Matrix Metalloproteinase-3 Differences in Oral and Skin Fibroblasts

S.T.W. McKeown, J.J. Barnes, P.L. Hyland, F.T. Lundy, M.J. Fray and C.R. Irwin

J DENT RES 2007; 86; 457
DOI: 10.1177/154405910708600513

RESEARCH REPORTS
Biological

S.T.W. McKeown[1†], J.J. Barnes[1†], P.L. Hyland[2], F.T. Lundy[1], M.J. Fray[3], and C.R. Irwin[1*]

[1]Oral Science Research Centre, School of Dentistry, Queen's University Belfast, Northern Ireland; [2]Centre for Cancer Research and Cell Biology, Queen's University, Belfast; and [3]Department of Discovery Chemistry, Pfizer Global Research and Development, Sandwich, Kent, England; [†]authors contributing equally to this work; *corresponding author, Department of Restorative Dentistry (Periodontology), Queen's University School of Dentistry, Royal Victoria Hospital, Grosvenor Road, Belfast, BT12 6BP, Northern Ireland; c.r.irwin@qub.ac.uk

J Dent Res 86(5):457-462, 2007

Matrix Metalloproteinase-3 Differences in Oral and Skin Fibroblasts

ABSTRACT

While skin wounds heal by scarring, wounds of oral mucosa show privileged healing with minimal scar formation. Our hypothesis was that phenotypic differences between oral and skin fibroblasts underlie these differences in healing. The aims of this study were to compare MMP-3 expression by oral and skin fibroblasts and investigate a role for MMP-3 in mediating collagen gel contraction. Oral fibroblasts induced significantly greater gel contraction than did paired skin cells. Inhibition of MMP activity significantly inhibited gel contraction by both cell types. Specific inhibition of MMP-3 activity reduced gel contraction by oral, but not skin, fibroblasts. Oral fibroblasts produced significantly higher levels of MMP-3 than did skin fibroblasts at all levels studied. TGF-β1 and -β3 isoforms stimulated MMP-3 expression at mRNA, protein, and activity levels by both fibroblast populations. Results suggest that increased MMP-3 production by oral fibroblasts may underlie the differences in wound-healing outcome seen in skin and oral mucosa.

KEY WORDS: wound healing, fibroblasts, MMP-3, TGF-β, wound contraction.

Received August 17, 2005; Last revision December 4, 2006; Accepted January 15, 2007

INTRODUCTION

Wound healing in the oral mucosa occurs in a privileged fashion, characterized by rapid wound remodeling and re-epithelialization with minimal scar formation, in contrast to skin repair, where the restoration of tissue continuity includes scar tissue formation (Whitby and Ferguson, 1991; Szpaderska *et al.*, 2003). Following injury, fibroblasts play a central role in the healing response, inducing wound contraction, depositing and remodeling granulation tissue, and stimulating re-epithelialization. Differences in healing outcome between oral mucosa and skin may reflect phenotypic differences between oral and skin fibroblasts (Irwin *et al.*, 1994, 1998; Stephens *et al.*, 1996; Knerer *et al.*, 1999).

Matrix metalloproteinases (MMPs) are a family of approximately 28 endopeptidases that play a role in many aspects of wound healing, including epithelial migration, granulation tissue remodeling, and growth factor/cytokine activation. Differences between oral and skin fibroblasts in terms of matrix metalloproteinase (MMP) expression have been previously described (Stephens *et al.*, 2001), suggesting that fibroblast phenotype and the regulation of local MMP levels in the wound site may determine the differences in healing between mucosal and dermal tissues. During dermal wound healing, MMP-3 is secreted by fibroblasts and has a broad range of ECM targets, primarily proteoglycans, such as decorin, biglycan, and versican, in addition to the glycoproteins laminin and fibronectin, and denatured collagens. MMP-3-induced degradation of decorin/biglycan results in TGF-β release from extracellular matrix stores and is thought to be important in both wound healing and tumor invasion (Imai *et al.*, 1997). Excisional wounds in MMP-3 knock-out animals showed impaired contraction compared with controls, while fibroblasts derived from the knock-out animals also exhibited reduced contractile activity (Bullard *et al.*, 1999a,b). This is of particular interest, since we and others have reported an accelerated contraction of oral wounds *in vivo*, coupled with an increased contractile phenotype of oral fibroblasts *in vitro* (Stephens *et al.*, 1996; Irwin *et al.*, 1998).

Transforming growth factor-β (TGF-β) is a well-recognized regulator of healing. TGF-β_1 is the predominant isoform in adult wound healing, and is closely associated with scar formation (Krummel *et al.*, 1988; Shah *et al.*, 1995). In contrast, TGF-β_3 is the predominant isoform during fetal wound healing and is known to promote scarless repair of fetal defects (Kohama *et al.*, 2002) and to reduce scarring in adult skin (Shah *et al.*, 1995). To date, no studies have compared the effects of TGF-β_1 and TGF-β_3 on skin and oral fibroblasts, and specifically their effects on metalloproteinase activity.

The aims of this study were therefore to investigate the role of MMP-3 in wound healing and to compare the effects of TGF-β_1 and TGF-β_3 on MMP-3 activity in oral and skin fibroblast populations.

MATERIALS & METHODS

Development of Cell Populations

Paired oral and skin fibroblasts were obtained by explant culture of tissue samples taken from six healthy adult donors. Ethical approval for the study was granted by the local Ethics Committee, and all donors gave informed consent to participate in the study. Fibroblast cultures were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FCS, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin sulphate (Invitrogen, Paisley, UK), in a 37°C humidified incubator with 5% CO_2.

Effects of Matrix Metalloproteinase Inhibitors on Gel Contraction

Type I rat tail collagen solution was extracted as previously described (Schor, 1980). Three paired oral and skin fibroblast cell lines were seeded into 2-mL gels in 30-mm dishes (Bibby Sterilin, Stafford, UK) at a concentration of 2.5 x 10^5 cells/mL. Polymerized gels were overlaid with DMEM supplemented with 5% FCS, containing (or not): (1) GM1489, a broad-spectrum MMP inhibitor (20 μM) (Calbiochem, Nottingham, UK); (2) UK356618, a specific inhibitor of MMP-3 (600 nM) (Pfizer, Tadworth, UK); or (3) DMSO vehicle (1:1000). Gel surface area was recorded over a seven-day period.

Effects of MMP Inhibitors on αSMA Expression

Quiescent oral and skin fibroblasts were seeded either on six-well plates at 3 x 10^5 cells/well, or into collagen gels as described above. Cultures were then overlaid with 2 mL of DMEM containing 5% FCS and MMP inhibitors as before. Following a seven-day incubation, monolayer cultures underwent lysis for 30 min on ice with a 1% SDS, 10 mM Tris (pH 7.4) buffer-containing protease inhibitor cocktail (Sigma, Poole, UK). In collagen gels, fibroblasts were harvested by collagenase digestion, pelleted, and subjected to lysis.

α-Smooth-muscle actin (αSMA) expression in lysate volumes equivalent to 2.5 x 104 cells was detected by Western blotting. Samples underwent electrophoresis on a NuPAGE 4-12% BIS-TRIS gel, and were transferred onto the nitrocellulose membrane and then incubated with a mouse monoclonal anti-αSMA antibody (1:1000) (Sigma, Poole, UK) for 1 hr at room temperature, followed by a rabbit anti-mouse peroxidase conjugate (1:1000) (Pierce, Cramlington, UK) for a further hour prior to chemiluminescence detection (Pierce, Cramlington, UK).

Effects of TGF-β Isoforms on MMP-3 mRNA Expression

Confluent fibroblast cultures were treated with TGF-β_1 or TGF-β_3 (Sigma, Poole, UK) over the concentration range 0.1-10 ng/mL. After 24 hrs, total cellular RNA was extracted by means of Trizol® reagent (Invitrogen, Paisley, Scotland). cDNA was synthesized from 1 μg of extracted RNA with the Superscript™ First Strand Synthesis System (Invitrogen, Paisley, UK) and an oligo dT primer mix, according to manufacturer's guidelines. cDNA was amplified with Platinum Taq DNA polymerase (Invitrogen, Paisley, UK) and 5 pmol of primers. Primer pairs used for amplification were as follows: MMP-3 (191 bp) GCCAGGGATTAATGGAGATGC (forward), ACAGGCGGAA CCGAGTCAGG (reverse); β-actin (394 bp) ATCTGGCACC ACACCTTCTACAATG (forward), GCTTCTCCTTAATGTCA CGCACGAT (reverse).

Amplification of both targets was carried out in separate but simultaneous reactions in a Perkin-Elmer Cetus Thermal Cycler for 27 cycles: 50 sec denaturation at 94°C, 25 sec annealing at 64°C, and 45 sec elongation at 72°C. We determined a cycle number of 27 to be within the exponential phase of amplication for both MMP-3 and β-actin targets, by screening specific MMP-3 and β-actin PCR reactions taken from the thermal cycler between 20 and 40 cycle numbers. Appropriate negative controls and a low-molecular-weight mass marker (Invitrogen, Paisley, UK) were used during all gel electrophoresis runs. PCR products were resolved by electrophoresis on a 2% agarose gel containing SyBr™ green, identified by size in a UV transilluminator and confirmed by sequence analysis. The net intensities of bands were measured with image analysis software, and MMP-3 expression levels were calculated relative to β-actin as described previously (McKeown *et al.*, 2003).

Effects of TGF-β Isoforms on MMP-3 Protein and Activity Levels

Confluent cultures of paired oral and skin fibroblasts were overlaid with serum-free DMEM containing TGF-β_1 or TGF-β_3 at 0.1, 1.0, and 10 ng/mL. Following a 48-hour incubation period, MMP-3 protein levels in the conditioned medium were determined by ELISA (Amersham Biosciences, Little Chalfont, UK), and activity levels by a Biotrak Activity Assay System (Amersham Biosciences, Little Chalfont, UK).

Statistical Analysis

Comparison of gel contraction and MMP-3 expression by paired skin and mucosal fibroblasts was performed by a paired *t* test. When analyzing the effects of TGF-β_1 and -β_3 on MMP-3 mRNA, protein, and activity levels, to diminish inter-strain variability, we normalized control values to 100% to allow for collective analysis of cytokine effects relative to controls. All results were subsequently analyzed by ANOVA, followed by the Duncan test, and values of $p < 0.05$ were considered statistically significant. The effects of MMP inhibitors on oral and skin contraction rates were also analyzed by ANOVA, followed by the Duncan test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Oral fibroblasts induced a significantly greater degree of contraction compared with skin counterparts at day 1 ($p < 0.05$), this trend continuing throughout the study (Fig. 1a). Gel contraction by both skin and oral fibroblasts was significantly reduced ($p < 0.05$) by the broad-spectrum MMP inhibitor at all time-points (Fig. 1). The MMP-3 inhibitor had no effect on gel contraction by skin fibroblasts. In oral fibroblast cultures, inhibition of MMP-3 activity resulted in a significantly reduced rate of contraction ($p < 0.05$), although this inhibitory effect was not as potent as that of the broad MMP inhibitor (Fig. 1b).

In control cultures, and under all 3 culture conditions, skin fibroblasts expressed higher levels of αSMA than did oral cells. This was particularly evident in the monolayer cultures (Fig. 2a). Both the broad-spectrum MMP inhibitor and the MMP-3 inhibitor significantly reduced αSMA expression in both cell types to a similar extent. In floating collagen gels populated with oral fibroblasts, both inhibitors reduced αSMA expression to below detectable levels.

At mRNA, protein, and activity levels, oral fibroblasts produced significantly greater levels of MMP-3 than did skin fibroblasts. Both TGF-β_1 and TGF-β_3 stimulated MMP-3 mRNA expression by oral and skin fibroblasts (Fig. 3). In skin

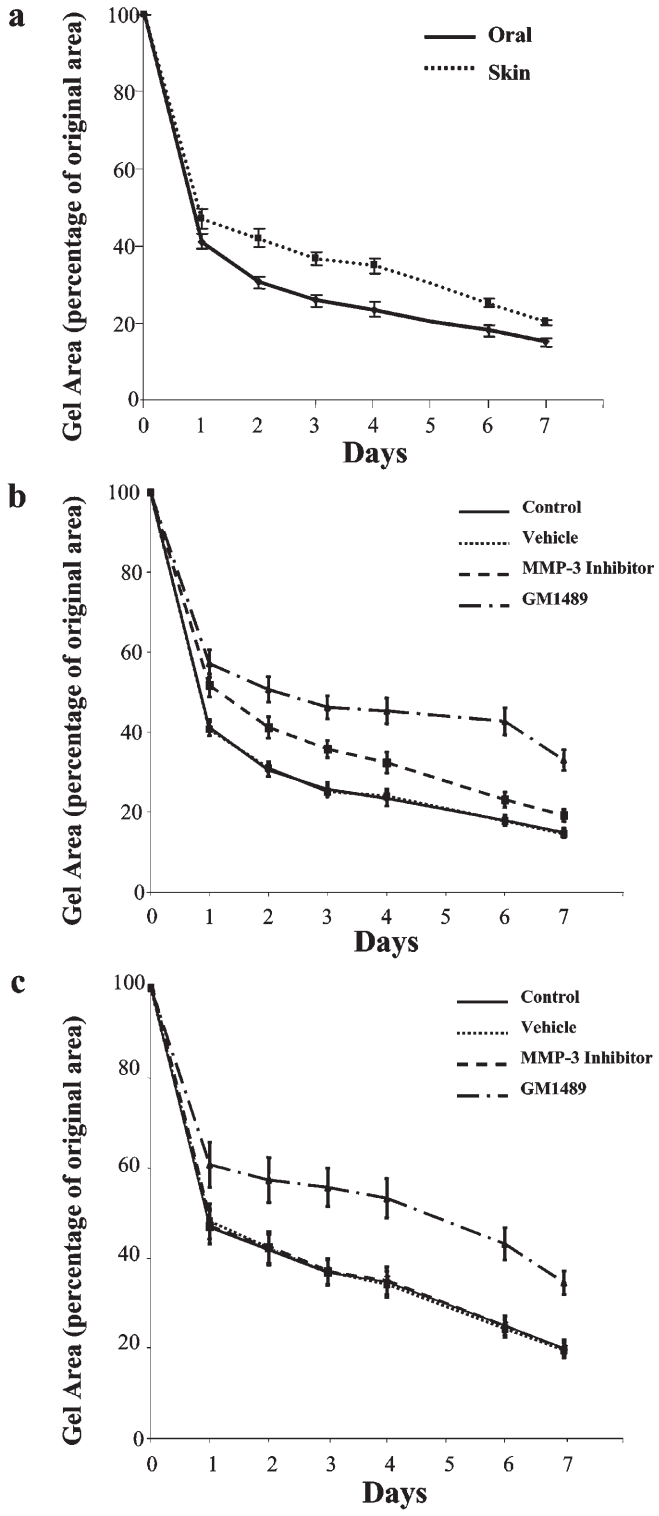

Figure 1. Kinetics of fibroblast-induced gel contraction. **(a)** Collagen gel contraction by paired oral and skin fibroblasts under control conditions. Oral fibroblasts induced significantly increased contraction compared with skin cells at all concentrations studied ($p < 0.05$). (b,c) Effect of total MMP (GM1489) and MMP-3 inhibition on collagen gel contraction kinetics by **(b)** oral and **(c)** skin fibroblasts over a seven-day study period. Both inhibitors significantly reduced gel contraction by oral fibroblasts at all time-points ($p < 0.05$); GM1489 significantly inhibited gel contraction by skin fibroblasts at all time-points ($p < 0.05$). Studies were carried out in triplicate on 3 paired skin and oral fibroblast populations. Data are expressed as mean ± SE.

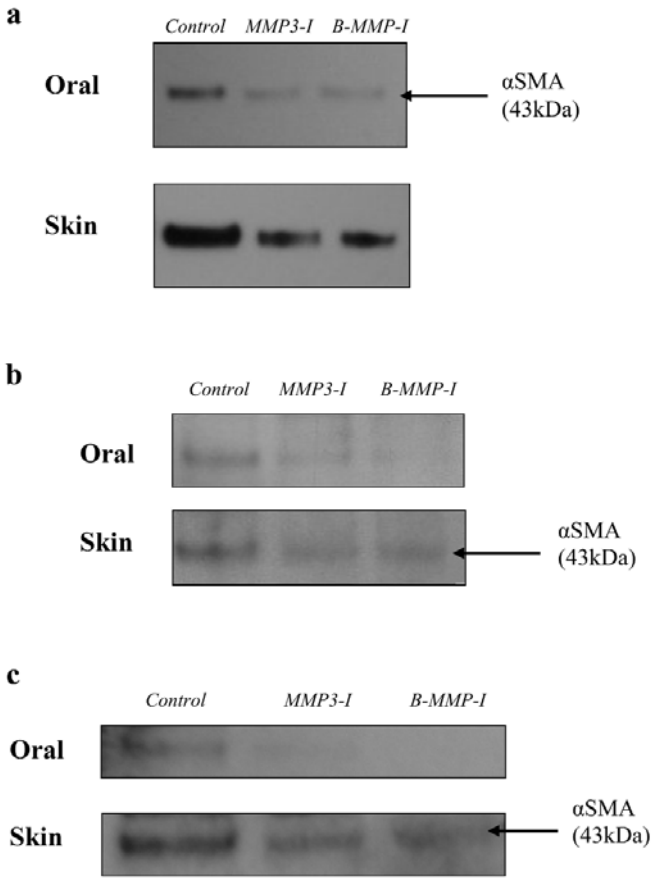

Figure 2. Effect of total MMP (B-MMP-I) and MMP-3 (MMP-3-I) inhibition on αSMA expression by oral and skin fibroblasts cultured **(a)** in monolayer, **(b)** in attached collagen gels, and **(c)** in floating collagen gels (representative of duplicate experiments for 3 paired oral and skin populations).

cultures, TGF-β_1 induced a greater increase in MMP-3 mRNA compared with TGF-β_3, while there was no marked difference in the level of stimulation seen in oral cultures (Fig. 3).

At both protein and activity levels, both growth factors again significantly stimulated MMP-3 expression ($p < 0.05$) (Fig. 4). The degree of effect on enzyme activity, normalized to control cultures, was significantly less than that seen at the protein level.

DISCUSSION

MMP levels are known to be markedly up-regulated both during wound healing *in vivo* and during fibroblast-induced gel contraction (Daniels *et al.*, 2003). In this study, inhibition of total MMP activity reduced collagen gel contraction by both skin and oral fibroblasts, supporting previous data indicating a role for MMPs in regulating the contraction process (Scott *et al.*, 1998; Rittie *et al.*, 1999; Daniels *et al.*, 2003; Mirastschijski *et al.*, 2004). This effect was not due to cytotoxicity: Indeed, fibroblast numbers were significantly increased in response to the MMP inhibitor (data not shown). Wound contraction *in vivo* is characterized by the differentiation of myofibroblasts within the granulation tissue. Myofibroblasts are contractile cells, sharing phenotypic characteristics of both fibroblasts and smooth-muscle cells,

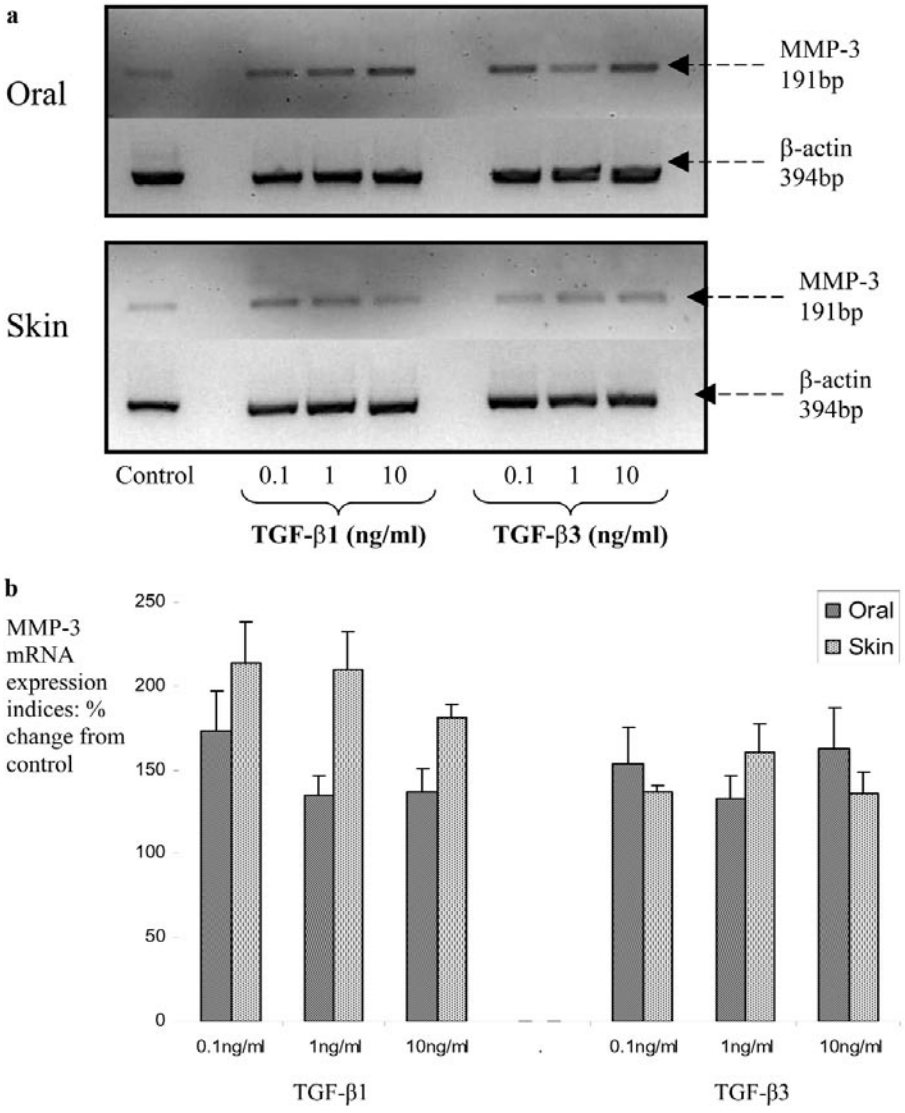

Figure 3. MMP-3 mRNA expression. **(a)** MMP-3 RT-PCR products in oral and skin fibroblasts relative to β-actin products for control and TGF-β-treated conditions. Representative of duplicate experiments for 3 paired oral and skin populations. Appropriate PCR-negative blanks and RT controls were used during all electrophoresis runs (data not shown). **(b)** Effects of TGF-β_1 and TGF-β_3 on MMP-3 mRNA expression indices. Data are expressed compared with control cultures following normalization of control values for each cell population to 100%. Treatment with both factors, at all concentrations, induced a statistically significant increase in MMP-3 expression ($p < 0.05$).

including the expression of αSMA. In this study, the inhibition of MMP activity in fibroblast cultures significantly reduced αSMA expression, indicating a reduction in myofibroblast differentiation, which in turn could account for the reduced levels of contraction. Myofibroblast differentiation is primarily induced by TGF-β_1 (Vaughan *et al.*, 2000). In a series of epithelial-fibroblast co-culture studies, Morishima *et al.* (2001) reported that epithelial cell promotion of myofibroblast differentiation involved the proteolytic cleavage of latent TGF-β binding protein (LTBP), and subsequent release of TGF-β from the extracellular matrix. These authors suggested that blockage of this proteinase-mediated release of TGF-β, thus inhibiting myofibroblast differentiation, provided a potential mechanism whereby inhibition of MMP activity would result in reduced wound contraction. Specifically, inhibition of the MMP-3-induced degradation of decorin and biglycan would also reduce TGF-β release from the extracellular matrix.

However, analysis of data reported in this study questions this proposed mechanism—indeed, questions the role played by myofibroblasts in gel contraction. First, both in monolayer and within collagen gels, skin fibroblasts expressed significantly higher levels of αSMA than did their oral counterparts, yet gel contraction rates were significantly lower in all skin cultures. Thus, the increased rate of gel contraction induced by oral fibroblasts was not due to a greater number of myofibroblasts in oral cultures. Second, MMP-3 inhibition significantly reduced the level of gel contraction in oral cultures, while having no effect in skin cultures. Again, this difference in response between cell types was not related to myofibroblast differentiation—inhibition of MMP-3 activity significantly reduced αSMA expression by both oral and skin fibroblasts. Furthermore, reduced contraction of skin wounds has been reported in MMP-3 knock-outs without affecting myofibroblast number at the wound site (Bullard *et al.*, 1999a,b). Analysis of the data, taken together, suggests that wound contraction and the effect of MMP-3 on the contraction process are not solely mediated through myofibroblast differentiation. Tractional forces generated by fibroblasts as they migrate into granulation tissue are also thought to regulate wound contraction (Ehrlich and Rajaratnam, 1990). Oral fibroblasts are known to show increased migration into and through collagen matrices compared with skin fibroblasts (Irwin *et al.*, 1994). MMPs, and specifically MMP-3, may be central to this preferential migration. Microscopic examination of fibroblast-populated collagen gels has shown areas of vacuolation next to fibroblasts, suggesting that proteolytic degradation of the matrix by fibroblast-derived factors stimulates cell migration. Thus, an MMP-3-induced accelerated migration of oral fibroblasts into granulation tissues, with the associated increase in early tractional forces, could account for both the accelerated contraction of oral wounds and the differential effect of the MMP-3 inhibitor on fibroblast-induced gel contraction by oral and skin cells.

The differential effect of the MMP-3 inhibitor does

indicate that the cellular mechanisms underlying wound contraction must differ between skin and oral mucosa. This is of particular interest, since oral wounds contract significantly faster than do skin wounds. This difference suggests, in effect, a more important role for MMP-3 in oral wounds. Significantly, we found increased MMP-3 expression by paired oral, compared with skin, fibroblasts at all levels studied, including protease activity. Increased MMP-2 activity in oral compared with skin fibroblast cultures has also been reported (Stephens *et al.*, 2001), while fetal skin fibroblasts also have been reported to show increased gelatinase activity (Gould *et al.*, 1997). The reduction in scarring seen in fetal and oral wounds may thus reflect an increase in MMP activity in these tissues.

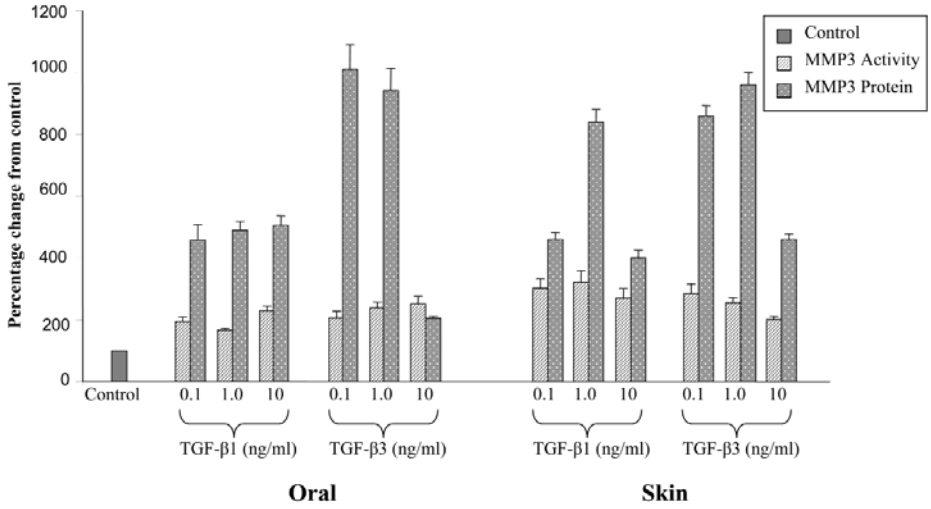

Figure 4. Effects of TGF-β_1 and TGF-β_3 on MMP-3 protein and activity levels in oral and skin fibroblast cultures. Each plot represents the mean ± SE of 3 paired fibroblast populations. Data are expressed as percentage change from control, where control values have been normalized to 100%. All culture conditions resulted in significantly increased protein and activity levels compared with controls ($P < 0.05$).

Shah *et al.* (1995) reported that application of TGF-β_3 or neutralizing antibodies to TGF-β_1 resulted in reduced scar formation in a rat model of wound healing. The mechanisms through which TGF-β_3 exerts anti-scarring effects on healing wounds remain to be fully elucidated. In fetal excisional wounds, both TGF-β_1 and TGF-β_3 had similar effects on myofibroblast differentiation and wound contraction (Lanning *et al.*, 2000). We have recently reported an equal stimulation of collagen gel contraction and αSMA production by fibroblasts in response to both TGF-β isoforms (Shannon *et al.*, 2006). In the present study, again, we found no significant difference between the two factors in their effect on MMP-3 expression levels. While TGF-β_3 clearly has anti-scarring effects, the cellular basis for these effects remains to be fully explained.

ACKNOWLEDGMENT

This study was funded by the Research and Development Office, Northern Ireland.

REFERENCES

Bullard KM, Lund L, Mudgett JS, Mellin TN, Hunt TK, Murphy B, *et al.* (1999a). Impaired wound contraction in stromelysin-1-deficient mice. *Ann Surg* 230:260-265.

Bullard KM, Mudgett J, Scheuenstuhl H, Hunt TK, Banda MJ (1999b). Stromelysin-1-deficient fibroblasts display impaired contraction in vitro. *J Surg Res* 84:31-34.

Daniels JT, Cambrey AD, Occleston NL, Garrett Q, Tarnuzzer RW, Schultz GS, *et al.* (2003). Matrix metalloproteinase inhibition modulates fibroblast-mediated matrix contraction and collagen production in vitro. *Invest Ophthalmol Vis Sci* 44:1104-1110.

Ehrlich HP, Rajaratnam JB (1990). Cell locomotion forces versus cell contraction forces for collagen lattice contraction: an in vitro model of wound contraction. *Tissue Cell* 22:407-417.

Gould LJ, Yager DR, Cohen IK (1997). In vitro analysis of fetal fibroblast collagenolytic activity. *Wound Repair Reg* 5:151-158.

Imai K, Hiramatsu A, Fukushima D, Pierschbacher MD, Okada Y (1997). Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor beta-1 release. *Biochem J* 322(Pt 3):809-814.

Irwin CR, Picardo M, Ellis I, Sloan P, Grey AM, McGurk M, *et al.* (1994). Inter- and intra-site heterogeneity in the expression of fetal-like characteristics by gingival fibroblasts: potential significance for wound healing. *J Cell Sci* 107(Pt 5):1333-1346.

Irwin CR, Myrillas T, Smyth M, Doogan J, Rice C, Schor SL (1998). Regulation of fibroblast-induced collagen gel contraction by interleukin-1beta. *J Oral Pathol Med* 27:255-259.

Knerer B, Formanek M, Temmel A, Martinek H, Schickinger B, Kornfehl J (1999). The role of fibroblasts from oropharyngeal mucosa in producing proinflammatory and mitogenic cytokines without prior stimulation. *Eur Arch Otorhinolaryngol* 256:266-270.

Kohama K, Nonaka K, Hosokawa R, Shum L, Ohishi M (2002). TGF-beta-3 promotes scarless repair of cleft lip in mouse fetuses. *J Dent Res* 81:688-694.

Krummel TM, Michna BA, Thomas BL, Sporn MB, Nelson JM, Salzberg AM, *et al.* (1988). Transforming growth factor-beta (TGF-beta) induces fibrosis in a wound healing model. *J Paediatr Surg* 23:647-652.

Lanning DA, Diegelmann RF, Yager DR, Wallace ML, Bagwell CE, Haynes JH (2000). Myofibroblast induction with transforming growth factor-beta1 and -beta3 in cutaneous fetal excisional wounds. *J Paediatr Surg* 35:183-188.

McKeown ST, Hyland PL, Locke M, Mackenzie IC, Irwin CR (2003). Keratinocyte growth factor and scatter factor expression by regionally defined oral fibroblasts. *Eur J Oral Sci* 111:42-50.

Mirastschijski U, Haaksma CJ, Tomasek JJ, Agren MS (2004). Matrix metalloproteinase inhibitor GM 6001 attenuates keratinocyte migration, contraction and myofibroblast formation in skin wounds. *Exp Cell Res* 299:465-475.

Morishima Y, Nomura A, Uchida Y, Noguchi Y, Sakamoto T, Ishii Y, *et al.* (2001). Triggering the induction of myofibroblast and

fibrogenesis by airway epithelial shedding. *Am J Respir Cell Mol Biol* 24:1-11.

Rittie L, Berton A, Monboisse JC, Hornebeck W, Gillery P (1999). Decreased contraction of glycated collagen lattices coincides with impaired matrix metalloproteinase production. *Biochem Biophys Res Commun* 264:488-492.

Schor SL (1980). Cell proliferation and migration on collagen substrata *in vitro*. *J Cell Sci* 41:159-175.

Scott KA, Wood EJ, Karran EH (1998). A matrix metalloproteinase inhibitor which prevents fibroblast-mediated collagen lattice contraction. *FEBS Lett* 441:137-140.

Shah M, Foreman DM, Ferguson MW (1995). Neutralisation of TGF-beta1 and TGF-beta2 or exogenous addition of TGF-beta3 to cutaneous rat wounds reduces scarring. *J Cell Sci* 108(Pt 3):985-1002.

Shannon DB, McKeown STW, Lundy FT, Irwin CR (2006). Phenotypic differences between oral and skin fibroblasts in wound contraction and growth factor expression. *Wound Rep Regen* 14:172-178.

Stephens P, Davies KJ, Al-Khateeb T, Shepherd JP, Thomas DW (1996). A comparison of the ability of intra-oral and extra-oral fibroblasts to stimulate extracellular matrix reorganization in a model of wound contraction. *J Dent Res* 75:1358-1364.

Stephens P, Davies KJ, Occleston N, Pleass RD, Kon C, Daniels J, *et al.* (2001). Skin and oral fibroblasts exhibit phenotypic differences in extracellular matrix reorganisation and matrix metalloproteinase activity. *Br J Dermatol* 144:229-237.

Szpaderska AM, Zuckerman JD, DiPietro LA (2003). Differential injury responses in the oral mucosal and cutaneous wounds. *J Dent Res* 82:621-626.

Vaughan MB, Howard EW, Tomasek JJ (2000). Transforming growth factor-beta1 promotes the morphological and functional differentiation of the myofibroblast. *Exp Cell Res* 257:180-189.

Whitby DJ, Ferguson MW (1991). The extracellular matrix of lip wounds in fetal, neonatal and adult mice. *Development* 112:651-668.