagen gels following a 7-day doxycycline treatment at 104 or 416 μM. We found no detectable toxicity effect for the drug at either concentration (Fig. 1D).

We have shown previously that fibroblast-mediated gel contraction is dependent on the ability of the cells to affect the organization of pericellular collagen fibers through both direct mechanical pulling on the fibers to align and compact them, as well as by matrix degradation through the release of MMPs.[23,24] To determine how doxycycline prevented gel contraction, we used confocal microscopy to assess cell morphology and pericellular matrix organization in the gels following doxycycline treatment. As all four cell lines behaved identically in terms of matrix contraction and response to doxycycline (data not shown), we selected two representative cell lines, F10 and F11, to perform these studies and further work. Trichiasis-derived fibroblasts had a stellate appearance in the gels, with long F-actin rich protrusions, as illustrated by the full projection of the cell volume[23] (Fig. 2, red staining). In agreement with the toxicity data, the overall morphology of the cells appeared unaltered by the doxycycline treatment. Consistent with our previous work on other types of fibroblasts, the high contractile profile of the trichiasis

FBS   DOX 416 uM

FIGURE 2. Doxycycline treatment prevents matrix degradation and remodeling. Trichiasis fibroblasts F10 and F11 were embedded in collagen gels in medium with/without 416 μM doxycycline. The gels were fixed and stained with rhodamine phalloidin after 7 days. Shown are representative images of cells embedded in the matrix: *red*, two-dimensional projection of the full cell F-actin volume; *white*, collagen matrix fibers viewed using confocal reflection microscopy. *Arrows* show pericellular collagen fibers compaction. *Arrowhead* radial alignment consecutive to cell dynamic activity. *Scale bar*, 10 μm.

fibroblasts was linked to extensive remodeling and degradation of the collagen matrix by day 7, as visualized by a lack of distinct collagen fibers following confocal reflection imaging.[8,23] Areas of dense, compacted, poorly resolved collagen clumps could be seen as a bright white aura around the cells (Fig. 2, arrows), while the rest of the matrix shows a fuzzy appearance, characteristic of MMP-mediated degradation.[23] By contrast, in presence of 416 μM doxycycline, the matrix fibers remained clearly defined and evidence of fiber alignment consecutive to active cell pulling on the matrix can be found surrounding most of the cells (Fig. 2, arrowhead).

Doxycycline Reduces MMP Expression During Collagen Matrix Contraction

Our morphological analysis of the cells and matrix during contraction strongly suggested that doxycycline could act through a modulation of matrix degradation and thus likely MMP release. MMPs have long been connected to scarring processes. We have previously shown that matrix remodeling by MMPs plays an important role in tissue contraction, both in in vitro,[8,23] ex vivo,[8] as well as in an ocular scarring model in the rabbit model of glaucoma filtration surgery.[27] In addition, our recent studies have suggested a role for MMPs in the development of the fibrotic phenotype in trachoma.[10,11] We thus used real-time PCR to evaluate MMP expression during matrix contraction. We chose to investigate levels of MMP1 as a well-known collagenase previously implicated in our standard collagen contraction assay[26]; MMP2 as a standard gelatinase; and MMP7, MMP9, and MMP12 as these particular MMPs have been found enriched in trichiasis samples.[12] The C_T values from the RT-PCR study demonstrated that all of the above MMPs were present in both F10 and F11 at day 0. MMP1 and

MMP2 were expressed at significant levels, while MMP7 and MMP9 were naturally low (Table). All MMPs showed an increased expression during contraction in the control group, although to a different extent and kinetics. While MMP1, MMP2, and MMP12 showed a sustained increase throughout the contraction kinetics, MMP9 expression peaked at day 3 (Fig. 3). Continuous treatment with 416 μM doxycycline did not significantly affect MMP2 expression (Figs. 3C, 3D). However, MMP1 (Figs. 3A, 3B), MMP9 (Fig. 3E, 3F), and MMP12 (Figs. 3G, 3H) all show a strong reduction in expression in the presence of the drug. We also observed a similar trend for MMP7 in F10 (Table, normalized expression data not shown), but could not confirm this effect in F11 due to its lower expression of MMP7 and the technical limitation of RT-PCR. To confirm that the effect of doxycycline on MMP gene expression led to a reduction in protein expression and activity, we measured the total MMP activity released in the medium during contraction. As expected, the total MMP activity releasable from the medium increased significantly during contraction, particularly in F10, matching the gene expression profile (Fig. 4). Treatment with doxycycline completely abrogated MMP activity, even in medium at day 0, suggesting that doxycycline affected both the MMP protein levels and the activity of the MMPs present in the medium.

DISCUSSION

Using our in vitro model of cell-mediated matrix contraction,[8,23–26] we found that doxycycline significantly reduced the contractile potential of primary fibroblasts isolated from the conjunctiva of patients with trachomatous trichiasis, while presenting only minimal toxicity. This low toxicity and strong effect on contraction compares favorably with previously studied inhibitors of matrix contraction targeting cell division,[28] matrix metalloproteinase activity,[8,23,29] or small Rho GTPases,[26] which have been found to prevent tissue contraction ex vivo[26,29] and scarring in vivo, both in animal models[27] and in the clinic.[30,31] Our results suggest that doxycycline's effect on contraction is at least partly mediated by its ability to inhibit MMP expression and activity, which we have shown is a major component of the contraction process.[8,23,26] In addition, doxycycline appears to selectively target the expression of MMP1, MMP7, MMP9, and MMP12, which have been linked to the fibrotic phenotype in trachoma. Doxycycline has previously been shown to both reduce MMPs expression levels[32,33] and affect MMP activity.[13,19,33] In particular, it reduced MMP2 and MMP9 activity during gel contraction in vitro[34] and fibrosis in vivo,[35] suggesting that doxycycline's effects on MMP underlies at least part of its strong effect on matrix remodeling in trachoma. While the doxycycline inhibition of MMP activity is known to involve zinc chelation, the mechanism by which doxycycline affects MMP gene expression is still unclear. However, as doxycycline appears to broadly affect the proinflammatory response, it could affect MMP expression through a downregulation of MMP-inducing proinflammatory cytokines.[21,22,32]

MMP1, one of the main collagenases, has been linked to pathological processes such as fibrotic diseases and cancer.[36] Although it has not been reported in association with trichiasis, our previous work with human tenons' capsule fibroblasts has shown that it is heavily expressed during matrix contraction in vitro and its reduction is linked to a decrease in contraction,[26] suggesting that it may also functionally facilitate the matrix remodeling process during trichiasis. MMP7 is expressed in epithelia and injured tissue. It plays an important role in inflammation.[37,38] MMP7 upregulation not only participates in ECM regulation, but also correlates with many fibrotic
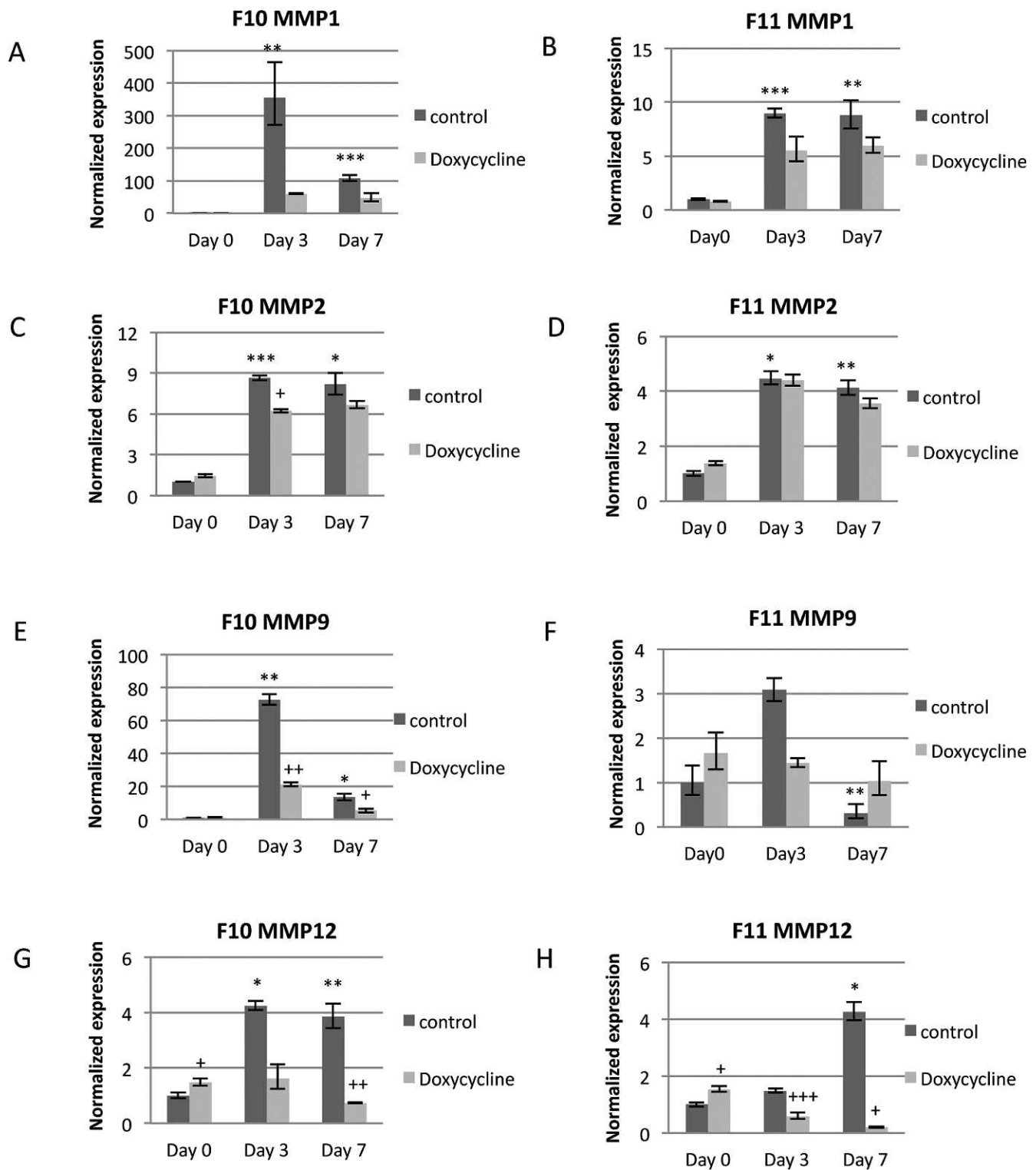
FIGURE 3. Doxycycline inhibits MMP expression during contraction. Quantitative RT-PCR for MMP1 (**A**, **B**), MMP2 (**C**, **D**), MMP9 (**E**, **F**), and MMP12 (**G**, **H**) mRNA expression in trichiasis fibroblasts F10 and F11 during contraction with/without 416 μM doxycycline. Significant differences in expression during contraction with reference to the value at day 0 are expressed as $*P < 0.05$. $**P < 0.01$. $***P < 0.001$. Significant differences between control and treated samples on the same day are expressed as $^+P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ (mean ± SEM, n = three repeats).

diseases,[39] including trachoma[11] and tumor metastasis.[40] MMP9 is a major component of ECM turnover during homeostasis and conjunctival scarring,[41] and its expression is closely linked to the degree of inflammation in the human conjunctival epithelium of children with active trachoma.[42,43] We found here that trichiasis-derived fibroblasts express low levels of MMP7 and MMP9. However, both MMP levels are increased transiently during the contraction process, suggest-

TABLE. Quantitative RT-PCR C_T Values for MMP mRNA Expression Levels During Gel Contraction

Gene	MMP1		MMP2		MMP7		MMP9		MMP12		HPRT1	
Dox	−	+	−	+	−	+	−	+	−	+	−	+
F10												
Day 0	31.1 ± 0.4	29.1 ± 2.1	25.1 ± 0.5	24.5 ± 0.6	39.6 ± 0.3	38.2 ± 0.9	39.0 ± 0.5	37.5 ± 1.0	34.8 ± 0.8	34.1 ± 0.8	29.8 ± 0.8	29.8 ± 0.8
Day 3	23.3 ± 0.7	24.0 ± 0.5	23.4 ± 0.4	22.8 ± 0.2	35.9 ± 0.4	36.7 ± 0.7	33.9 ± 0.5	34.0 ± 0.7	31.7 ± 0.8	32.9 ± 0.7	30.9 ± 0.8	30.4 ± 0.5
Day 7	23.0 ± 0.4	25.9 ± 0.7	22.1 ± 0.2	22.2 ± 1.2	36.3 ± 0.2	37.2 ± 1.0	34.6 ± 0.5	35.5 ± 0.9	30.3 ± 0.8	32.7 ± 0.9	30.0 ± 0.7	30.1 ± 1.0
F11												
Day 0	30.4 ± 0.4	29.4 ± 0.1	26.5 ± 2.3	27.7 ± 3.4	37.0 ± 0.1	n/a	40.4 ± 0.9	37.0 ± 2.6	34.4 ± 1.3	34.4 ± 1.0	31.9 ± 0.9	32.1 ± 0.8
Day 3	26.3 ± 0.2	27.0 ± 0.5	23.5 ± 0.2	21.9 ± 0.9	41.3 ± 1.4	39.2 ± 0.6	39.0 ± 0.2	39.7 ± 0.5	34.1 ± 1.7	37.0 ± 0.4	32.0 ± 0.4	30.3 ± 1.2
Day 7	25.6 ± 0.5	27.6 ± 0.7	23.2 ± 0.2	22.4 ± 1.3	40.8 ± 0.9	40.5 ± 0.7	39.9 ± 0.6	41.1 ± 1.3	33.6 ± 1.4	38.5 ± 0.6	32.0 ± 0.6	30.6 ± 1.6

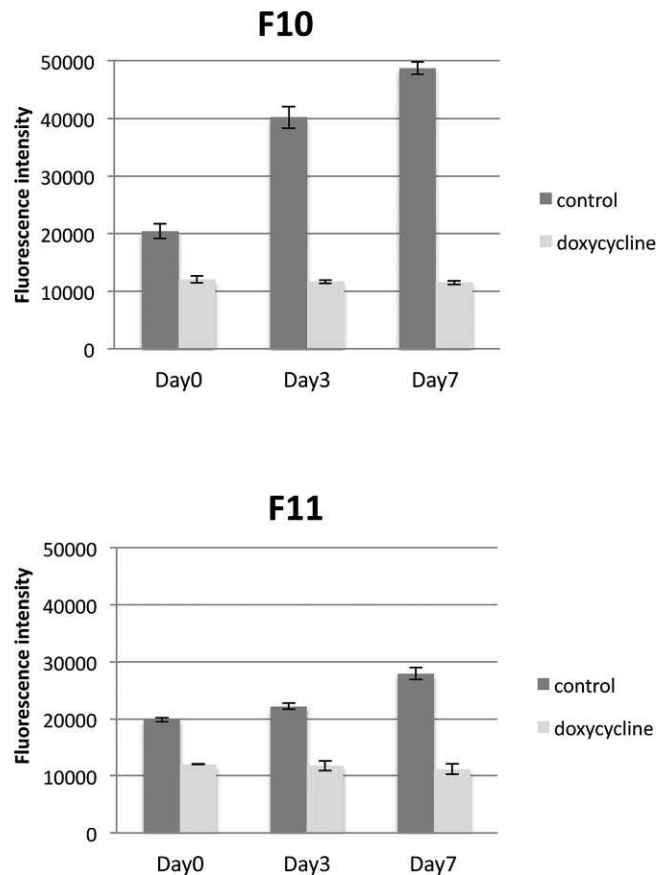C_T values are averaged from $n \geq 3$ experiments.

FIGURE 4. Doxycycline inhibits MMP activity during contraction. The total MMP activity released in the medium by F10 and F11 cells with/without doxycycline treatment was measured at day 0, 3, and 7 during contraction using a FRET-based assay. MMP activity is expressed as fluorescence levels (mean ± SEM; F10, $n = 3$; F11, $n = 2$).

ing that these MMPs may be functional and activated mostly at the initial stage. The extremely low expression of MMP7 in F11 might be the result of the natural biological variation of F11, together with the technical limit of semi-quantitative RT-PCR. MMP12, on the other hand, is mainly produced by macrophages, its main function including degrading elastin and taking part in proinflammatory processes.[44] Increased MMP12 expression has been reported in the scarred conjunctiva of people with trichiasis either with or without inflammation.[12] Our results showed MMP12 has a modest but consistent increasing during the matrix contraction both in F10 and F11, suggesting that it could directly contribute to matrix remodeling in trichiasis. Interestingly, though doxycycline treatment was shown to significantly inhibit MMP12 expression in both cell lines studied, it was significantly more efficient in preventing the contraction of F11, which did not express significant levels of MMP7 and MMP9. This suggests that in the absence of MMP7 and MMP9, MMP12 might be a significant factor driving trichiasis fibroblast-mediated contraction.

Doxycycline's potential as an MMP inhibitor has been extensively documented and it has proved useful in clinical settings,[14,20] with many reporting its strong effect on MMP9.[13,14,19] Recent work suggests that it can also modulate inflammation,[21,22] thus making it a good candidate to prevent the immunofibrogenic process that underlies recurrent trachomatous trichiasis. We present here evidence that doxycycline

prevents matrix remodeling and contraction by trichiasis-derived fibroblasts and leads to a significant downregulation in MMP expression in these cells. The in vitro model of tissue contraction used here has already proved essential to the development of treatments for the prevention of scarring following glaucoma filtration surgery and a reasonable predictor of the clinical potential of anti-scarring treatments.[30] In the absence of any animal model for trichiasis development and recurrence, this in vitro model may facilitate the translational pathway to modeling the pathogenesis of trachoma and evaluating the effectiveness of new treatments in advance of clinical trials. In view of our results and the wider use of doxycycline in clinical settings, we propose that doxycycline might be useful as a treatment to prevent recurrence following trichiasis surgery.

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