MMP7 in Periodontitis with Type 2 Diabetes Mellitus

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Running title: Matrilysin in diabetic periodontitis

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Abstract

Background and Objective: Elevated levels of matrix metalloproteinase-7 (MMP7) have been observed in serum samples of subjects with type 2 diabetes mellitus (T2DM) and in gingival tissues of subjects with periodontitis. The aim of the present study was to collect *in vivo* and *in silico* evidence on the role of MMP7 in the interplay between T2DM and generalized periodontitis. **Materials and Methods:** The extent of MMP7 expression and localization were immunohistochemically analyzed in gingival tissues of generalized periodontitis patients with T2DM (T2DM/GP, n=11), systemically healthy generalized periodontitis patients (GP, n=7), and systemically and periodontally healthy controls (n=11). An *in silico* network model was built to determine the interactions between MMP7 and T2DM pathways. Regulation of neutrophil transmigration by MMP7 was analyzed in a knock-out mice model. **Results:** In human gingival tissues, the proportion of cells with robust MMP7 expression was elevated in T2DM/GP patients in comparison to controls (p=0.014). According to the *in silico* analysis, "Hydroxyl radical" and "Hydrogen peroxide" compounds were among the most central nodes of the network, and were within the shortest paths connecting "Glucose" to "MMP7". In MMP7 knock-out mice, an intense accumulation of neutrophils was observed in the gingival epithelium as compared to wild-type mice (p=0.0001). **Conclusion:** An elevated MMP7 expression in gingival tissues of T2DM/GP patients is related to the activation of reactive oxygen species by hyperglycemia. Suppression of MMP7 expression results in impaired neutrophil transmigration in gingiva.

Introduction

Type 2 diabetes mellitus (T2DM) results from the combination of defective expression of and increased cell resistance to endogenous insulin, leading to elevated long-term blood glucose levels without autoimmune-derived destruction of β-cells. T2DM is known to induce several adverse effects in the periodontium via decreased collagen turnover, and impaired neutrophil chemotaxis and phagocytosis, thus exposing to increased periodontal destruction [1].

Matrix metalloproteinase-7 (MMP7) is a neutral proteinase, which is expressed in the sulcular and junctional epithelium of the gingiva [2-4]. MMP7 can degrade various tissue proteins, including laminin, fibronectin, type IV collagen, gelatin, and elastin [3]. Elevated MMP7 levels have been reported from saliva, gingival crevicular fluid, and gingival tissue samples of periodontitis patients [2-5]. In individuals with T2DM, there is a correlation between elevated serum levels of MMP7 and diabetic complications, such as renal disease and diastolic dysfunction [6]. In *in vitro* conditions, high glucose levels in the cell culture environment enhance lipopolysaccharide-induced expression of MMP7 mRNA in histiocytes [7]. To date, however, levels or expression profiles of MMP7 in gingival samples from periodontitis patients with T2DM have not been studied.

How MMP7 contributes to immune response has both beneficial and destructive effects. MMP7 stimulates re-epithelialization and transepithelial migration of neutrophils, thereby being involved in wound healing and inflammation, respectively [8-10]. In the lungs, MMP7 takes part in the influx of neutrophils to the site of infection; when MMP7 is depleted in null mutants, neutrophil migration is abolished [9]. In the present study, we hypothesize that MMP7 acts as a regulator protein in T2DM-associated generalized periodontitis by interacting with disease-associated molecular pathways and by

modulating neutrophil recruitment into periodontal tissues. As previously demonstrated, *in silico* analyses enable to view shared molecular targets, interactomes, and pathophysiologies of clinically distinct diseases and to define the central points that control the communication within the disease network [11, 12]. The overall aim of the present study was to collect *in vivo* and *in silico* evidence on the role of MMP7 in the pathogenic interplay between two chronic diseases, T2DM and generalized periodontitis.

Materials and Methods

Ethical Guidelines

The study protocol was approved by the Ethical Committee of Hacettepe University, Ankara, Turkey (Protocol no: LUT 12: 129/22) in accordance with Helsinki Declaration 1975, as reviewed in 2000 [13].

Study Population and Clinical Examinations

Human gingival tissues were collected at the University of Hacettepe, Turkey, from three different subject groups: 1) 11 T2DM patients (five males and six females; age range 39-66 years) suffering from generalized periodontitis (T2DM/GP), 2) seven systemically healthy individuals (three males and four females; age range 45-64 years) with generalized periodontitis (GP), and 3) 11 systemically and periodontally healthy subjects (five males and six females; age range 18-57 years; control). Only individuals with medically diagnosed diabetes [13, 14] were included in the T2DM/GP group; the mean duration of diabetes was 8.36±7.5 years, mean blood glucose 166 ml/dl (min-max values 106-225 ml/dl), and mean HbA1c 7.34% (6.25%-8.6%). Of the 11 diabetics, two used intramuscular insulin injections for treatment. Altogether seven subjects were

smokers (<10 cigarettes/per day; one in the T2DM/GP, two in the GP, and four in the control groups). Periodontal examinations were performed by a single calibrated examiner (D.Y.). Radiographic evidence of alveolar bone loss was evaluated in panaromic tomographs (Orthopantomograph OP 100, Sirona Orthophos XG5, NY, USA). Probing pocket depth (PPD), clinical attachment level (CAL), plaque index (PI) [15], gingival index (GI) [16], and bleeding time index (BTI) [17] were measured from six sites *per* tooth (mesio-buccal, midbuccal, disto-buccal, disto-lingual/palatinal, mid-lingual/palatinal, and mesio-lingual/palatinal) by using a manual periodontal probe (#PCP-15, HuFriedy, Chicago, IL, USA). PPD is defined as the distance between the base of the gingival pocket and the gingival margin, and, CAL as the distance between the base of the gingival pocket and the cemento-enamel junction. BTI scores were based on the occurrence of gingival bleeding after gingival stimulation, as described in detail elsewhere [17].

Diagnosis of periodontitis was based on having at least four teeth with one or more site(s) with PPD >3 mm and CAL >3 mm, which bled upon probing and had radiographic evidence of alveolar bone loss. As the distribution of periodontitis-affected teeth did not show a clear pattern and was over 30% of all teeth, subjects with periodontitis are categorized as generalized periodontitis [18]. Periodontally healthy participants had no sites of PPD >3 mm with clinical signs of inflammation.

All periodontitis patients received oral hygiene motivation sessions and non-surgical periodontal therapy, which were consisted of mechanical instrumentation of the supra- and subgingival root surfaces. Three weeks after the completion of the non-surgical therapy, sites with ≥5 mm residual pockets with bleeding on probing (moderate to severe periodontitis) were directed to periodontal surgery.

Immunohistochemical Quantification of MMP7 on Human Gingiva

In periodontitis patients (GP and T2DM/GP groups), the samples were collected during the surgical treatment of periodontal disease, with a surgical incision longitudinally targeted the sulcular epithelium and the bottom of the pocket. From control group, the samples were collected during tooth extractions made for orthodontic reasons or crown lengthening procedures. Same examiner (D.Y.) performed non-surgical periodontal therapies, surgical treatments, and tissue sampling. Tissue samples were immediately fixed in 4% formalin and embedded into paraffin blocks, which were sent to the University of Turku, Institute of Dentistry, Finland, for immunohistochemistry.

The embedded samples were sectioned to yield 5 μm-thick specimens and mounted onto slides for immunohistochemistry procedures. After deparaffinization the specimens were immunostained for MMP7 (cat nr. AF907, R&D Systems, Minneapolis, MN, USA) by using routine procedures with an immunostainer (TechMate, Dako, Glostrop, Denmark). When tested in direct ELISAs, this antibody cross-reacted with recombinant human (rh) MMP-8 and rhMMP-12 with less than 1%, according to the product catalog information. Briefly, antigen retrieval in a microwave (twice for 5 min) in 1 mmol/L citrate buffer (pH 6.0) was followed by endogenous peroxidase blocking with 3% H_2O_2. The primary antibody was detected by a biotinylated secondary antibody, coupled with streptavidin-horseradish peroxidase (HRP), and visualized with 3,3'diaminobenzidine tetrahydrochloride in HRP buffer (Dako). Control stainings were performed by omitting the primary antibody from the incubations. The image analysis system comprised of a true-color-red-green-blue video camera (Leica DC 300V 2,0 Leica, Wetzlar, Germany, 2088×1550 pixels) connected to a microscope (LeicaDMLB, Leica). High definition images of different sections were acquired and evaluated at a 40X magnification. The

intensity of immunohistochemical MMP7 stainings was semiquantified using the following scale: ST- (negative staining), ST+ (weak or moderate staining for the nucleus or cytoplasm), and ST++ (strong positive staining for both the nucleus and cytoplasm).

In Silico MMP-T2DM Network Model

The network model ("MMP-T2DM") for protein-protein, compound-compound, and protein-compound interactions between members of the T2DM pathway (map04930; source: KEGG Pathway, http://www.genome.jp/kegg/pathway.html) and MMPs/TIMPs was developed by using the STITCH 4.0 database with ''Experiments'' and ''Databases'' as input options and a confidence score of 0.600 [19]. The selected list of genes/proteins and compounds (Supp. Table 1) was applied into the STITCH database and the links (interaction strength) between two different nodes (genes/proteins and compounds) were saved in data files and handled by utilizing the Cytoscape open source software platform (http://www.cytoscape.org/). Centrality values (Supp. Table 2) were also determined by using this software and the SPs with the ShortestPath plugin (http://apps.cytoscape.org/apps/shortestpath).

Quantification of Neutrophils in Gingival Tissue Specimens of MMP7 Knock-out Mice

Frozen heads of eight wild-type (WT) and eight MMP7 KO -/- mice (8-10 weeks old) were generously provided by Dr. William Parks, Center for Lung Biology and Institute for Stem Cell and Regenerative Medicine, University of Washington [20]. The heads were fixed in 4% formaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 2 days, dissected to separate the mandible and maxilla, and then decalcified in phosphate

buffered saline (PBS, pH 7.4) containing 0.4 M EDTA and 2% formaldehyde. The solution was changed every other day for 6 weeks. After that, tissues are washed with water and dehydrated gently by immersion in increasing concentrations of alcohol for dehydration. The decalcified samples were then embedded in paraffin, and samples were sectioned (6 µm) in the mesio-distal direction using routine protocols for paraffin embedded samples. Every 10^{th} slide from each block of sections was stained with hematoxylin and eosin to assess overall histology.

The mouse sections were deparaffinized and incubated in 0.3% H_2O_2 in methanol for 30 min to block endogenous peroxidase activity, followed by rinsing in PBS and incubation with normal blocking serum (Vectastain; Vector Laboratories Inc., Burlington, ON, Canada) at a room temperature for 60 min. The sections were incubated with monoclonal rat anti-mouse neutrophil antibody (1:100; Thermo Fisher Scientific, Inc., Rockford, IL, USA) at 4°C for 18 h, followed by rinsing and incubation with a biotinylated anti-rat antibody for 1 h. After rinsing, the sections were incubated with ABC avidin-peroxidase reagent (Vectastain Elite kit, Vector Laboratories Inc.) for 30 min. For the color development, the sections were reacted with the Vector VIP substrate (Vector Laboratories Inc.) for a standardized time. Control stainings performed by omitting the primary antibody from the incubations, did not show any immunoreactivity. The sections were mounted with the Entellan mounting medium (EMD Millipore, Darmstadt, Germany), and standardized digital images were obtained using a Nikon TS100 bright-field microscope (Nikon Canada, Mississauga, ON, Canada) equipped with a Nikon Coolpix 995 digital camera (Nikon Canada). The number of neutrophils *per* unit area of the epithelium and connective tissue of the interdental papilla at molars in the mandible and maxilla were calculated in 2 to 5 tissue sections from the papillae in each of the 8 wild-type and MMP7 knock-out (KO **-/-)** -

mice. The calculations were performed by two independent, blinded investigators, and the results were averaged for each tissue section.

Statistical Analyses

Differences in age, number of teeth, and periodontal parameters between the T2DM/GP, GP, and control groups were statistically evaluated by one-way ANOVA with Bonferroni post-hoc test. Differences in MMP7 expression levels between the groups were statistically compared by using univariate general linear model by adjusting the confounding factors (age, gender, and smoking). Differences between the wild type and MMP7 KO -/- mice were analyzed by an unpaired t-test. A p-value of <0.05 was considered statistically significant.

Results

Demographic and clinical periodontal parameters of the three subject groups are provided in S3 Table. No significant gender difference was found between the groups ($p=0.993$), whereas there were significant differences in age ($p<0.001$) and the number of teeth ($p<0.004$) between the control group vs. the T2DM/GP and GP groups. Clinical periodontal parameters (PPD, CAL, GI, PI, and BTI) differed significantly ($p<0.001$) between the study groups (Supp. Table 3).

Positive immunoreactivities to MMP7 were detected in the nucleus and also in the cytoplasm of gingival epithelial cells (Fig 1, A-F). The proportion of MMP7-positive cells with strong staining for both the nucleus and cytoplasm was significantly higher (34.41%) in the samples collected from T2DM/GP patients as compared to those from controls ($p=0.014$) (Fig 2). No difference was observed between the systemically healthy generalized periodontitis and control groups in terms of their MMP7-positive

cell numbers in the gingival epithelium. Hematoxylin & eosin stained sections for tissue characteristics are given in Supp. Fig. 1. Negative control sections incubated with no primary antibody did not show any staining (Supp. Fig. 2).

In silico model interconnected a total of 56 genes/proteins and five compounds (S1 Table). "Hydrogen peroxide" and "ABCC8 (ATP-binding cassette, sub-family C (CFTR/MRP), member 8)" presented betweenness values (but not degree values) over the thresholds (falling within one standard deviation of the mean), thus being non-hub-bottlenecks in our MMP-T2DM model. Both connectivity and betweenness values over the thresholds revealed "Hydroxyl radical", "Glucose", "MMP2", and "RKCD (Protein kinase C, delta)" as hub-bottlenecks of this network. The shortest paths or the routes with a minimum length in nodes that connected "Glucose" and "MMP7" are 1) Glucose-Hydrogen peroxide-MMP2-MMP7, 2) Glucose-Hydrogen peroxide-MMP9-MMP7, and 3) Glucose-Oxygen-MMP9-MMP7 (Fig. 3B). Actions view representation between the shortest paths network nodes is additionally given (Fig. 3C).

A significantly increased number of neutrophils was found in the gingival epithelium of the MMP7 KO -/- mice compared to wild type mice (p=0.0001) (Fig 4 A-B), whereas the number of neutrophils did not differ in connective tissue (p=0.321) (Fig 4 A-B).

Discussion

To our knowledge, the present study is the first to demonstrate an expression profile of MMP7 in gingival tissues of diabetics suffering from periodontitis. In gingival tissue samples, MMP7 expressing cells were localized at all epithelial layers and the proportion of cells with strong MMP7 expression was elevated in T2DM/GP patients compared to healthy controls. The *in silico* model, which integrated MMPs, TIMPs, and members of the T2DM pathway into one single network of interactions, revealed

“Hydrogen peroxide” and “Oxygen” as central nodes of the MMP-T2DM model and within the shortest paths between “Glucose” and “MMP7”. In MMP7 KO -/- mice, there was an intense accumulation of neutrophils in the gingival epithelium compared to wild type mice.

In the pathogenesis of periodontitis, *MMP7* has been found as the second most highly upregulated gene [21]. As regards the expression profile of MMP7 in gingiva, contradictory results have been presented in the literature. On one hand it has been reported that its expression was limited to suprabasal cells of the junctional epithelium, and no MMP7 expression occurred in resident cells of the connective tissue [2]. On the other hand, it has been shown that the basal cell layer of the sulcular epithelium is the main source of MMP7 in gingiva, while endothelial cells, fibroblasts, and stromal cells of connective tissue give positive immunoreaction to MMP7 [3]. Recently, an elevated MMP7 expression was found in the connective tissue, but not in the epithelium [21]. In our study, we found elevated numbers of MMP7-positive cells in the gingival epithelium of systemically healthy generalized periodontitis group in comparison to the control group, however, this difference was not significant. The controversy between the present results and those of previous studies may be due to differences in the health status of the sampling site, as it significantly affects tissue MMP7 levels. Moreover, uneven distribution of smokers between the groups and younger age of the control group may act as confounding factors in the results of the present study. Finally, panaromic tomographs, which are used in detection of bone loss, have limitations in their diagnostic capabilities due to the inconsistent magnifications and geometric distortions.

In the present study, MMP7 expression was mainly localized in the gingival epithelium, but also, cells of the connective tissue contributed to MMP7 expression. Strong positive

staining of the nucleus was observed in samples collected from the T2DM/GP group as compared to those from the control group. Nuclear localization of MMP7 has been associated with aggressive migration of cancer cells and decreased wound healing in cancer tissues [22]. Although pathogenesis of cancer and periodontitis are not comparable, cell migration (pocket formation) and decreased wound healing are observed in periodontitis patients with T2DM as well. Therefore, extensive nuclear localization of MMP7 may be linked to increased periodontal complications of T2DM.

Here we tested the feasibility of an *in silico* model, which enables to integrate MMPs, TIMPs, and members of the T2DM pathway into a single network. According to our results, both connectivity and betweenness values over the thresholds revealed "Hydroxyl radical", "Glucose", "MMP2", and "RKCD (Protein kinase C, delta)" as hub-bottlenecks of this network. It is noteworthy that the shortest paths linking glucose and MMP7 within the MMP-T2DM model necessarily involved the interactions between "Hydrogen peroxide" and "MMP2", "Hydrogen peroxide" and "MMP9", and finally "Oxygen" with "MMP9" nodes. This observation indicates that "Hydrogen peroxide" and "Oxygen" are the first upstream points in the cross-talk between glucose and MMP7 signaling. Thus, hyperglycemia-derived effects on MMP7 levels may be secondary at changes on hydrogen peroxide and oxygen levels. Diabetes-related complications include angiopathies and atherosclerotic events that lead to an inefficient blood flow and oxygen supply and, further, to the development of ischemic episodes and hypoxic conditions (low oxygen tension) characterized by an increased production of reactive oxygen species in different tissues and organs [23,24]. *In vitro* studies have shown that myeloperoxidase generates hypochlorous acid (HOCl) by using reactive oxygen species, such as hydrogen peroxide, and hydrogen peroxide activates MMP7 expression and HOCl converts pro-MMP7 to active MMP7 [25,26]. This mechanism

may explain the elevated MMP7 expression found in gingival tissues of periodontitis patients with T2DM.

A key process is the inflammatory response to periodontal infection and tissue destruction is the recruitment of neutrophils from the circulation into infected areas [27]. This involves their adhesion to endothelial cells, crawling to detect locations for transmigration, extravasation into the tissues, and migration to the site of infection in response to chemotactic factors. Using a MMP7 knock-out mice model, Swee and coworkers [8] demonstrated that the absence of MMP7 induces a delayed re-epithelization and an impaired neutrophil migration. However, lack of MMP7 does not affect the macrophage migration, indicating that MMP7 regulates specifically neutrophil migration [8]. In line with these results, the present findings revealed a disrupted transmigration and an enhanced accumulation of neutrophils in the gingival epithelium of MMP7 knock-out mice. The mechanism explaining how elevated MMP7 levels seen in T2DM patients could be involved in the pathogenesis of periodontitis remains elusive but may depend on an altered neutrophil recruitment and/or MMP7-mediated proteolytic disruption of the epithelial barrier function. It has been demonstrated that inhibition of tumor necrosis factor (TNF)-α reduces fibroblast apoptosis and caspase-3 activity in diabetic mice [28]. Moreover, MMP7 has been shown to be a regulator of TNF-α release in peritoneal macrophages [29]. MMP7-regulated TNF-α induced fibroblast apoptosis may explain the link between MMP7 and destruction of periodontal tissues. In addition, tumor growth factor-β regulates MMP7-activated fibronectin accumulation in diabetic kidney [30]. *MMP7* was found to be upregulated in human cadaver pancreases of T2DM patients, together with other genes associated with cell cycle and apoptosis [31]. Taken together, extensive expression of MMP7 in periodontal

tissues of subjects with T2DM is most probably an outcome of systemic inflammatory stage, rather than a local host response against infection.

In conclusion, in periodontitis patients with T2DM, there is an elevated MMP7 expression in gingival tissues that may be related to the activation of reactive oxygen species by hyperglycemia. Lack of MMP7 expression suppresses neutrophil transmigration in the gingiva.

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Figure legends

Fig 1. MMP7 was detected in both the nucleus and cytoplasm of gingival epithelial cells in controls **(A-B)**, in generalized periodontitis patients, GP **(C-D)**, and in type 2 diabetes mellitus patients with generalized periodontitis, T2DM/GP **(E-F)**. Regions of interests (white rectangles in figures A, C, and D) are shown at a 100X magnification in figures B, D, and F. White arrow indicates a non-stained cell, blue arrow indicates a cell with weak or moderate staining of the nucleus or cytoplasm, and yellow arrow indicates a cell with strong positive staining of the nucleus and cytoplasm.

Fig 2. The intensity of MMP7 staining was quantified based on the following scale: ST- (negative), ST+ (weak or moderate staining of the nucleus or cytoplasm), and ST++ (strong positive staining of the nucleus and cytoplasm)**.**

Fig 3. *In silico* network model of interactions between members of the type 2 diabetes mellitus (T2DM) pathway and metalloproteinases (MMPs)/tissue inhibitors of metalloproteinases (TIMPs). **(A)** "MMP-T2DM" is composed by the interaction of 56 proteins and five compounds. **(B)** Shortest association paths between "Glucose" and "MMP7" nodes are shown in the inset. **(C)** Actions view representation of nodes within the shortest paths.

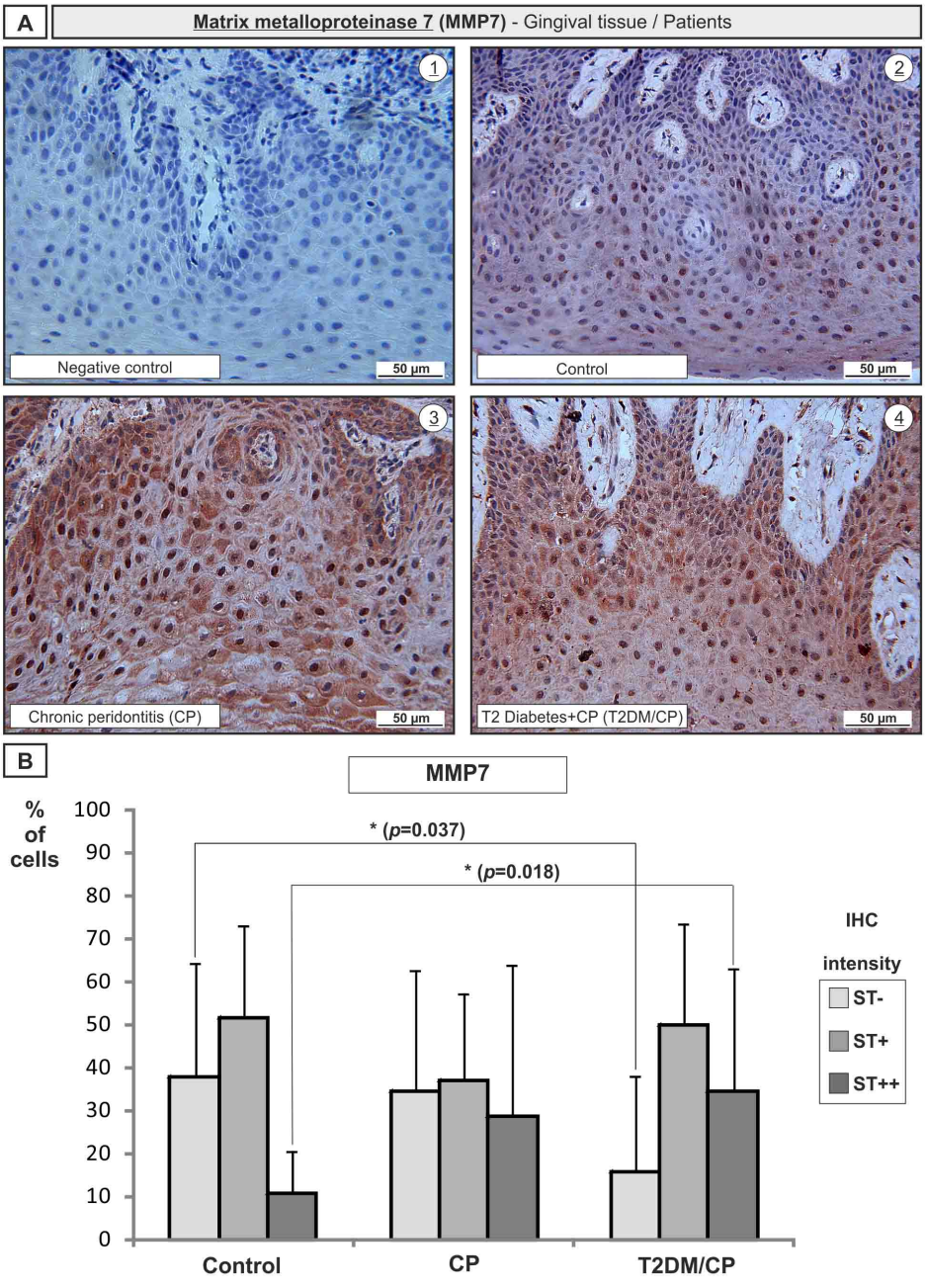
Fig 4. (A) Representative images of mouse interdental papilla sections from WT mice (+/+) and MMP7 knock-out (KO -/-) mice stained with an antibody against neutrophils (E, epithelium; CT, connective tissue). **(B)** MMP7 knock-out (KO -/-) mice had a significantly higher number of neutrophils in the gingival epithelium as compared to

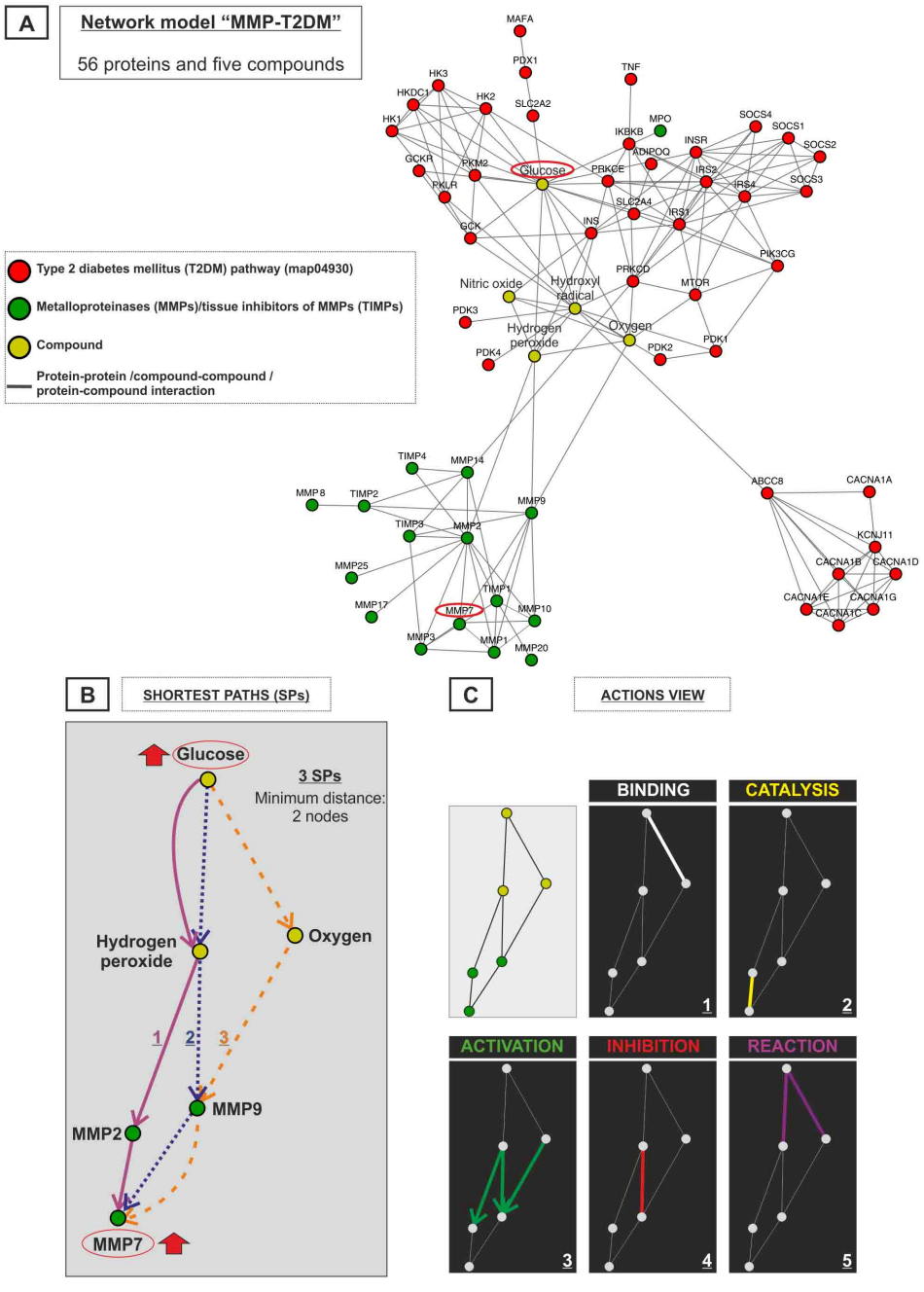
wild type mice while the number of neutrophils in the gingival connective tissue did not differ between the mouse groups.

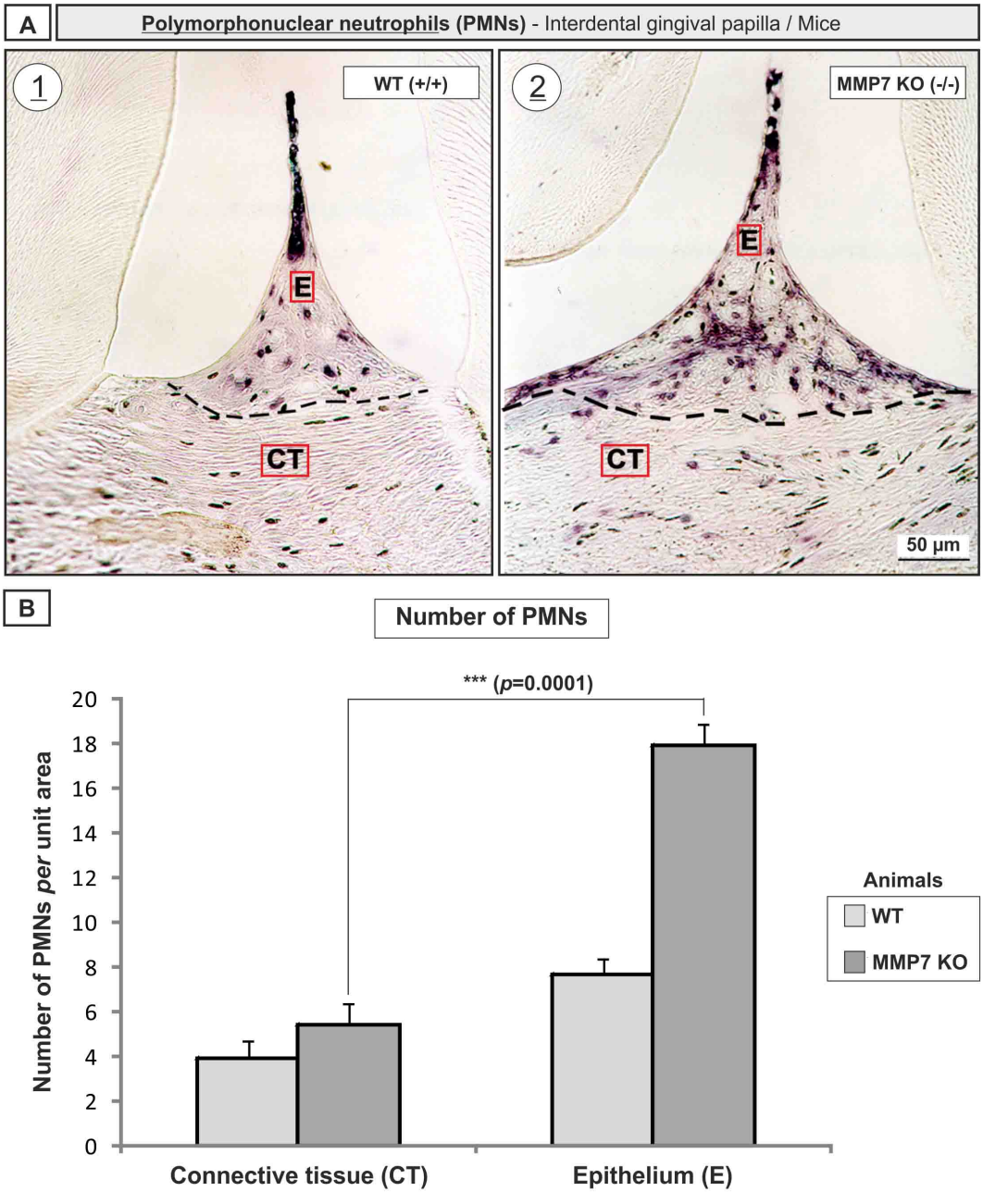
Supplementary Figure Legends

Supplementary Figure 1: Hematoxylin & Eosin stained sections of the control, generalized periodontitis (GP), and generalized periodontitis with type 2 diabetes mellitus (T2DM/GP) groups. Tissue samples and region of interests are identical in figure 1 and supplementary figure 1.

Supplementary Figure 2: Negative control stainings of human and mice tissues performed by omitting primary antibodies.


A
Matrix metalloproteinase 7 (MMP7) - Gingival tissue / Patients
1
Negative control
50 µm
2
Control
50 µm
3
Chronic peridontitis (CP)
50 µm
4
T2 Diabetes+CP (T2DM/CP)
50 µm
B
MMP7
% of cells
100
90
80
70
60
50
40
30
20
10
0
* (p=0.037)
* (p=0.018)
Control
CP
T2DM/CP
IHC intensity
ST-
ST+
ST++


A
Network model "MMP-T2DM"
56 proteins and five compounds
Type 2 diabetes mellitus (T2DM) pathway (map04930)
Metalloproteinases (MMPs)/tissue inhibitors of MMPs (TIMPs)
Compound
Protein-protein /compound-compound / protein-compound interaction
MAFA
PDX1
TNF
HK3
HKDC1
HK2
SLC2A2
HK1
MPO
IKBKB
SOCS4
SOCS1
SOCS2
INSR
ADIPOQ
GCKR
PKM2
Glucose
PRKCE
IRS2
IRS4
SOCS3
PKLR
SLC2A4
IRS1
INS
GCK
PIK3CG
PRKCD
Nitric oxide
Hydroxyl radical
MTOR
PDK3
Hydrogen peroxide
Oxygen
PDK1
PDK4
PDK2
TIMP4
MMP14
MMP8
TIMP2
MMP9
TIMP3
MMP2
MMP25
TIMP1
MMP17
MMP7
MMP10
MMP3
MMP1
MMP20
ABCC8
CACNA1A
KCNJ11
CACNA1B
CACNA1D
CACNA1E
CACNA1G
CACNA1C
B
SHORTEST PATHS (SPs)
Glucose
3 SPs
Minimum distance:
2 nodes
Oxygen
Hydrogen peroxide
1
2
3
MMP9
MMP2
MMP7
C
ACTIONS VIEW
BINDING
CATALYSIS
1
2
ACTIVATION
INHIBITION
REACTION
3
4
5


A
Polymorphonuclear neutrophils (PMNs) - Interdental gingival papilla / Mice
1
WT (+/+)
E
CT
2
MMP7 KO (-/-)
E
CT
50 µm
B
Number of PMNs
*** (p=0.0001)
Number of PMNs per unit area
20
18
16
14
12
10
8
6
4
2
0
Connective tissue (CT)
Epithelium (E)
Animals
WT
MMP7 KO