Active matrix metalloproteinase-8 (aMMP-8)  
- a biomarker for inflammatory destructive processes in periodontitis and peri-implantitis

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Nomenclature:

MMP-8: Matrix-metalloproteinase-8, derived from human host neutrophilic cells (PMNs: polymorphonuclear leukocytes), synonym: Collagenase 2.

aMMP-8: activated form of MMP-8, as quantitatively detected by specific patented monoclonal antibodies (Mabs 8708 & 8706, cf. Sorsa et al. 2002).

Collagenase activity: Activity of MMP-8 (Collagenase 2) as measured via cleavage of collagen fibres into smaller components (e.g. Lee et al. 1995).  
NOTE: In publications dealing with periodontitis and peri-implantitis, it was proven concomitantly (by immunoblotting / Western Blot) that this 'collagenase activity' resembles the action of MMP-8.

PISF: Peri-implant sulcus fluid.
1. General Aspects

1.1 Relevance of matrix metalloproteinases in health and disease

Fifty years ago research started on matrix metalloproteinases (MMPs; Woessner 2002), when Gross und Lapière (1962) tried to answer the question how the disappearance of tadpole tissue during metamorphosis works. Regarding the scientific relevance the tadpole became a prince: „a tail of a frog that became a prince” (Brinckerhoff and Matrisian 2002).

Matrix metalloproteinases are zinc-dependent enzymes which are involved in any destructive or remodeling processes of `matrix molecules´ like collagen or elastin. If overexpression of MMPs occurs due to an imbalance between MMPs and their inhibitors, the `Tissue Inhibitors of Metallo-Proteinases´ (TIMPs; Woessner 2002), quite a lot of pathologies may occur. A few representative examples are shown in the following table (adapted from Potempa et al. 2000):

<table>
<thead>
<tr>
<th>Disease</th>
<th>The cause of unregulated proteolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>Overexpression of matrix metalloproteinases</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>Either enhanced expression of matrix metalloproteinases, or release of neutrophil serine proteinases</td>
</tr>
<tr>
<td>Septic shock</td>
<td>Uncontrolled activation of the zymogens of coagulation and fibrinolysis cascades, and/or neutrophil degranulation</td>
</tr>
<tr>
<td>Tumor invasion and metastasis</td>
<td>Excessive expression of matrix metalloproteinases</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Release of large amounts of elastase and matrix metalloproteinases by neutrophils accumulating at the site of infection</td>
</tr>
</tbody>
</table>
1.2 Progression of the periodontal inflammatory process

Periodontitis is described as chronic inflammation culminating in the destruction of tissue and/or bone. Presented schematically and extremely simplified the inflammatory process proceeds as follows:

(1) **Triggering stimulus**
   - e.g. supra- or subgingival bacteria

(2) **Release of bacterial mediators**
   - mainly lipopolysaccharide (LPS)

(3) **Attraction and activation of inflammatory cells**
   - e.g. macrophages, polymorphonuclear granulocytes (PMN)

(4a) **Release of tissue (autologous) mediators**
   - such as prostaglandins and interleukins

(4b) **Activation of matrix-metalloproteinases (MMPs)**
   - i.e. of tissue-destroying collagenases

This process is controlled or inhibited by

(5a) **Regulation of MMPs by “tissue inhibitors of metalloproteinases” (TIMPs)**

(5b) **Suppression of MMPs with sub-dose doxycycline (SDD, LDD)**

(6) **Periodontal therapy** by removing the triggering stimulus (1),
   the bacterial biofilm.

These highly complex interrelationships are described in various review articles (e.g. Reynolds 1996, Page & Kornman 1997, Birkedahl-Hansen 1993, 1998, Sorsa et al. 2014; etc.).
1.3 MMP-8: The oral matrix metalloproteinase in sulcus fluid

It is undisputed that MMP-8 represents the most important detectable matrix-metalloproteinase in gingival crevicular fluid (GCF) for periodontopathic processes (Birkedal-Hansen 1998, Sorsa et al. 2006, [Sorsa et al. 2014 in preparation], and others), see corresponding citations from review articles:

- **Reynolds et al. 1994** "members of the family of MMPs are key enzymes in matrix degradation"
- **Teronen et al. 1997** "Of the known interstitial collagenses … MMP-8 is known to be predominant in the most common form of periodontal disease, adult periodontitis (AP) …"
- **Sorsa et al. 1999** "MMPs, especially collagenase-2 (MMP-8), are key mediators of irreversible tissue destruction associated with periodontitis and peri-implantitis. MMP8 is known to exist in elevated amounts and in active form in the gingival crevicular fluid (GCF) and peri-implant sulcular fluid (PISF) from progressing periodontitis and peri-implantitis lesions and sites, respectively."
- **Kinane 2000** "matrix metalloproteinase activity is associated with inflammation related to both gingivitis and periodontitis."
- **Sorsa et al. 2004** "The role of collagenase, especially MMP-8, in periodontitis and peri-implantitis is the best-known example of the unwanted tissue destruction related to increased presence and activity of MMPs at the site of disease"
1.4 Different forms of matrix-metalloproteinases

MMP-8 is stored in sub-cellular, specific granules of mature human PMNs in peripheral blood (Hanemaaijer et al. 1997). The PMN leukocytes liberate their MMPs by granule release within seconds (Birkedahl-Hansen 1993). This means that these proteinases are available in an inactive pre-form and are very quickly activated on demand. In simplified terms, MMPs are actually available in 3 different forms (Nagase 1997, Palosaari 2003, Palosaari et al. 2003, Sorsa et al. 2004):

(a) in a latent, inactive pro-enzyme or zymogene form
(b) after cleavage of a C-terminal peptide in an active form,
(c) which is deactivated by TIMPs (inactive form).

Fine tuning of the tissue destruction is therefore dependent on the relationship of MMPs to TIMPs (Nomura et al. 1998, Page et al. 1997, Sorsa et al. 2014), which is described as a “delicate balance”. The MMPs may be enhanced due to the inflammatory processes in relation to the TIMPs already existing in the tissue. In this respect it should be noted that only the active form of the enzyme is of significance in periodontal tissue destruction. In order to detect this singularly important form of MMP-8 monoclonal antibodies have been produced (Mabs 8708 and 8706; Hanemaaijer et al. 1997, Sorsa et al. 1999, 2002), which recognize mainly the active collagenase.

Concerning this digest the active form of MMP-8 will be named aMMP-8.

2. aMMP-8 in Gingival Crevicular Fluid (GCF) – Periodontitis

2.1 Differentiation of patient groups

During the last decade it was proven in several independent international publications that the quantitative site-specific assessment of aMMP-8 from GCF allows the researcher and the clinician to distinguish significantly between healthy sites, sites with gingivitis and those with periodontitis (Mäntylä et al. 2003, Prescher et al. 2007, Leppilahti et al. 2014, Sorsa et al 2014). Because, in detail, different technologies and calculation regimes where used by different research groups (Sorsa et al. 2010) the mere values of aMMP-8 from GCF are not always directly comparable.

Unequivocally, however, any of the corresponding studies has shown
- Very low aMMP-8 values at healthy sites;
- Slightly elevated, physiological aMMP-8 levels in case of gingivitis;
- Strongly increased pathologic aMMP-8 values in cases of (untreated) periodontitis (Prescher et al. 2007), with quite high standard deviations (Mäntylä et al. 2003);
- and a significant decline of aMMP-8 levels after periodontal therapy (cf. paragraph 4.)

2.2 Predictive value of aMMP-8

It should be noted that GCF-derived aMMP-8 levels possess a predictive value. Sorsa et al. (2010) presented a comparison of methods to assess MMP-8, mainly the "immune-fluorometric assay" (IFMA) used in all investigations from the Sorsa group and with the dagnostics system (for examples see Munjal et al. 2007, Prescher et al. 2007, Kraft-Neumärker et al. 2012). In this context, periodontitis patients were examined over a course of 12 months at 2 month intervals. In these patients it was possible to clearly differentiate "stable sites" from "unstable sites":

- "Stable sites": Improvement in pocket depth (PD) and attachments (AL: attachment loss) were continuously preserved after treatment, similarly the aMMP-8 values were and remained consistently low;
- "Unstable sites": No improvement or only temporary improvement in PD and AL were found, in parallel aMMP-8 only improved shortly after treatment, followed by an immediate re-increase in the aMMP-8 values.

Moreover Reinhardt et al. (2010) state, for example, that increases in MMP-8 during the first year of periodontal maintenance are associated with increased odds of subsequent periodontal attachment loss. Thus these authors conclude that elevated biomarkers of inflammation and bone resorption identify patients vulnerable to progressive periodontitis.
3. aMMP-8 in Peri-implant Sulcular Fluid (PISF) – Peri-Implantitis

3.1 Assessing peri-implantitis and peri-implant mucositis

Ma et al. (2000) looked for collagenases (i.e. aMMP-8) in different categories of peri-implant vertical bone loss. According to the short table of results shown below, the authors summarize: “The loosening of dental implants is associated with vertical bone loss … we measured collagenase-2 (MMP-8) (MABs 8706, 8708) … in PISF in 49 implant sites….Gingival Index did not correlate with the category of bone loss (p > 0.05). Collagenase-2 (was) higher (p < 0.05) in the group which had lost > 3 mm of bone than in the two other groups. Gingival Index is not a clinically important marker for bone loss, but collagenase-2 … in peri-implant sulcus fluid …. “

<table>
<thead>
<tr>
<th>Category Bone Loss:</th>
<th>&lt; 1 mm</th>
<th>1-3 mm</th>
<th>&gt; 3 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>0.6</td>
<td>0.6</td>
<td>1.2</td>
</tr>
<tr>
<td>MMP-8</td>
<td>861</td>
<td>1265</td>
<td>2021</td>
</tr>
</tbody>
</table>

Another study conducted by Xu et al. (2008) clearly underlines the significance of the aMMP-8 biomarker in peri-implantitis (data from the results section and discussion from Xu et al. 2008):

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Gingiva Index</th>
<th>Pocket depth</th>
<th>Bone loss*</th>
<th>Collagenase activity (rel. units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>0.6</td>
<td>3.3</td>
<td>none</td>
<td>1</td>
</tr>
<tr>
<td>Moderate periodontitis</td>
<td>1.6</td>
<td>5.6</td>
<td>all</td>
<td>80</td>
</tr>
<tr>
<td>Severe periodontitis</td>
<td>2.0</td>
<td>5.9</td>
<td>all</td>
<td>78</td>
</tr>
<tr>
<td>Healthy implant</td>
<td>1.0</td>
<td>2.4</td>
<td>none</td>
<td>1</td>
</tr>
<tr>
<td>Peri-implantitis</td>
<td>2.0</td>
<td>5.0</td>
<td>all</td>
<td>971</td>
</tr>
</tbody>
</table>

* Bone loss: Radiographically visible bone loss in none (healthy) or in all (periodontitis / peri-implantitis) for determination of collagenase activity selected sites.

The difference factor of 80 between “healthy” and “periodontitis” is in line with the spectrum of values as found in diverse research groups. In the case of peri-implantitis, a further spread
by an additional factor of 10 times could be calculated. The authors draw the conclusion: “… this study … is the basis for MMP-8 based … peri-implantitis diagnostics.”

### 3.2 Experimental peri-implant mucositis

The purpose of the investigation of Salvi et al. (2012) was to estimate the concentrations of MMP-8 in GCF of healthy teeth and corresponding PISF at healthy implants in the same 15 patients during the course of a so called “Experimental Gingivitis” (EG), i.e. a time of 21 days of neglected mechanical tooth brushing. Because peri-implant samples were also compared, this study is also an “Experimental Mucositis” (EM) investigation. Moreover the authors measured the concentrations of MMP-8 after reestablishment of the mechanical oral hygiene.

**Outcome:**

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td>72</td>
<td>303</td>
<td>507</td>
<td>884</td>
<td>282</td>
<td>308</td>
<td>158</td>
</tr>
<tr>
<td>MG</td>
<td>438</td>
<td>988</td>
<td>1762</td>
<td>1678</td>
<td>476</td>
<td>948</td>
<td>822</td>
</tr>
</tbody>
</table>

Legend: EG: Experimental Gingivitis: No mechanical oral hygiene starting day 0 until day 21, thereafter restart of oral hygiene; MG: Experimental Mucositis analogous to EG. Values are medians of MMP-8 in pg / site.

- At day 0 the MMP-8 values in healthy (!) PISF are much higher than in healthy GCF.
- In both “groups” the MMP-8 values increase only due to non-brushing of 21 days. Thus MMP-8 monitors even this slight challenge accordingly.
- In case of EG this increase is reversible (according to the definition of gingivitis / Experimental Gingivitis), what holds only partly true in the case of EM.

**In sum,** the investigations of Salvi et al. (2012) and Xu et al. (2008) compared GCF from teeth directly with PISF from implants. In any case, peri-implant mucositis or peri-implantitis, the implant-related MMP-8 values are markedly higher than these related to teeth / periodontitis.
3.1 Peri-implantitis and peri-implant mucositis: relating MMP-8 and clinical findings

Generally it is very important to recognize these high concentrations of aMMP-8 in PISF as a tremendous challenge. It is to deduct therefrom that inflammatory peri-implant processes should run faster than those in periodontitis. In fact that is in line with clinical findings as reviewed and reported in the “Consensus of the 7th European Workshop on Periodontology” (Lang and Berglundh 2011, Berglundh et al. 2011), where the authors state that a ´self-limiting´ process exists in the tissues around teeth, resulting in a protective capsule. However, such a ´self-limiting´ does not occur in peri-implant tissues, so the lesions extend to the bony crest. It is claimed furthermore that peri-implant disease, in general, progress faster than periodontitis. The action of MMP-8 offers the physiologic explanation for the clinical outcome.

4. aMMP-8 concentrations during therapeutic measures – Reaction of the aMMP-8 concentration in GCF regarding therapy

SRP (Scaling / Root Planing) is the most frequently practiced method and the general first step in initiating periodontal therapy. Hence there are several studies relating the aMMP-8 concentration to the treatment outcome. Unequivocally it was proven in all of these studies that the levels of aMMP-8 drop significantly after therapy (Chen et al. 2000, Kinane et al. 2003, Mäntylä et al. 2003, Emingil et al. 2004; for review cf. Sorsa et al. 2014). For example Chen et al. (2000) found significant reductions in the MMP-8 per sample two weeks after SRP. MMP-8 and elastase activity correlated significantly. Significant correlations of both enzymes/activities existed with the clinical parameters GI (Gingiva Index) and BI (Bleeding Index), and partly with PD (pocket depth). As well, Kinane et al. (2003) established significant reductions in the MMP-8 concentrations (p < 0.005) 6-8 weeks after SRP. Following further periodontal therapy the MMP-8 values improved even more, after 3 months highly significant (p < 0.001) differences existed to the initial findings. Last not least Mäntylä et al. (2003) reported significant reductions in MMP-8 concentrations after SRP among periodontitis patients, including a sub-group with positive BOP. Thus the quantitative aMMP-8 diagnosis represents a strong tool to monitor any treatment effect.
5. aMMP-8 concentrations in saliva and/or mouth rinse samples

The ability to discriminate healthy subjects from patients with gingivitis / periodontitis holds also true for saliva specimen and / or mouth rinse samples. However, less work was done in this research area, and most of the studies deal with saliva (e.g., Gursoy et al. 2010, 2011, 2013, Salminen et al. 2014). Ramseier et al. (2009), assessing different biomarkers in saliva, stated that salivary MMP-8 is the most relevant biomarker concerning periodontitis. As well Leppilahti et al. (2011) used different methods to quantify aMMP-8 from mouth rinse samples. Moreover they calculated a Periodontal Inflammatory Burden Index (PIBI) to categorize the tested subjects. ROC analysis clearly showed that the aMMP-8 quantification differentiated the study group with strong periodontal inflammatory burden from those with lower levels.

Just recently Nwhator et al. (2014) used a qualitative lateral flow (LF) immunoassay (Perio Marker®, Dentognostics GmbH, Jena, Germany) containing MABs 8708 and 8706 (as already described in section 1.4) to discriminate diverse patient groups. In strong accordance with Leppilahti et al. (2011) the LF point-of-care immunoassay differentiated patients from “good”, “fair” and “poor” oral hygiene categories. A 96 % sensitivity was established by these authors regarding poor oral hygiene, as well as a 95 % sensitivity for chronic periodontitis (defined as at least two sites per person with periodontal pockets). From both studies it is to conclude that mouth rinse samples could be used to reveal patients in risk for acute or further ongoing periodontitis.
Literature


Palosaari H: Matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) in mature human odontoblasts and pulp tissue. PhD Thesis, Oulun Yliopisto, Oulu 2003


Sorsa et al. 2014 – in preparation, submitted


Woessner JF Jr. MMPs and TIMPs – An historical perspective. Molecular Biotechnology 2002; 22: 33-49