Expression Changes of Gelatinases in Human Osteoarthritic Knee and the Arthroscopic Débridement

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Running title: *GELATINASE IN HUMAN KNEE OA & ARTHROSCOPY*

ABSTRACT

Purpose: To quantify the expression changes of gelatinase-A & B (matrix metalloproteinase-2 & 9, MMP-2 & 9) in a series of chondral, meniscal, and synovial cultures of knee osteoarthritis (OA) for investigation of the possible roles of the cartilage, menisci and synovia and the efficacy of arthroscopic débridement. **Type of Study:** Case series. **Methods:** In consecutive 43 patients with knee OA to receive arthroscopic débridement, we examined the amount of MMP-2 & 9 in a series of chondral, meniscal and synovial cultures. We also compared the gene expressions of MMP-2 & 9 and membrane-type 1 MMP (MT1-MMP) in the chondral, meniscal, and synovial cultures using reverse transcription polymerase chain reaction (RT-PCR). **Results:** Latent & activated forms of MMP-2 were produced in all series of chondral, meniscal, and synovial cultures and their activities in lesional cultures were significantly higher than that in paralesional ones ($P < .001$). Moreover, the latent form of MMP-9 (proMMP-9) appeared in 29 of 37 series of synovial cultures and in 13 of 40 series of meniscal cultures. In meniscal cultures after 24 hours incubation and synovial cultures after 3 and 24 hours incubation, the activity of proMMP-9 in lesional cultures was significantly higher than that in paralesional ones ($P < .001$). The activated form of MMP-9 appeared in 10 of 37 series of synovial cultures and its activity in lesional cultures was significantly higher than that in paralesional ones (*P*

< .05). Furthermore, MMP-2, -9 and MT1-MMP mRNA levels of lesional areas also showed the increased expression in RT-PCR. **Conclusions:** Our data confirm that tissue repair of OA is ascribable to enzymic digestion of the ECM *ex vivo*. When technically appropriate, arthroscopic débridement for the pathologic lesions of OA, such as meniscal tears, chondral lesions, and hypertrophic villi, may be beneficial to the process of early cases. Still, it should be carefully studied for its overall effect and mechanism *in vivo*.

Key words: Gelatinase—Knee—OA—Arthroscopy.

INTRODUCTION

Osteoarthritis (OA) is the most prevalent joint disease in humans and is characterized by degenerative changes due to a gradual loss of extracellular cartilage matrix.[1] Matrix metalloproteinases (MMPs) are a family of proteinases that together can degrade all extracellular matrix (ECM) components. Their activity is controlled by their inhibitors, known as tissue inhibitors of metalloproteinases (TIMPs). Both MMPs and TIMPs are considered important for the chondrolytic processes that contribute to the degenerative changes in OA cartilage.[2-4]

Type IV collagenases (gelatinases) are members of the family of MMPs and are thought to play an important role in degradation of extracellular components. The gelatinase subclass can be divided into gelatinase-A (MMP-2) and gelatinase-B (MMP-9) and they are capable of degrading types IV and V collagens, elastin, and gelatin.[5-7] The MMPs are secreted as latent precursor enzymes and can be activated by limited proteolysis, which results in a loss of molecular weight of about 10 kDa. Moreover, membrane-type 1 MMP (MT1-MMP) is able to degrade different ECM components like fibronectin, vitronectin, laminin, and gelatin and also capable of activating the latent form of MMP-2 and MMP-13 (proMMP-2 and proMMP-13) and thereby contributing to matrix degradation.[8-10]

Thus far, the presence of latent form of MMP-2 & 9 was demonstrated in synovial fluid of patients with arthritis.[11,12] Recently, the presence of the latent and activated forms of MMP-9 in synovial fluid samples aspirated from the joints of patients with arthritis reflected the inflammatory condition of the joints.[13] A positive correlation between the production of MMP-9 and a rapid destruction of the hip joint with OA was found.[14] The zymographic profile and MMP expression can be a useful diagnostic adjunct in patients with OA, providing precise information on the condition of the articular cartilage and the breakdown of its matrix.[15,16] However, little is known regarding the synthesis and secretion of MMPs in human OA joint *in vivo*. Moreover, there is little available information regarding the effectiveness of

arthroscopic débridement for the treatment of knee OA and they are controversial.[17-21] The aim of this study was to analyze the expression changes of gelatinases in a series of chondral, meniscal, and synovial cultures of the knee joint with OA for investigation of the possible roles of the cartilage, menisci, and synovia and making conjectures on the efficacy of arthroscopic débridement for knee OA.

MATERIALS AND METHODS

Patients

Table I shows the clinical data for the consecutive 43 patients with 58 knees of OA that fulfilled the ACR criteria in half a year.[22] The patients were all grade II-III in anteroposterior weight-bearing and lateral radiographs of knee according to the Kellgren and Lawrence grading scale for the medial, lateral and patellofemoral compartments.[23] They received arthroscopic débridement that included partial meniscectomy (40), chondral shaving (58), subchondral drilling (1), osteophyte excision (41), loose body removal (1), medial patellar plica excision (12), limited synovectomy (37), and lateral retinacular release (43) using standard portals as well as medial plication (1) and modified Elmslie-Trillat procedure (3) for the mechanical problems of meniscus and cartilage including patellofemoral joint at our hospital by the same author. All patients gave informed consent for their effusion samples and surgical specimens to be studied.

Materials

If the knee had effusions, arthrocentesis was performed by puncture with a 19-gauge needle into the suprapatellar pouch from anterolateral approach before arthroscopy. Pre-operative blood samples and heparinized effusion samples were centrifuged for 10 minutes at 3000 rpm, and aliquots were prepared for gelatin zymography.

Tissue Cultures

Using the Arthroforce® III forceps (Karl Storz, Germany), more than 100 mg specimens of diseased cartilage (lesional) and near normal cartilage (paralesional) were obtained separately during arthroscopic chondral shaving for chondral ulcer and fibrillation of femoral condyles, tibial plateaus, and patella (Fig 1). If the knee had meniscal degenerative tear, more than 100 mg specimens of diseased meniscus (lesional) and near normal meniscus (paralesional) were obtained separately during arthroscopic partial meniscectomy. If the knee had effusions, arthroscopic limited synovectomy was performed. More than 100 mg specimens of hypertrophic (lesional)

and paralesional villi were obtained separately.

Both of the lesional and paralesional specimens were weighted equally 100 mg, then transferred into 24 tissue culture flasks respectively and incubated at 37°C under a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% penicillin-streptomycin (10,000 U/ml) and 10 mg/ml streptomycin. Individual culture media were obtained at various times (3 and 24 hours) and prepared for gelatin zymography.

Gelatin Zymography

Gelatin zymography was performed according to a protocol developed by Kleiner *et al*.[24] Of each effusion sample or culture medium, 20 μl of effusions or media containing 10 μg of total protein was loaded onto a precast sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) containing 0.1% gelatin. After electrophoresis, gels were processed as described by Chou *et al*.[25] Nonstaining bands representing the activities of latent and activated forms of MMP-2 & 9 were quantitatively measured by spot density measurement using a digital imaging analysis system (Alpha Innotech, Mt. Prospect, IL). Results were expressed as integrated density value (% of 100%). IDV is the sum of all the pixel values after background correction, i.e., IDV = Σ(each pixel value – background value).

Preparation of RNA and RT-PCR

Total RNA was extracted from meniscal, chondral and synovial tissue at the 24 hours after meniscal, chondral and synovial cultures following the protocol described by Chormczynski and Sacchi.[26] Procedures of cDNA synthesis and PCR amplification were described by Kuo *et al*.[27] The sequence of primers was as follows: sense of MMP-2, 5'-GGCCCTGTCACTCCTGAGAT-3'; antisense of MMP-2, 5'-GGCATCCAGGTTATCGGGGA-3'; sense of MMP-9, 5'-CAACATCACCTATTGGATCC-3'; antisense of MMP-9, 5'-CGGGTGTAGAGTCTCTCGCT-3'; sense of MT1-MMP, 5'-CTCCTGCTCCCCCTGCTCACG-3'; antisense of MT1-MMP, 5'-CTCACCCCCATAAAGTTGCTG-3; sense of GAPDH, 5'-CGGAGTCAACGGATTTGGTCGTAT-3'; antisense of GAPDH, 5'-AGCCTTCTCCATGGTTGGTGAAGAC-3'.

Statistical Analysis

The linear regression analysis was employed for the correlation of the activity of proMMP-2 between effusions and sera. The nonparametric Wilcoxon signed-rank test was performed for comparisons of paired measures within groups (lesional vs.

paralesional). Statistical significance was set at $P < .05$.

RESULTS

Gelatinase Activity in Sera and Effusions

There were 43 serum samples and 39 effusion samples aspirated before arthroscopy. The latent form of MMP-2 & 9 was presented in all sera and only proMMP-2 appeared in all effusions (Table 1). Actually, the proMMP-9 could be detected as a very small white zone in all effusions those were not diluted. The comparison of the activity of proMMP-2 between sera and effusions revealed that there was no correlation (r = .016, $P = .951$).

Expression Changes of Gelatinase Activity in Relation to Serial Tissue Cultures

After 24 hours incubation of lesional and paralesional chondral cultures of femoral condyles, tibial plateaus, and patella, 58 paired chondral samples obtained at various times (3 and 24 hours) were quantified for the MMP-2 & 9 activities by gelatin zymography (Fig 2). The activities of latent and activated forms of MMP-2 in lesional cultures were significantly higher than that in paralesional ones ($P < .001$).

After 24 hour incubation of lesional and paralesional meniscal cultures, 40 paired samples obtained at various times (3 and 24 hours) were quantified for the MMP-2 & 9 activities by gelatin zymography. Latent & activated forms of MMP-2 were produced in all and proMMP-9 appeared in 13 meniscal cultures (Fig 3). The activities of the latent & activated forms of MMP-2 in lesional cultures were significantly higher than that in paralesional ones ($P < .001$). The activity of proMMP-9 in lesional cultures was significantly higher than that in paralesional ones after 24 hours incubation ($P < .001$). Nevertheless, there were no significant differences between lesional and paralesional cultures after 3 hours incubation ($P = .109$).

After 24 hours incubation of lesional and paralesional synovial cultures, 37 paired samples obtained at various times (3 and 24 hours) were quantified for the MMP-2 & 9 activities by gelatin zymography. Latent & activated forms of MMP-2 were produced in all and latent & activated forms of MMP-9 appeared in 29 and 10 serial synovial cultures, respectively (Fig 4). Their activities in lesional cultures were significantly higher than that in paralesional ones (proMMP-2 & 9 and activated MMP-2: $P < .001$; activated MMP-9: $P < .05$).

Changes of MMPs mRNA Expression in Serial of Tissue Cultures

MMP-2 & 9 and MT1-MMP mRNA levels in various cultures after 24 hours

incubation were examined. Data presented in Fig 5 showed that the MMPs mRNA levels all increased in lesional cultures. The results indicated that the pathologic lesions of OA did change the expressions of MMP-2 & 9 and MT1-MMP mRNA in the cartilage, meniscus and synovium.

DISCUSSION

OA has complex etiology and causes degenerative changes of articular cartilage or subchondral bone and loss of function. There are two pathways for cartilage metabolism. Firstly, an intrinsic pathway by which chondrocytes themselves degrade cartilage ECM and, secondly, an extrinsic pathway by which tissues or cells other than chondrocytes, such as inflamed synovium, pannus, and infiltrated inflammatory cells, break down the ECM of cartilage mostly through synovial fluid. However, they are ascribable to enzymic digestion of the ECM.

Based on their structures and substrate specificities, MMP family members are classified into five groups composed of interstitial collagenase, gelatinases, stromelysins, membrane-type MMPs (MT-MMPs), and other MMPs. Most are secreted from cells as a latent proenzyme and are activated in ECM, except for stromelysin-3 (MMP-11) and MT-MMPs. MMP-2 is produced by stromal cells in the sublining synovial layer and chondrocytes; whereas MMP-9 is secreted by neutrophils, macrophages, synovial cells, and chondrocytes.[28-31]

Most MMP genes are expressed only when active physiological or pathological tissue remodeling takes place. An exception is MMP-2, which is widely expressed, in apparently quiescent tissues at significant levels, and therefore other levels of regulation, such as activation and inhibition of the enzyme, might be more important.[32,33] There is strong circumstantial evidence that MMP-2 participates in the turnover of normal cartilage matrix, whereas MMP-9 and some MMP-2 facilitate the progressive destruction of the cartilage matrix in OA. In this study, they were all early cases of knee OA according to the Kellgren and Lawrence grading scale. We virtually found that the latent and activated forms of MMP-2 were present in all tissue cultures and proMMP-2 appeared in all 37 effusions. Moreover, the activity of proMMP-2 in effusions did not significantly correlate with the level of it in sera. It also confirmed that the activities of MMPs in early OA were all apart from the serum levels those might reflect the systemic condition of inflammation.[34]

In arthritic joints, spatially and temporally controlled expressions of MMPs and their inhibitors have been confirmed in proteolytic degradation of damaged ECM components in order for repair tissue to integrate with surrounding healthy materials.[35] MMP-2 can degrade denatured collagen as well as gelatin, fibronectin

and elastin. MMP-9 has been implicated in the cellular migration and invasion in conditions such as inflammation, tumor invasion and metastasis.[36] In addition, recent studies suggest that MMP-9 can digest cartilage proteoglycans and also play a role in osteoclastic bone resorption[37, 38] without compensatory bone formation[14]. In this present study, proMMP-9 was in 13 of 40 serial meniscal cultures and its activity in lesional cultures was significantly higher than that in paralesional ones after 24 hours incubation. Latent & activated forms of MMP-9 appeared in 29 and 10 serial synovial cultures, respectively. Their activities in lesional cultures were also significantly higher than that in paralesional ones. Furthermore, increased MT1-MMP mRNA also suggests that this proteinase may contribute to degradation of the ECM by activating MMP-2.

In well selected middle-aged patients with knee arthritis, arthroscopic débridement may be a valuable method for providing transient relief of symptoms.[17-20] Although the repair of fibrillated cartilage was not promoted, its degeneration was prevented by correction of the mechanical status.[39] Nevertheless, the true outcomes of most of these arthroscopic procedures are difficult to determine, as most investigators have used nebulous inclusion criteria, inadequate study designs, short-term follow-up, as well as limited outcome-based analyses. However, normal turnover of ECM is a conservative process in which the rate of breakdown and release of fragments from the tissue does not exceed the rate at which it is replaced by newly synthesized molecules. In pathology, an increased rate of degradation results in a net loss of ECM, and the tissue becomes thin and mechanically weak and may form a barrier to integration of repair tissue with viable tissue.[35] Not only does arthroscopic management remove the pathologic area, which is required to be proteolytic degradation, and decrease the production of MMPs which may attack the normal ECM and the mechanical stress due to impingement and uneven contact surface.

Our data confirm that tissue repair of human OA is ascribable to enzymic digestion of the ECM *ex vivo*. When technically appropriate, arthroscopic débridement for excising and smoothing the pathologic lesions of OA, such as meniscal degenerative tear and chondral lesion may lengthen the useful lifetime of a knee joint. Arthroscopic partial synovectomy for out of control synovitis seems to be useful during arthroscopy for associated disorders diagnosed and treated. Still, further *in vivo* studies are required to elucidate its overall effect and the mechanism of action on ECM proteolysis.

Acknowledgement

This work was supported by grant from the Research Section of Chung Shan Medical University (CSMU 90-OM-B-016) and National Science Council, Taiwan

(NSC91-2314-B-040-019).

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TABLE 1. Characteristics of the 43 Patients and Their 58 Knees. Results Are Shown as Range (Median).

Patient	Sex (F/M)	Age	Right/left	Serum		Effusion		
				Pro-MMP-2	Pro-MMP-9		Pro-MMP-2	Pro-MMP-9
43*	31/12	46-86 (63)	35/23	25.4-48.1 (32.3)	32.6-48.5 (38.4)	2-38 ml (8 ml)	23.0-62.3 (33.1)	ND

* Fifteen with both knees, including 12 female and 3 male.

ND = not detectable (see "Results")

There was no correlation in the activity of proMMP-2 between sera and effusions (r = 0.016, *P* = .951).

FIGURE LEGENDS

FIGURE 1.

Arthroscopic débridement for excising and smoothing the pathologic lesions of OA. (A) Intraoperative view of the lesional (arrow) and paralesional (arrowhead) areas of chondral ulcer and fibrillation. (B) Arthroscopic chondral shaving and osteophyte excision. (C) Intraoperative view of the lesional (arrow) and paralesional (arrowhead) areas of meniscal degenerative tear. (D) Arthroscopic partial meniscectomy and chondral shaving. (E) Intraoperative view of the lesional (arrow) and paralesional (arrowhead) areas of hypertrophic villi. (F) Arthroscopic limited synovectomy.

FIGURE 2.

Gelatinase activities in a series of chondral cultures of patients with knee OA at various times (3 and 24 hours) were quantified by gelatin zymography. (A) Latent and activated forms of MMP-2 appeared in serial chondral cultures. (B) Their activities in lesional cultures were significantly higher than that in paralesional ones and increased in time dependent manner ($P < .001$).

FIGURE 3.

Gelatinase activities in a series of meniscal cultures of patients with knee OA at various times (3 and 24 hours) were quantified by gelatin zymography. (A) Latent & activated forms of MMP-2 and proMMP-9 appeared in serial meniscal cultures. (B) The activities of the latent & activated forms of MMP-2 in lesional cultures were significantly higher than that in paralesional ones and increased in time dependent manner ($P < .001$). The activity of proMMP-9 in lesional cultures was significantly higher than that in paralesional ones after 24 hours incubation ($P < .001$). Nevertheless, there were no significant differences between lesional and paralesional cultures after 3 hours incubation ($P = .109$).

FIGURE 4.

Gelatinase activities in a series of synovial cultures of patients with knee OA at various times (3 and 24 hours) were quantified by gelatin zymography. (A) Latent and activated forms of MMP-2 & 9 appeared in serial synovial cultures. (B) Their activities in lesional cultures were significantly higher than that in paralesional ones and increased in time dependent manner (proMMP-2 & 9 and activated MMP-2: $P < .001$; activated MMP-9: $P < .05$).

FIGURE 5.

MMP-2 & 9 and MT1-MMP mRNA levels in serial (A) chondral, (B) meniscal, and (C) synovial cultures after 24 hours incubation were examined and all increased in lesional cultures.

Fig 1

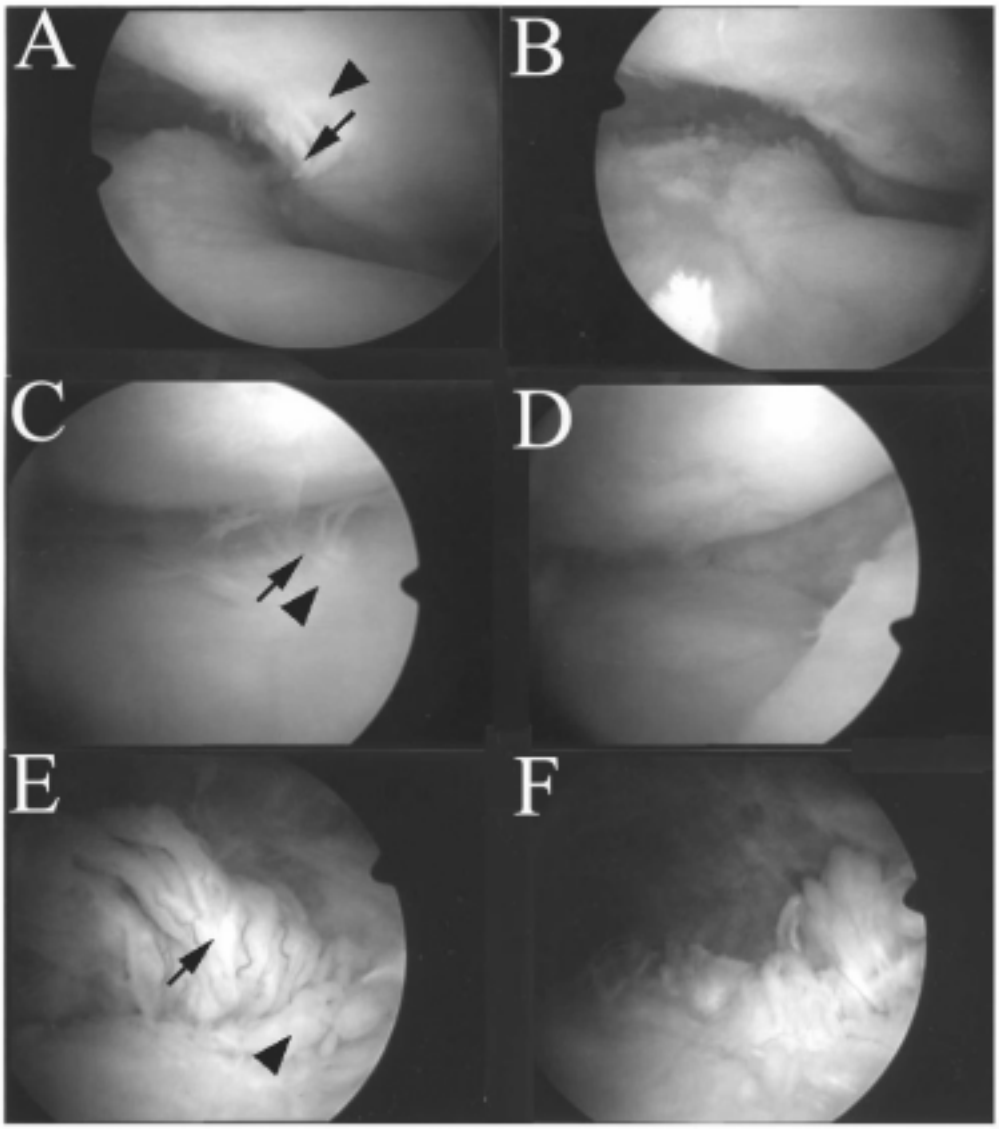
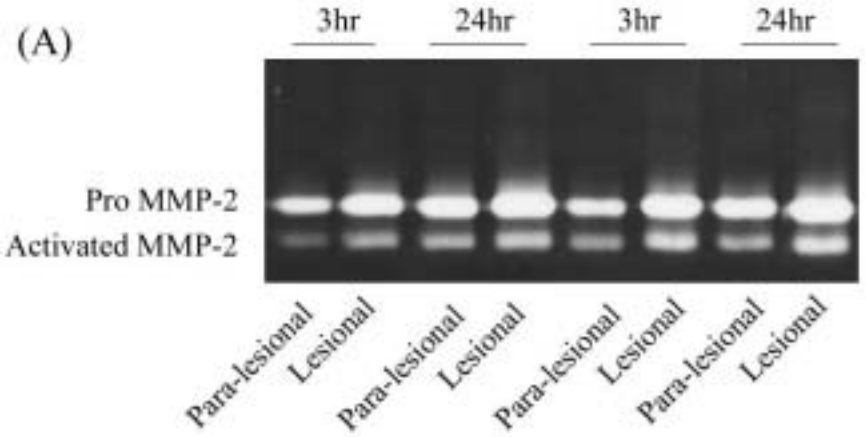


Fig 2

(A)


3hr
24hr
3hr
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Pro MMP-2
Activated MMP-2
Para-lesional
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Para-lesional
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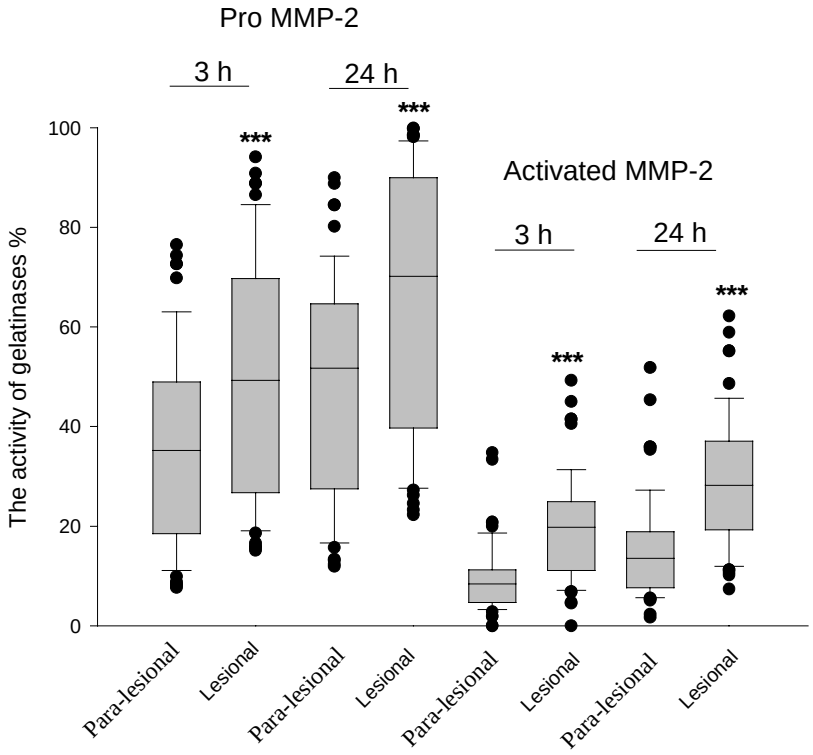
Para-lesional
Lesional

(B)


Pro MMP-2
3 h
24 h
***
***
Activated MMP-2
3 h
24 h
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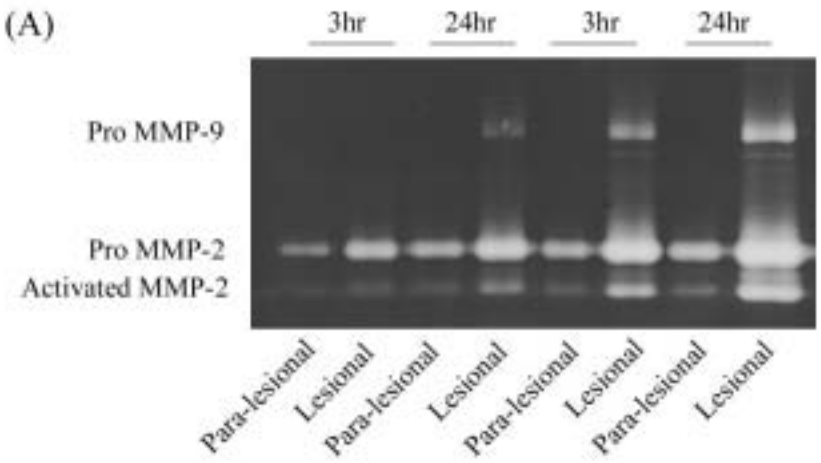
***
The activity of gelatinases %
100
80
60
40
20
0
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional

Fig 3


(A)
3hr
24hr
3hr
24hr
Pro MMP-9
Pro MMP-2
Activated MMP-2
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional

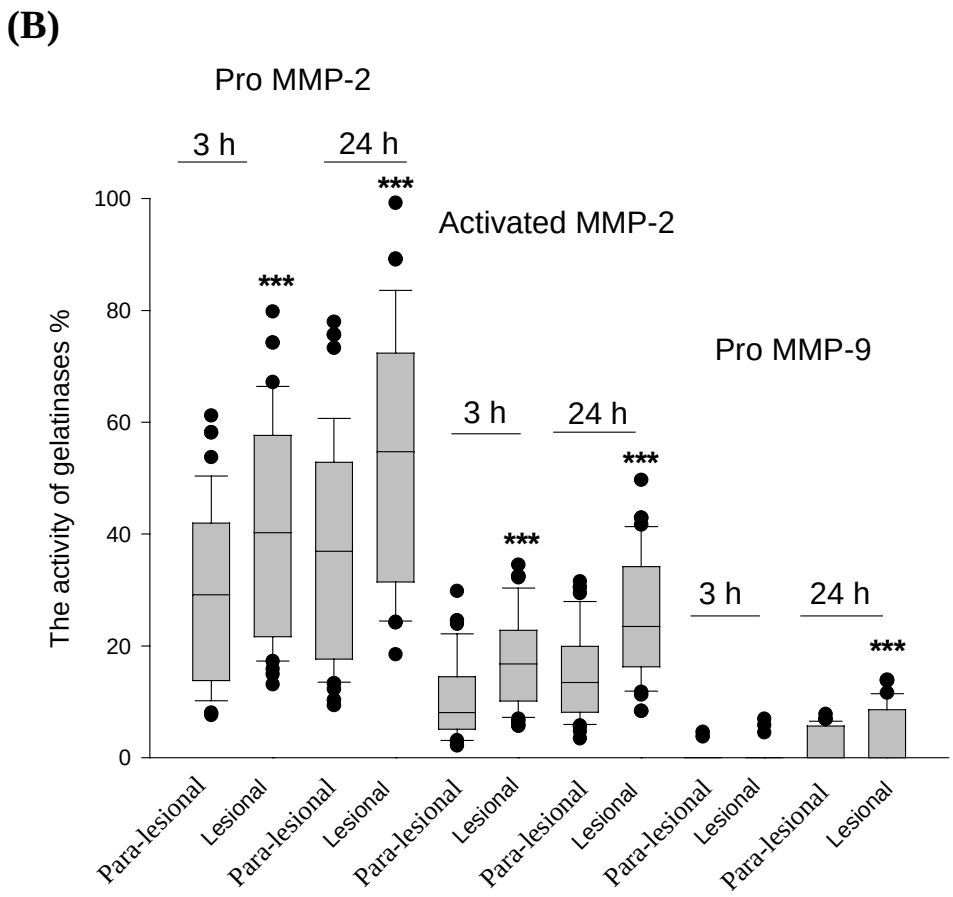
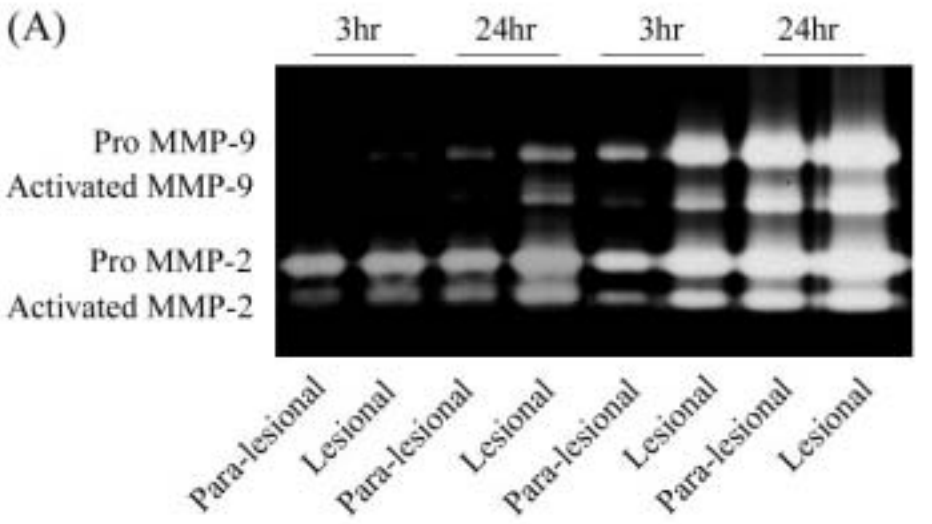

(B)
Pro MMP-2
3 h
24 h
Activated MMP-2
Pro MMP-9
3 h
24 h
3 h
24 h
The activity of gelatinases %
100
80
60
40
20
0
***
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional

Fig 4


(A)
3hr
24hr
3hr
24hr
Pro MMP-9
Activated MMP-9
Pro MMP-2
Activated MMP-2
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional

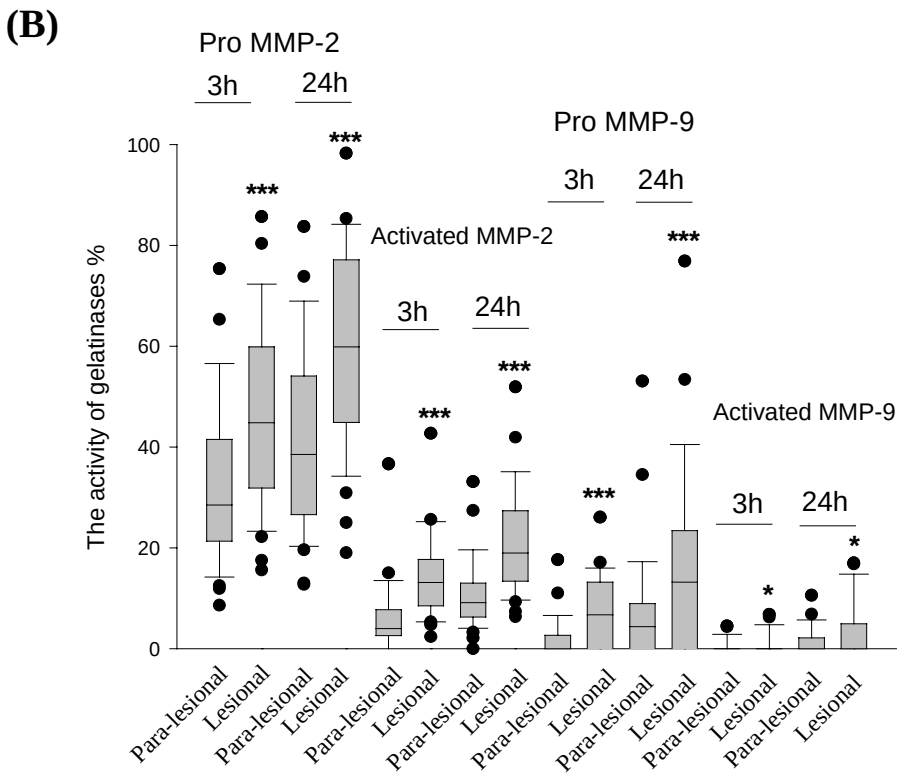
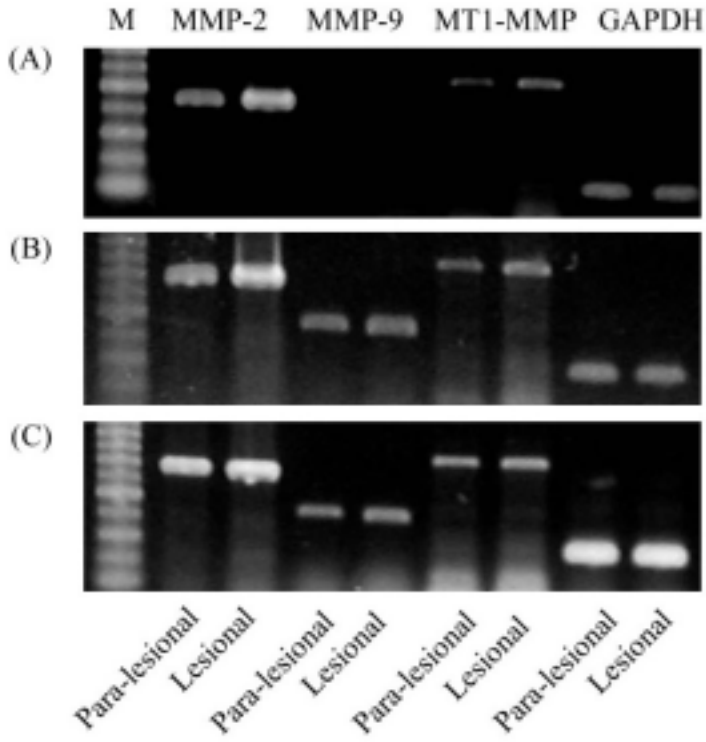

(B)
Pro MMP-2
3h
24h
Pro MMP-9
3h
24h
Activated MMP-2
3h
24h
Activated MMP-9
3h
24h
The activity of gelatinases %
100
80
60
40
20
0
***
*
Para-lesional
Lesional

Fig 5


M
MMP-2
MMP-9
MT1-MMP
GAPDH
(A)
(B)
(C)
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional
Para-lesional
Lesional