Modulation of fibroblast activity by collagens

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CHAPTER 5

Collagen types I, III and V differently modulate synthesis and activation of MMPs by periosteal, gingival and periodontal ligament fibroblasts

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Chapter 5

Abstract

In the present study we investigated whether the collagen types I, III and V modulate the production and activation of the matrix metalloproteinases (MMPs) gelatinase A (MMP-2), interstitial collagenase (MMP-1) and stromelysin (MMP-3) by fibroblasts obtained from three different soft connective tissues: gingiva, periodontal ligament (PDL) and periosteum.

Each collagen type rapidly promoted attachment and spreading of the fibroblast subsets. Irrespective of the collagen type used, the release of MMP-1 and MMP-3 decreased, whereas expression and activation of MMP-2 increased. These effects were more pronounced with the collagen types I and III than with collagen type V. Differences between the three fibroblast populations were found with respect to the level of MMP-expression and -activation. Compared to the PDL and periosteal cells, gingival fibroblasts expressed the highest level of MMP-1 and MMP-3. The collagen-induced stimulation of active MMP-2, on the other hand, was most pronounced in the PDL cells. Analysis of the amount of collagen broken down during culturing revealed that this was highest with the PDL fibroblasts.

The present study demonstrated that MMP profiles of gingival, PDL and periosteal, fibroblasts are up- (MMP-2) or down-regulated (MMP-1, -3) by the collagen types I, III and V, and that considerable differences exist in the level of expression of these enzymes among the different fibroblast populations.

Introduction

The fibroblast is the predominant cell type in soft connective tissues and is essential for tissue homeostasis. Although all fibroblasts fulfill a comparable role in that they synthesise and degrade extracellular matrix (ECM) constituents, numerous studies have shown that there is considerable heterogeneity among fibroblast populations. Phenotypic variations include differences in size, shape, attachment, proliferation and collagen synthesis, enzyme production, collagen turnover, and cytokine production, but also the reaction pattern towards cytokines, growth factors, hormones and bacterial products appears to depend on the tissue from which the fibroblast originates.

Several data indicate that extracellular matrix components like collagens and fibronectin strongly influence cell activity by modulating a variety of cellular functions such as gene-expression, protein synthesis, proliferation and cell migration. It may thus be hypothesised that the composition of the matrix somehow directs the functional properties and behaviour of
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cells. Thus, it is of considerable interest to determine to which extent different fibroblast populations respond differently to extracellular matrix components. In the present study we have investigated the behaviour of fibroblasts cultured gingiva, periodontal ligament (PDL) and periosteum. The cells were maintained on culture plates coated with the collagen types I, III, or V (15 µg/well). We analysed cellular attachment and spreading, actual breakdown of the collagen coating, and the production and activation of matrix metalloproteinases (MMP-1, -2 and -3). In addition, we investigated the influence of TGF-β on MMP-modulation. This growth factor has been shown to influence both the level of MMP-1 and MMP-2, in that it down-regulates the former\(^\text{18-21}\) and up-regulates the latter\(^\text{20}\).

**Materials and methods**

**Materials**

Iscove's Modified Dulbecco's Medium (IMDM), Dulbecco's Modified Eagle Medium (DMEM) penicillin, streptomycin, amphotericin were purchased from Gibco (Gibco Lab., Grand Island, NY). Bovine serum albumin (BSA, fraction V), sheep IgG and purified human type V collagen were from Sigma (Sigma Chemical Co., St. Louis, MO). L-[\(^{35}\)S]methionine for in vivo cell labelling was from Amersham (Amersham Life Science Ltd., Buckinghamshire, UK). Transforming growth factor-β (TGF-β) was purchased from BTI (Biochemical Technologies Inc., Stoughton, MA). Culture flasks and multi-well culture dishes were from Costar (Cambridge, MA), Euthesate from Apharmo (Apharmo B.V., Arnhem, NL), gelatin (from porcine skin, 250 bloom) from Fluka (Fluka Chemie, Buchs, Germany) and 40% acrylamide/Bis. 37.5:1 from Bio-Rad (Hercules, CA). Human leiomyoma purified collagen type I and III were a generous gift from P. Teeling (Dept. of Pathology, Academic Medical Center, Amsterdam). Drs. R. Hembry and G. Murphy (School of Biological Sciences, University of East Anglia, Norwich, UK) kindly donated a sheep polyclonal antibody (IgG fraction) to rabbit MMP-1. All other reagents were of analytical grade.

**Cell isolation and culture**

One week old Chinchilla rabbits were killed with Euthesate and decapitated. Mandibular molars were carefully dissected, pulp and crown were removed and the roots were cut into smaller pieces. Free and attached gingiva was removed from the mandibular molars; periosteum was removed from the calvaria and cut into smaller pieces. All explants were washed in IMDM.
supplemented with 10% FCS and 10 x antibiotics: amphotericin (2.5 mg/ml), streptomycin (1 mg/ml) and penicillin (1000 U/ml), placed in 6 well culture plates and incubated in IMDM supplemented with 10% FCS and 10 x antibiotics in a humidified atmosphere containing 5% CO₂ in air at 37 °C for one day after which the medium was changed with growth medium (IMDM supplemented with 10% FCS and 1 x antibiotics). The growth medium was changed twice a week until the cells, grown out of the tissue explants, were confluent. The cells were then trypsinised with 0.1% ethylenediaminetetraacetic acid (EDTA) and 0.25% trypsin (pH 7.3), transferred to 25 cm² culture flasks and designated as 'passage one' (P1). The cells of P1 and subsequent passages showed typical fibroblastic characteristics with trapezoid morphology when cultured confluent. For all experiments, cultures between P3 and P8 were used. Four days prior to the onset of the experiments the medium was changed with IMDM supplemented with 5% FCS and antibiotics, which was changed after three days with IMDM supplemented with 4 mg/ml BSA and antibiotics. The cells (3 x 10⁴ cells/well) were incubated with 300 μl IMDM (+ 4 mg/ml BSA) in collagen-coated 24-well culture plates and cultured in a humidified atmosphere containing 5% CO₂ in air at 37 °C.

Coating of culture plates

Native human type I, III and V collagen were characterised as purified collagens by SDS polyacrylamide gel electrophoreses analysis. The collagens were dissolved in 0.01 M acetic acid in a concentration of 50 μg/ml. 24 well culture plates were coated by incubating the wells with 500 μl diluted collagen. The culture plates were air-dried in a laminar flow cabinet at ambient temperature and subsequently sterilised by UV light for 20 min. The wells were washed with HBSS before the cells were seeded. In preliminary experiments the amount of collagen coated to the plates (after air-drying and washing) was established by measuring the hydroxyproline content in the wells, which was performed by using a microassay developed by Creemers et al. This approach showed that equal amounts of each of the three collagen types attached to the cultured plates and that an initial coating of 50 μg/ml (25 μg/well) collagen resulted in a final coating of 15 μg/well.

Cell attachment and cell extraction

Cells were cultured in non-coated or collagen-coated wells. For the biochemical analyses conditioned media were collected after a culture period of 48 h and attached cells were extracted in 150 μl extraction buffer (10 mM sodium cacodylate, 1 M NaCl, 0.01% Triton X-100 and 1
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raM ZnCl₂, pH 6.0; Eeckhout et al.²³) or in 150 µl DNA-lysis buffer (50 mM Tris, 150 mM NaCl, 1% Triton-X-100, pH 10) and frozen at −20 °C. Both conditioned media and cell extracts were analysed for the activity and protein level of MMP-1, -2 and -3.

To establish specific cell-collagen attachment properties, wells were (after coating, air-drying, sterilisation and washing) incubated for 30 min with a BSA-containing buffer (50 mM Tris, 110 mM NaCl, 5 mM CaCl₂, 0.1 mM PMSF, 1% BSA). Cells (3 x 10⁴ cells/well) were then seeded in 300 µl IMDM supplemented with BSA (4 mg/ml). After a culture period of 1.5, 3, 6, 24 or 48 h the cells were washed with PBS and fixed and stained with Giemsa Azur-Eosin-Methyleneblau. Micrographs were taken from two predetermined fields and printed at a final magnification of 750 x, coded and randomised. Counting all cells on each micrograph assessed the number of attached cells. The results shown are representative for all experiments.

DNA assay

The amount of DNA was assessed fluorometrically using a Perkin Elmer spectrofluorometer (extinction 400 nm, emission 505 nm) according to the method of Janakidevi et al.²⁴.

MMP-1, -2 and -3 analysis

Western blotting was used to analyse MMP-1. To this end aliquots of conditioned medium (15 µl) or cell extracts (equalised with respect to their DNA-level) were diluted 1:1 with sample buffer (0.1 M Tris-HCl, 4% SDS, 20% glycerol, 0.005% BFB, 10 mM EDTA) and electrophoresed through a 10% polyacrylamide gel. Following electrophoresis, proteins were transferred onto a nitrocellulose membrane (0.45 µm, Bio-Rad) with a Trans-Blot cell system (Bio-Rad Laboratories). The blots were preincubated for 1 h with PBS, 0.05% Tween 20 and 5% Protifar (Nutricia, The Netherlands), followed by an incubation with sheep anti-rabbit MMP-1 (80 µg/ml, 5% Protifar/PBS) for 2 h at 37 °C. The blots were washed and incubated with a peroxidase-conjugated donkey anti-sheep IgG (20 µg/ml, 5% Protifar/PBS) for 60 min. After washing (in 5% Protifar/PBS) the blots were stained with H₂O₂ and 3,5,3',5'-tetramethylbenzidine (Pierce, Rockford, IL) as chromogen.

Gelatin zymography was performed to analyse gelatinolytic activity. To this end aliquots of conditioned medium (5 µl) and cell supernatant (equalised according to their DNA-content) were diluted 1:1 with sample buffer (0.1 M Tris-HCl, 4% SDS, 20% glycerol, 0.005% BFB, 10 mM EDTA) and electrophoresed through a 10% polyacrylamide gel containing 2% gelatin.
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After electrophoresis, gels were washed for 15 min in a buffer containing 50 mM Tris-HCl, 2.5% Triton X-100 and 5 mM CaCl$_2$ (pH 7.5) and transferred to incubation buffer consisting of 50 mM Tris-HCl, 1% Triton X-100 and 5 mM CaCl$_2$. After an overnight incubation at 37 °C, the gel was stained for 1 h at 60°C with 0.1% Coomassie Brilliant Blue in 10% acetic acid, 50% methanol and destained with 7% acetic acid, 5% methanol. To ascertain that these bands reflected gelatinase activity, a number of samples was preincubated with an activity blocking anti-gelatinase antibody which resulted in the disappearance of the lytic bands.

Densitometric analyses of the gelatinolytic bands were performed using a Quantimet 500 (Leica, Cambridge Ltd., UK) image analysis system and the Qwin v02.00B software program. In preliminary experiments we established that a linear relationship exists from 0 up to 3 mU recombinant MMP-2. The zymographic analyses were performed within this range.

For the analysis of MMP-3 activity conditioned media or cell extracts were analysed by using the fluorogenic substrate TNO003 according to the method of Beekman et al.$^{25}$.

Isotopic labelling

For these experiments fibroblasts (3 x 10$^4$ cells/well) were cultured in 300 µl DMEM (+ 4 mg/ml BSA) in 24 well culture plates coated with a final concentration of 15 µg/well collagen in a humidified atmosphere containing 5% CO$_2$ in air at 37 °C. After an initial culture period of 3 h, the medium was changed with 300 µl DMEM without cysteine/methionine and incubated for 1.5 h. Metabolic protein labelling was performed by the inclusion of 10 µCi/ml [-$^{35}$S]-methionine in the medium. The culture medium was harvested after a period of 20 h and the cells were washed with PBS and subsequently extracted with extraction buffer (see Cell extraction). Total incorporation of radiolabel was analysed by TCA precipitation of the cell extracts and the radioactivity was counted on a LKB liquid scintillation counter. The de novo synthesis of MMP-2 was determined by immunoprecipitation, which was performed with antibodies to gelatinases bound to CNBr-activated Sepharose® 4B (Pharmacia, Uppsala, Sweden) beads. After precipitation and washing with PBS, a part of the MMP-2-antibody-beads complex was counted on an LKB liquid scintillation counter and part of the complex was electrophoresed through a 10% polyacrylamide gel. To this end the samples were equalised to their protein-level and diluted 1:1 with sample buffer (see Western blotting). Following electrophoresis, using a phosphor-imager (Molecular Dynamics, Image QuantTM, Sunnyvale, CA) performed autoradiography.

Hydroxyproline assay

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The amount of hydroxyproline released in medium was established and used as a parameter of collagen degradation. Preliminary studies using α,α-dipyridyl (which inhibits the synthesis of collagen) demonstrated that, under the culture conditions employed, the hydroxyproline content in the culture media reflected degradation of collagen and not release of newly synthesised collagen. Using a microassay developed by Creemers et al., hydroxyproline analyses were established. In a series of experiments it was demonstrated that MMPs were required for the degradation of the coated collagens in our model, since inhibition of this class of enzymes by using the selective MMP-inhibitor CT1166 reduced the hydroxyproline release for at least 50%.

Statistical analysis

The attachment, densitometric, enzymatic and hydroxyproline data were presented as mean ± SD. Statistical analyses were performed by a Mann-Whitney test (densitometric/enzymatic/hydroxyproline data) and by a Bonferroni multiple comparison test (attachment data). P<0.05 (two-tailed) was assumed to indicate statistical significance.

Results

I. Cell attachment

Fibroblasts of the three populations cultured on plates not coated with collagen did hardly attach and spread after 24 h of culturing (Fig. 1A). On collagen-coated plates, however, they attached quite rapidly. A maximal attachment of the cells was already found within 90 min (Fig. 2). The cells spread within 24 h on collagen (Fig. 1B-D). All three collagen types had a similar attachment- and spreading-promoting effect on the different fibroblast populations.

II. MMP-2

Gelatin zymographic analysis of the extracted periosteal, gingival and PDL fibroblasts, cultured for 48 h, revealed the presence of inactive and active MMP-2 (Fig. 3). When cultured on collagen coatings all three populations expressed a higher level of both the inactive and active form of MMP-2. There were, however, differences with respect to the ratio inactive/active MMP-2 among the three cell populations. Compared to periosteal and gingival fibroblasts, PDL fibroblasts revealed the highest ratio active to inactive MMP-2 when cultured on collagen.
Fig. 1. Light microscopy showing periosteal (A, B), gingival (C) and PDL (D) fibroblasts cultured for 24 h in wells coated (B-D) or not (A) with collagen type V. Note spreading of cells seeded on collagen. Giemsas Azur-Eosin-Methylblau. x750.
Fig. 2. The number of periosteal, gingival and PDL cells attached to type V collagen after a 1.5, 3, 6, or 24 h culture period. The wells were preincubated with a buffer containing 1% BSA to block aspecific cell attachment. No significant differences were found among the attachment properties of the cells: neither between the various populations nor for the various collagens. Data are expressed as mean (± SD) number of attached cells per area (n = 4). One area is the sum of two microscopic fields.

Fig. 3. Gelatin zymogram of cell extracts of periosteal (PER), gingival (GF) and PDL (PDL) fibroblasts that were cultured for 48 h in non-coated (control) or collagen-coated (type III and V; 15 μg/well) wells. The bands at 72 and 66 kDa represent the inactive and the active form of MMP-2, respectively. Note that the amount of active MMP-2 had increased when the cells were cultured on collagen. This effect was found for all three collagen types.
Densitometric analyses of the gelatin zymograms, demonstrated that the collagen-induced increase of MMP-2 (both inactive plus active MMP-2) was statistically significant when the cells were cultured on type I and type III collagen (Fig. 4). Under these conditions the level of MMP-2 proved to be 3 to 5 times higher than the amount produced by cells cultured on plastic. Although also in the presence of collagen type V a higher level of MMP-2 was apparent, this increase was not statistically significant.

![Graph](image)

**Fig. 4. Densitometric analysis of the MMP-2 bands of zymograms.** Extracts of fibroblasts that were cultured for 48 h in wells coated with collagen type I- or type III collagen (15 μg/well) revealed a significant increase of MMP-2 expression in comparison with those cells that were cultured in non-coated (control) wells. Cells cultured on type V collagen did not significantly enhance their MMP-2 production. Values are expressed as the ratio experimental versus control (E/C ratio) of 8 samples per condition. *: p<0.05; **: p<0.01 versus control.

![Graph](image)

**Fig. 5. Gingival and PDL fibroblasts were cultured for 24 h in wells coated with type I, III or V collagen (15 μg/well). [35S]-methionine incorporation was determined for the last 17 h of culturing. MMP-2 synthesis increased in comparison with cells cultured in non-coated (control) wells. Values are expressed as the mean ratio experimental versus control (mean E/C ratio ± SD) of 2 experiments.**
The de novo synthesis of MMP-2 was determined in gingival and PDL fibroblasts by $[^{35}\text{S}]$-methionine incorporation and immunoprecipitation with anti-MMP-2/MMP-9 antibodies. This approach demonstrated an increase of newly synthesised MMP-2 within 24 h when cultured on collagen (Fig. 5). The radiolabel proved to be MMP-2 since autoradiographic analysis revealed one band of 72 kDa (not shown).

**III. MMP-1 and MMP-3**

Western blot analysis of MMP-1 in media conditioned by periosteal, gingival and PDL cells cultured on not-coated plates showed the presence of three immunoreactive fractions of MMP-1: an inactive (51 kDa), an intermediate (43 kDa) and an activated fraction (41 kDa) (Fig. 6A and B). The highest level of MMP-1 was found in media obtained from gingival cells (Fig. 6A). The three collagen types induced a decrease of this MMP in each of the three fibroblast populations. The down-regulating effect, however, was less pronounced for cells cultured on type V compared to the other collagens (Fig. 6B). In line with these findings were collagenolytic data.
assessed with a $[^{14}C]$-fibrillar collagen assay. The total amount of collagenolytic activity was significantly lower in media from cells cultured on the collagens, and again media of fibroblasts kept on type I and type III collagen revealed a more pronounced decrease of collagenolytic activity than media obtained from cells cultured on type V collagen.

A sensitive activity assay using the fluorogenic MMP-3 specific substrate showed that collagen coatings were associated with a decreased enzyme activity (Fig. 7). Media conditioned by gingival fibroblasts revealed the highest MMP-3 activity.

![MMP-3 activity in the culture media of fibroblasts maintained for 48 h in non-coated (control) or collagen-coated wells. Note that the collagens were associated with a decreased activity of MMP-3 in gingival and PDL fibroblasts. MMP-3 activity was higher for gingival cells than for periosteal and PDL fibroblasts (non-coated wells). The data are expressed as mean (± SD) of 4 experiments. *: p < 0.05, **: p < 0.01 versus control.](image)

**Fig. 7.** MMP-3 activity in the culture media of fibroblasts maintained for 48 h in non-coated (control) or collagen-coated wells. Note that the collagens were associated with a decreased activity of MMP-3 in gingival and PDL fibroblasts. MMP-3 activity was higher for gingival cells than for periosteal and PDL fibroblasts (non-coated wells). The data are expressed as mean (± SD) of 4 experiments. *: p < 0.05, **: p < 0.01 versus control.

### IV. Effects of TGF-β

Gelatin zymographic analysis of media conditioned by gingival fibroblasts revealed the presence of inactive and active MMP-2 and inactive MMP-9 (at 92 kDa). Addition of 10 ng/ml TGF-β increased the level of both enzymes in cells that were cultured on plastic (Fig. 8A), whereas the
growth factor decreased the level of MMP-1 in cells cultured on plastic or collagen (Fig. 8B). These effects were observed with all three populations.

V. Collagen breakdown

In order to investigate whether different levels of digested collagen reflected differences in MMP-profiles, the amount of collagen released in the medium was assessed. To this end the amount of hydroxyproline in the media was determined after a 48 h culture period. Comparison of the three cell types revealed that the highest level of this imino acid was found with the PDL cells (Fig. 9). The highest levels of degraded collagen were found in media obtained from cells cultured on type I collagen; digestion of type V collagen was lowest, irrespective of the cell type analysed (Table I).
Discussion

The present data demonstrate for the first time that the production of several MMPs may differ considerably among distinct populations of fibroblasts. The highest level and/or activity of MMP-1 and MMP-3 was expressed by gingival fibroblasts. Active MMP-2, on the other hand, was most markedly produced by PDL fibroblasts when cultured on collagen. These data indicate that tissue-related differences exist in the release of these enzymes and may suggest site-specific differences in MMP-mediated remodelling of extracellular matrices. Recent data indicate that MMP-2 activity is essential for remodelling and turnover of collagens under physiological conditions whereas MMP-1 activity is probably less important under these conditions. The findings of the present study are in line with the alleged role of MMP-2 since high levels of its active fraction were found in fibroblasts that were isolated from the PDL, a tissue characterised by a rapid turnover of collagen. In this respect it is also of interest to note that the digestion of collagen coated to the culture plates was higher with the PDL fibroblasts than with the gingival (or periosteal) cells and that the digestion correlated positively with the level of active MMP-2. The latter data strongly suggest that also under these conditions MMP-2 was more important for collagen digestion than MMP-1 or MMP-3. Despite relatively high levels of active MMP-1 and -3 in the gingival cultures, this did not reflect in the amount of digested collagen. However, since we have not analysed the collagen-induced modulation of the tissue inhibitors of
Table I

Breakdown of collagen coating as assessed by hydroxyproline (HP) content in the media

<table>
<thead>
<tr>
<th>Collagen type</th>
<th>Collagen coating</th>
<th>HP content</th>
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<tbody>
<tr>
<td></td>
<td>% HP periosteal cells</td>
<td>% HP gingival cells</td>
</tr>
<tr>
<td>I</td>
<td>15±5*/; 11±7*/</td>
<td>35±19*/; 19±12*/</td>
</tr>
<tr>
<td></td>
<td>33±7*; 3±2*</td>
<td>52±4*; 3±1*; 42±4*;</td>
</tr>
<tr>
<td></td>
<td>11±3*; 14±4*</td>
<td>98±37*; 48±8*</td>
</tr>
<tr>
<td>III</td>
<td>3±2*; 22±11*;</td>
<td>3±2*; 47±9*; 6±5*;</td>
</tr>
<tr>
<td></td>
<td>8±8*;</td>
<td>2±5*; 5±3*</td>
</tr>
<tr>
<td>V</td>
<td>3±1*; 8±6*</td>
<td>10±5*; 3±4*</td>
</tr>
</tbody>
</table>

The data are given as percentage (mean±SD) of at least 6 samples per condition of 7 experiments (A-G). The fibroblasts were cultured for 48 h on type I, III, or V collagen. The hydroxyproline content in the conditioned media in several experiments were below detection limit of the assay, particularly for type V collagen. Note that in 6 out of 7 experiments PDL cells revealed the highest level of hydroxyproline in their culture media.

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Metalloproteinases (TIMPs), inhibitors that proved to be produced by PDL fibroblasts, or other proteolytic enzymes in these cultures, further studies are needed to elucidate this in more detail.

Not only differences between the various fibroblast populations were found when cultured on plastic, they also responded differently to the extracellular matrix on which they were seeded (type I, III or V). In general, MMP-1 and -3 were down-regulated by the collagen coatings. MMP-2, however, was up-regulated. Not only did the total amount of this enzyme increase, also the active fraction was higher. This latter effect was particularly obvious for cells derived from PDL.

Type I and III collagen proved to have comparable effects on the expression of MMPs, whereas type V collagen had a more moderate effect resulting in an intermediate amount of enzyme. These data not only indicate that collagens strongly affect production of collagenolytic enzymes, but also that the ratio between the different collagen types in the matrix is important.

The present data indicate differences in the reaction patterns of the different fibroblast populations. In line with this are data presented by others who showed that PDL and periosteal fibroblasts on one hand and gingival fibroblasts on the other differ in their expression of the membrane-bound enzyme alkaline phosphatase. The former cells express high levels of this...
enzyme whereas gingival fibroblasts produce significantly less\(^5,31\). Differences were also reported with respect to the expression of MMP-2, both \textit{in vitro} (this study) and \textit{in vivo}\(^28\).

One of the few growth factors known to influence the level of MMP-1 and MMP-2 is TGF-\(\beta\). In the present study we found, in line with data presented by others, an up-regulation of MMP-2\(^20\) and a down-regulation of MMP-1\(^18,21\). The up-regulation of MMP-2 was most pronounced when fibroblasts were kept on plastic. When the cells were cultured on collagen, however, TGF-\(\beta\)-induced effects were largely prevented, especially when the cells were cultured on type V collagen. Based on these results we suggest that cells attaching to collagen become less susceptible for TGF-\(\beta\). Support for this assumption was the finding that the increased synthesis of gelatinases and matrix components by TGF-\(\beta\) was reduced in fibroblasts that were kept in a collagenous gel\(^22\). Furthermore, the finding that collagen has the capacity to counteract the TGF-\(\beta\)-effect is in line with recent findings in our group, showing that this growth factor does not affect the production of MMP-2 by periostal fibroblasts that are still embedded in their tissue environment. Isolated periostal fibroblasts, on the other hand, appeared to respond rapidly by secreting higher levels of MMP-2\(^33\). Although these observations suggest that the collagens somehow prevent a reaction toward TGF-\(\beta\), several data seem to indicate that this applies for MMP-2 but not for MMP-1. Down-regulation of MMP-1 under the influence of TGF-\(\beta\) was not only found with isolated cells seeded on plastic\(^{first study,20,21}\) but also with cells in their normal tissue environment\(^{18,19}\). Taken together these observations would seem to suggest that cells express different TGF-\(\beta\)-receptors: receptors that are involved in MMP-2 production interact with collagen and those that participate in MMP-1 modulation do not interact with collagen.

In conclusion, the data presented in this study indicate that fibroblasts obtained from different connective tissues express different levels of MMPs and that the collagen types I, III and V greatly affect the expression of these enzymes. Our data strongly suggest that the composition of the extracellular matrix plays an essential role in the modulation of enzyme production and that this modulation is perhaps tissue-specific.

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27. Kerkvliet, E. H. M., Jansen, D. C., Beertsen, W., and Everts, V. Collagen type I, III and V differently modulate production and activation of matrix metalloproteinase 1 (MMP-1) and matrix metalloproteinase 2 (MMP-2) by periodontal ligament fibroblasts. This thesis; Chapter 4.
CHAPTER 6

Synthesis of DNA by fibroblasts is modulated by the collagen types I, III and V.