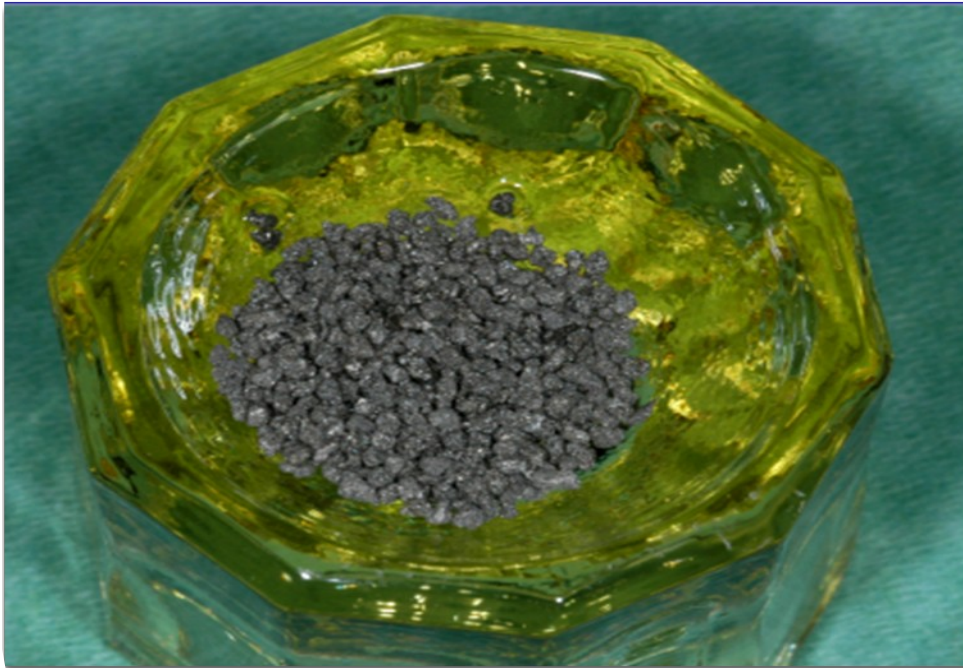


## Surgical treatment of peri-implantitis and its effects on Matrix metalloproteinase 8 (MMP-8) levels



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**Objective:** A case-control study was performed to examine possible differences in MMP-8 levels before and after surgical treatment of peri-implant osseous defects with porous titanium granules. **Method:** Subjects with osseous defects around dental implants ( $n = 36$ ) were treated with two different methods of peri-implantar therapy. One control group ( $N=17$ ) were treated with conventional peri-implantar operation (open flap debridement), whilst the case group ( $N=19$ ) was treated with porous titanium granules in addition to open flap debridement. Samples of peri-implantar sulcus fluid were collected before treatment and at one-year follow-up. The level of MMP-8 were tested in each samples using ELISA. The results are presented as the ratio between MMP-8 and total protein in each individual sample. **Results:** There was a significant reduction in MMP-8 levels after one year compared to before surgery ( $N = 35$ ), however the observed 25% reduction within each group (case and control) failed to be significant. There was no difference in the MMP-8 levels between the control and case group after one year. **Conclusions:** There is a reduction in MMP-8 levels in peri-implantar sulcus fluid after surgical treatment; however there was no difference between the two treatment strategies.

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## 1 Introduction/ background

Peri-implantitis is an inflammation round the supporting tissue of dental implants, with resulting destruction of this surrounding tissue and eventually loosening of the implant. A correlation between peri-implantitis and other diseases as endocarditis has also been described<sup>1</sup>.

Between three and five percent of dental implants are lost during the first five years after installation. The main risk factors for implant loss are smoking, short implant length<sup>2</sup> and history of periodontitis<sup>3</sup>.

The inflammation may aetiologically be considered as a mixed bacterial infection, where the subsequent inflammation through time destroys the surrounding tissue<sup>4</sup>. This is due to the hosts response to microbial endo- and/ or exotoxins. This loss has several inflammatory causes, including

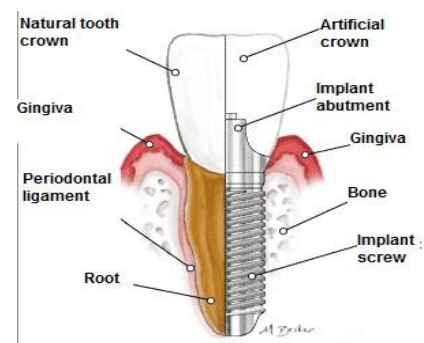
- Destruction of bone through Receptor Activator for Nuclear Factor  $\kappa$  B Ligand (RANK-L)- activated osteoclasts (several inflammatory mediators acts as RANK-ligand)
- Destruction of collagen by MMP-8/neutrophil collagenase from polymorphonuclear leukocytes (PMN)
- Destruction of collagen through bacterial enzymes.

It has been done more research on periodontitis than peri-implantitis regarding terms of bone degradation and biofilms, but there are great similarities between the two diseases. For example, studies of biofilms around dental implants and teeth have not shown any significant differences between their structure and composition, neither in cross-sectional nor longitudinal studies<sup>5</sup>.

### 1.1 Concepts

The following terms are widely used in the paper, and are reviewed in the following sections

Dental implant: Artificial and tissue friendly substitute for a tooth. It consists of a fixture (usually made of titanium) screwed into jaw bone. The bone modulates around it, so that the fixture is osseointegrated. After a few months without load, an abutment (short extendor pipe) is integrated with this system, after which fixed prosthodontics (crowns, bridges et cetera) can be attached on top (figure 1)<sup>6</sup>.



*Figure 1: Comparison between dental implant and natural tooth (basis from <http://chandigarhdentist.com/implants/images/PartsOfImplant.gif>)*

<sup>1</sup> [Loesche WJ, Journal of Dental Research 1996; 75: 1432](http://jdr.sagepub.com/cgi/reprint/75/7/1432.pdf) (<http://jdr.sagepub.com/cgi/reprint/75/7/1432.pdf>) (last visited 13.5.2010)

<sup>2</sup> Baelum, V. and Ellegaard, B, The Journal of Periodontology 2004; 75 (10); 1404-1412

<sup>3</sup> Roos-Jansåker A-M. et al, The Journal of Clinical Periodontology 2006; 33; 283-289

<sup>4</sup> Lindhe, J. (2003) *Clinical Periodontology and Implant Dentistry* (4.th edition). Oxford: Blackwell Publishing Ltd page 163

<sup>5</sup> Lindhe, J. (2003) *Clinical Periodontology and Implant Dentistry* (4.th edition). Oxford: Blackwell Publishing Ltd page 98

<sup>6</sup> Øyri, A. (2003) *Norsk medisinsk ordbok* (7.th edition). Oslo: Det Norske Samlaget. Entry: «Implantat»

Endotoxins: Toxins inside the bacterial bodies<sup>7</sup>.

Exotoxines: Toxins on the bacterial surface<sup>8</sup>.

Receptor Activator for Nuclear Factor  $\kappa$  B Ligand (RANK-L) also known as TNF-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL), and ODF (osteoclast differentiation factor), is a molecule important in bone metabolism. This natural and necessary surface-bound molecule found on osteoblasts serves to activate osteoclasts, which are the cells involved in bone resorption (Yasuda, H. et al 1998 March 31; 95(7): 3597–3602)<sup>9</sup>.

Osteoclast: Multi-nucleated giant cells which contributes to bone degradation by proteolytic digestion (hydrolysis of peptide bonds)<sup>10</sup>.

Biofilm: Coating/ plaque on surface, consisting of bacteria, their products and particles/ matter from the host/ the surroundings<sup>11</sup>.

PMN (PolyMorphoNuclear leukocytes): A subgroup of leukocytes (white blood cells), filled with granules of toxic chemicals that enable them to digest micro-organisms by phagocytosis. These cells are important contributor to the innate immediate immune system. Move towards the site of inflammation by means of chemotaxis (movement against a chemical gradient).<sup>12</sup>.

Inflammation: Tissue response to infection and trauma characterized by the four cardinal signs *dolor* (pain), *calor* (heat), *rubor* (redness) and *tumor* (swelling). In addition, sometimes *functio laesa* (loss of function) is added. The clinical symptoms are due to increased blood perfusion and vasopermeability, and increased leakage from the capillary walls because of greater extravasal concentration of proteins and leucocytes (white blood cells)<sup>13</sup>.

PISF (Peri-Implantar Sulcus Fluid): GCF (Gingival Crevicular Fluid)- analogue around implants, id est various plasma proteins, tissue proteases, inhibitors and breakdown products and leukocyte enzymes<sup>14</sup>.

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<sup>7</sup> Øyri, A. (2003) *Norsk medisinsk ordbok* (7.th edition). Oslo: Det Norske Samlaget. Entry: «Endotoksin»

<sup>8</sup> Øyri, A. (2003) *Norsk medisinsk ordbok* (7.th edition). Oslo: Det Norske Samlaget. Entry: «Eksotoksin»

<sup>9</sup> Yasuda, H. et al, Proceedings of the National Academy of Sciences of U S A. 1998 March 31; 95(7): 3597–3602 (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC19881/>)

<sup>10</sup> Øyri, A. (2003) *Norsk medisinsk ordbok* (7.th edition). Oslo: Det Norske Samlaget. Entry: «Osteoclast»

<sup>11</sup> Øyri, A. (2003) *Norsk medisinsk ordbok* (7.th edition). Oslo: Det Norske Samlaget. Entry: «Biofilm»

<sup>12</sup> <http://www.hon.ch/Library/Theme/Allergy/Glossary/wbc.html>, Last visited 18.5.2010

<sup>13</sup> Øyri, A. (2003) *Norsk medisinsk ordbok* (7.th edition). Oslo: Det Norske Samlaget. Entry: «Betennelse»

<sup>14</sup> Lindhe, J. (2003) *Clinical Periodontology and Implant Dentistry* (4.th edition). Oxford: Blackwell Publishing Ltd page 58

## 1.2 Anatomy of the peri-implantium

The peri-implantium consists of the gingiva and the alveolar bone proper. Histologically these tissues are made up of the following main cells and structures:

Gingiva (its lamina propria): Usually it consists of approximately 40 percent epithelium and 60 percent connective tissue. The connective tissue have the following components: 60 percent collagen (type I, II and III), 13 percent fibroblasts, seven percent vessels and 20 percent other tissues (intercellular matrix and nerves et cetera)<sup>15</sup>

Alveolar bone proper (id est the part of bone surrounding the implant, that is the bone-implant interface): A mineralized structure consisting of inorganic and organic matter. The organic part is composed of collagen type I and ground substance (different types of proteins)<sup>16</sup>.

## 1.3 MMPs

Matrix MetalloProteinases (MMPs) makes up a group of zinc and calcium dependent endopeptidases which degrades different types of ExtraCellular Matrix (ECM) depending on type of MMP. They have different physiological tasks, which range from natural tissue remodeling to pathological conditions like cancer.

The proteolytic activity of MMPs are regulated by activation of proenzymes and inhibition of active enzymes by endogen inhibitors,  $\alpha$ 2- macroglobulin and Tissue Inhibitors of MetalloProteinases (TIMPs)<sup>17</sup>.

MMP-8 (Matrix MetalloProteinase- 8) is a zink dependent collagen splitting MMP which is found in connective tissue all over the body, and is secreted as inactive proenzymes.

There are higher concentrations of MMP-8 in saliva than exempli gratia wound fluid, and precautions have to be made not to contaminate PISF- samples with this<sup>18</sup>.

The enzymes get activated by autolysis (by the enzyme itself) or other factors as oxygen radicals and proteinases in secondary granules of neutrophils.

MMP-8 splits collagen I, II and III<sup>19</sup>.

## 1.4 Enzymatic diagnostics

Enzyme diagnostics is a relatively recent field in medicine, and measures intra cellular enzymes released to tissue fluid and plasma during tissue damage. This is done to tell something about

- which organ/ organ system that is affected
- the extent of the disease and/ or
- monitor progression/ development of a disease<sup>20</sup>

<sup>15</sup> Lindhe, J. (2003) *Clinical Periodontology and Implant Dentistry* (4.th edition). Oxford: Blackwell Publishing Ltd page 19

<sup>16</sup> Janqueira, L. C. (1998) *Basic Histology* (9.th edition). Rio deJaneiro: Editoria Guanaharu Koogan S. A. page 137

<sup>17</sup> <http://www.rndsistemas.com/pdf/dmp800.pdf> page 3 (last visited 13.5.2010)

<sup>18</sup> <http://www.rndsistemas.com/pdf/dmp800.pdf> page 13 (last visited 13.5.2010)

<sup>19</sup> <http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=4317> (last visited 13.5.2010)

<sup>20</sup> Stokke, O. (2006). *Klinisk biokjemi og fysiologi* (3.rd edition). Oslo: Gyldendal Norsk Forlag AS page 37-38



The idea is that deviations from normal enzyme patterns can give a hint about the reason for the deviation, how serious the deviation is and/ or give the disease a quantifiable parameter (in the shape of one or more enzymes) to follow its progression.

The MMP-8 system consists, as mentioned, of a constitutive expressed extra cellular proenzyme that lyses by different influences, and not intra cellular enzymes that are released during tissue damage. It is therefore not enzyme diagnostics in narrow sense of the term. It is still appropriate to use this terminology, because one uses enzyme changes to assess extent and progression of disease

## 1.5 ELISA

ELISA (Enzyme-Linked Immuno Sorbent Assay) is a biochemical survey method which is used for quantitative measurements of proteins and other molecules that can stick to an enzyme. ELISA is considered a good method amongst other biochemical laboratory techniques in terms of sensitivity and specificity<sup>21</sup>.

The technique involves microtiter wells that are covered by monoclonal antibodies that are specific for the substance that are to be analyzed. When the samples are added to these wells, the substance of interest will stick to the antibodies; thereafter another monoclonal antibody is added. This binds to another epitope (molecular site), and is connected with yet another molecule (with specific light absorbing qualities) for later measurement of absorbance.

The sensitivity is thus partly dependent on the antibodies' affinity to the epitopes. The colour change that occurs is proportional to the amount of bound protein, and the concentration of this is determined by comparison with standard curve of known concentrations of cytokines.

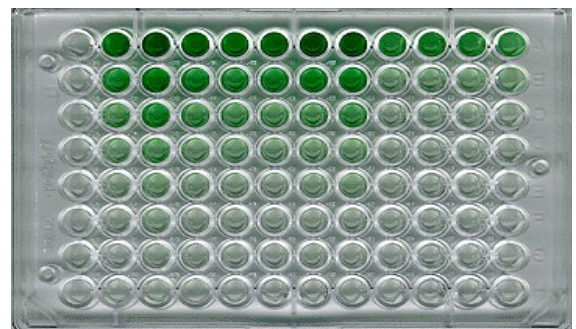


Figure 2 Microtiter plate (from [http://www.biology.arizona.edu/immunology/activities/elisa/elisa\\_intro.html](http://www.biology.arizona.edu/immunology/activities/elisa/elisa_intro.html))

<sup>21</sup> <http://www.mabtech.com/main/Page.asp?PagelD=26&PageName>About+ELISA> (last visited 13.5.2010)

## 1.6 Surgical treatment and porous titanium granules

Conventional surgical procedure for treatment of peri-implantitis is to elevate a flap at the implant site, and clean the surface («open flap debridement») by means of hand instruments and H<sub>2</sub>O<sub>2</sub>.

In the later years several additional methods and techniques have been developed to treat and heal osseous defect. Amongst these are

- regenerating enzymes (emdogain and others)
- bone grafts from other species (bio-oss and others)
- allotransplantation (bone grafts from the same patient)
- different materials to support osteoconduction (porous titanium granules and others)

Porous titanium granules are swamp-like «rocks» that are thrombogenic and able to make blood clot. When mixed with exempli gratia blood, the granulae stick to each other. The granulae-clot- complex promotes osteoblast colonization and creates a pattern for new bone growth<sup>22</sup>.



*Figure 3. Osseous defect filled with porous titanium granules (with permission from Johan Caspar Wohlfahrt)*

<sup>22</sup> <http://www.biodent-systems.com/TigranBrochure.pdf> (last visited 13.5.2010)

## 2 Materials and methods

PISF samples were taken from 36 patients distributed in two groups (19 case patients and 17 controls) before surgical treatment and on one year follow-up. This was performed by dipping standard endodontic paper points size 50 in the peri-implantar sulcus for ten seconds in every (buccal, mesial, distal and palatal/ lingual) aspect of the implant. The implant area was air dried and isolated with cotton rolls prior to sampling.

Each sample was collected by the same operator, to achieve approximately similar sampling parameters, and minimize inter-operator bias.

The samples were coded, immediately frozen after collection, and stored at minus 80 degrees celcius. The analyses were performed by another operator.

During analysis an operator error to one of the samples, which was later revealed as a control sample, reduced the number of samples in the control group to 16, resulting in 35 paired samples (pre-surgery and one year follow-up), a total of 70 samples.

All tests were performed at Oral research laboratory at the Faculty of Dentistry in Oslo

### 2.1 MMP-8

«Quantikine® Human Total MMP-8 Immunoassay» (DMP800) from R & D Systems was used.

Standards in the range from 0 to 10 ng/ ml was prepared and pipetted to two duplicate wells<sup>23</sup>.

The PISF samples were slowly thawed and centrifuged for one minute, after which it was possible to extract 10µl sample fluid. These were diluted 1/20 with RD5-10 (190 µl RD5-10 to 10 µl sample), of which 50 µl was delivered into marked ELISA wells on same plate as the standard solution. The rest was preserved for measurements of total protein (see later) and future reference.

To each sample-RD5-10- solutions (50 µl) was pipetted 150 µl RD 1-52. The plate was then incubated for two hours with gentle shaking, whereafter the wells were emptied and washed four times with wash buffer. Now 200 µl MMP-8- conjugate was added each well, and incubation pluss gentle shaking in additional two hours. Then 200 µl substrate solution was added, and yet another incubation (30 minutes) was initiated. Finally 50 µl stop solution was added to each<sup>24</sup>.

The microtiter plate was then immediately analyzed using a Cobas Mira chemistry analyzer (Roche Diagnostics, Germany) and absorbance read at 450 nm. The absorbance is given in table 1. A regression line based on the absorbance of the standard solution (figure 5), and the concentrations of MMP-8 was calculated from this using the values of the different wells. It was compensated for dilution (table 1).

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<sup>23</sup> <http://www.rndsystems.com/pdf/dmp800.pdf> page 7 (last visited 13.5.2010)

<sup>24</sup> <http://www.rndsystems.com/pdf/dmp800.pdf> page 8 (last visited 13.5.2010)



		0,03	2,4	0,15	2,43	0,86	0,03	1,4	2,55	0	0,04
10	0,73	0,18	0,27	1,53	3,07	3,19	2,36	0,87	0,12	3,55	
5	0,29	0,33	2,33	0,6	1,68	2,6	0,73	1,54	0,09	-0,01	
2,5	0,18	0,03	0,01	3,24	3,18	3,24	0,52	3,24	0,01	-0,02	
1,25	0,08	1,06	0,33	2,41	0,43	0,48	1,87	0,23	0,18	-0,01	
0,63	0,02	3,17	0,01	0,47	0,06	0,87	3,36	2,95	0,05	-0,02	
0,31	0,01	0,09	0,21	3,2	0,19	1,3	2,79	0,14	3,27	-0,02	
0,16	0,01	0,29	0,15	3,24	0,58	2,69	0,73	3,19	0,1	-0,02	

Table 1. The absorbance values for MMP-8 in each individual samples. As we see, several of the values are above the values of the standard curve [0,73, 0,01]. These values are therefore extrapolated according to the graph.

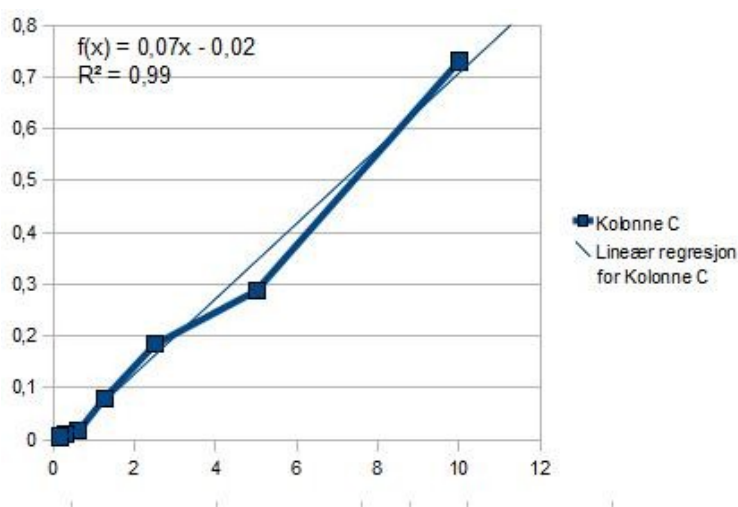


Figure 4. The regression line and calculation formula for MMP-8, based on the absorbance of the standard solutions.

## 2.2 Total proteins

«Pierce BCA Protein Assay Kit» (23225) from Thermo Scientific was used to quantify the amount of proteins in each sample.

Standards in the range from 0 to 2000 µg/ ml was prepared and pipetted to two duplicate wells<sup>25</sup>.

25 µl from each of the previously prepared wells was pipetted onto a new microtiter plate. Substrate solution was prepared by mixing the BCA reagent B and BCA reagent A in a 1/50- relation (380 µl BCA-B to 18620 µl BCA-A). 200 µl of this mixture was added to each well and mixed 5 times by pipetting.

The solution was incubated under gentle shaking and measured for absorbance.

The measured values were beyond the standard, and it was decided to dilute the samples another ten times using BCA-A and-B, to a total dilution of 1 / 80.

The new values were within the standard (Table 2). Another regression line was computed (Figure 6), and the amount of total protein was calculated based on this. It was compensated for the dilution (Table 3)<sup>26</sup>.

<sup>25</sup> <http://www.piercenet.com/files/1296as8.pdf> page 2 (last visited 13.5.2010)

<sup>26</sup> <http://www.piercenet.com/files/1296as8.pdf> page 2-3 (last visited 13.5.2010)

0	0	0,1	1,64	2,11	1,88	1,85	1,92	1,98	2,04	2,02
0,11	0,12	1,97	1,78	1,95	1,96	1,91	1,61	2,02	2,02	1,91
0,21	0,23	1,54	1,72	1,94	1,77	1,9	1,9	2	1,97	2,01
0,35	0,38	1,55	1,77	1,94	1,72	1,8	1,9	2,02	1,96	1,96
0,72	0,72	1,58	1,76	1,98	1,91	1,88	1,79	1,87	1,88	2,16
1,22	1,1	1,88	1,78	1,78	1,8	1,9	1,85	2,03	1,97	2,18
2,35	2,37	1,66	1,78	1,92	1,91	2,1	1,94	1,98	1,77	2,11
0	0	1,53	1,82	1,92	2	1,89	1,96	2,08	2,02	2,13

Table 2: Absorbance (total protein)

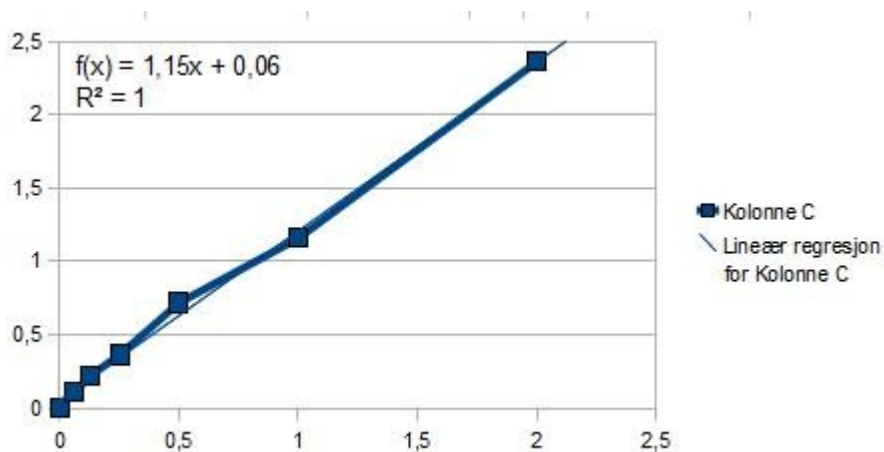


Figure 5: Regression line for absorbance of standard solution (total protein)

## 2.3 Statistics

All calculations were performed using Sigma plot (Sigma Diagnostics St.Louis, USA). The ratio between MMP-8 and total protein content was calculated for each sample and the difference before and after treatment was performed using a paired t-test, whereas the difference between the groups were tested using non-parametrical test (Mann-Whitney Rank Sum Test). If normality test passed, Student t- test was performed. A P-value < 0.05 was considered significant.

### 3 Results

The case and control groups were comparable with respect to age, sex, diabetes, stress level and other relevant factors for peri-implantitis level. The analysis was done blindly by one operator collecting the samples, whilst the analysis was done by an operator who did not know who the samples came from.

There was a significant reduction ( $P = 0.043$ ) in MMP-8/ total protein levels between before (pre) and after surgery (post) for the whole study population ( $n = 35$ ) (figure 6).

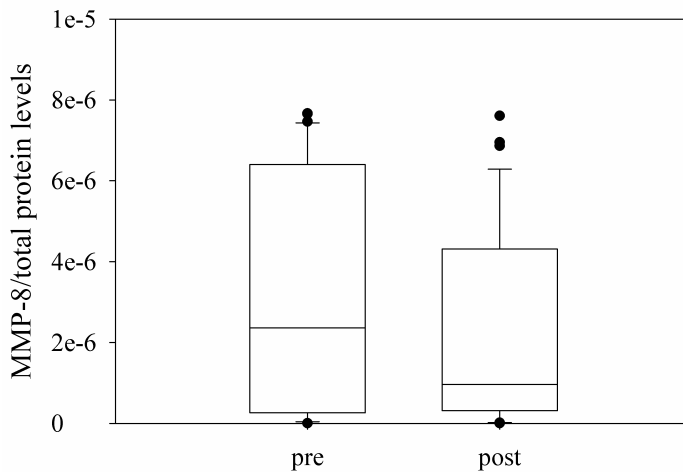


Figure 6. The MMP-8/ total protein levels before (pre) and one year after (post) surgery.

There was no difference between the samples collected before or after the two surgical methods (case and control) (figure 7). It seemed to be a difference in the median value for the two groups prior to surgery, but this was not significant.

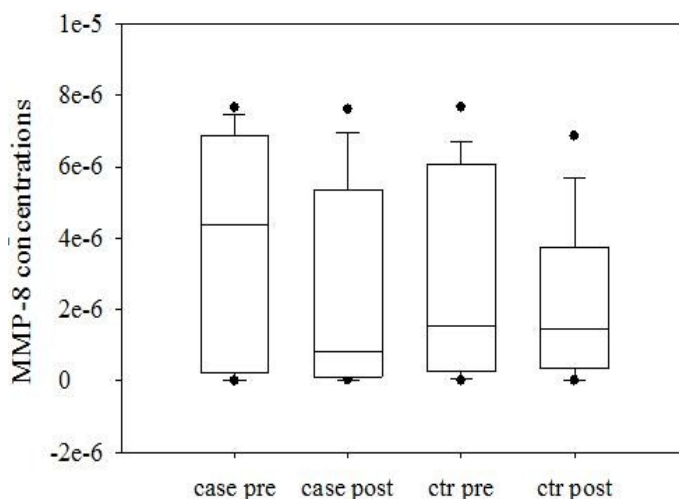
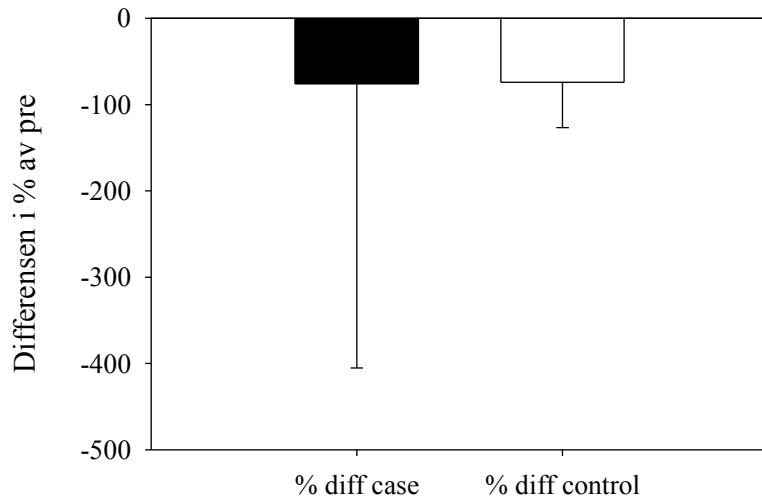


Figure 7: The MMP-8/ total protein levels before (pre) and after (post) the two different surgical procedures (case and control).

There was not a difference in the change in MMP-8/ total protein levels (pre-post) between the two groups (case and control) (Fig. 8).



*Figure 8: The change in MMP-8/ total protein ratios presented in % of level prior to surgery*

## 4 Discussion

We observed a significant reduction in the relative MMP-8 level in samples collected a year after a surgical procedure compared to before in peri-implantitis patients; however we found no difference in MMP-8 levels between the two different surgical methods; conventional surgical procedure (control) and the use of titanium granules (case).

MMPs are catalytic proteins, enzymes capable of cleaving almost all extracellular matrix (ECM) and basement membrane (BM) proteins. MMPs are involved in many physiological processes such as bone formation, tooth eruption, and wound healing. On the other hand, MMP expression and activation is disturbed in many pathological conditions such as cancer<sup>27</sup>, oral cysts<sup>28</sup>, and periodontitis<sup>29</sup>. An enhancement in MMP-8 due to severity of peri-implantitis or oral pathological conditions has been reported; however this is to the authors' knowledge the first observation in reduced MMP-8 levels one year after surgery.

Our results demonstrate that there are changes in PISF composition before and after surgery, and that it may be possible to find markers for the degree of peri-implantar decomposition, early detection of such based on this and/ or monitor treatment outcome. MMP-8 may be such a marker, but this study doesn't say anything about this, since the results were not evaluated with respect to therapeutically outcome.

As suggested by Kuula<sup>30</sup> and Teronen, O. et al<sup>31</sup>, further investigations are needed to determine whether there is a corresponding reduction in MMP-8 levels in terms of periodontal surgery in relation to peri-implantitis surgery.

The cause of MMP-8's candidacy for such a diagnostic role is due to enhanced levels of MMP-8 in peri-implantitis sites. This is associated with extravasations and migration of PMN to sulcus / pocket of inflammation, and that the amount of this is related to the number of activated PMN, id est. the degree of inflammation. A pilot study by Xu et al. from 2008, suggests that PISF have higher MMP-8 levels and activity than GCF from similar deep pockets<sup>32</sup>. None of these studies have, however, examined MMP-8 changes over time.

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<sup>27</sup> Sabeh, F et al, *The Journal of Cell Biology* 2004; 167 (4): 769-781

(<http://jcb.rupress.org/content/167/4/769.abstract>) (last visited 18.5.2010)

<sup>28</sup> Verstappen, J. and Von den Hoff, JW, *Journal of Dental Research* 2006; 85 (12): 1074-1084

(<http://jdr.sagepub.com/cgi/content/abstract/85/12/1074>) (last visited 18.5.2010)

<sup>29</sup> Wahlgren, J. et al, *The Journal of Pathology* 2001; 194 (2); 217-224

(<http://www.ingentaconnect.com/content/jwspath/2001/00000194/00000002/art00854>) (last visited 18.5.2010)

<sup>30</sup> Kuula 2009 (<https://oa.doria.fi/bitstream/handle/10024/45113/periodon.pdf?sequence=1>) (last visited 18.5.2010)

<sup>31</sup> Teronen, O. et al, *Journal of Dental Research* 1997; 76 (9); 1529-1537

<http://jdr.sagepub.com/cgi/content/abstract/76/9/1529> (last visited 18.5.2010)

<sup>32</sup> Xu, L. et al, *Acta Odontologica Scandinavica* 2008; 66 (4); 219-224

(<http://www.informaworld.com/smpp/content~content=a794876592&db=all>) (last visited 18.5.2010)

We found no differences between the two surgical strategies, although a mechanism for an eventually greater reduction in MMP-levels in the case-group might be due to lower levels of PMNs at the implant site because of the titanium granules' anti-inflammatory function<sup>33</sup>. Such an effect might take place because of the formation of titanium dioxide (TiO<sub>2</sub>), which is formed spontaneously on exposed titan. Titanium dioxide has the ability to scavenge Reactive Oxygen Species (ROsS)<sup>34</sup>, which are one of the main activators of MMP-8, and result in less amounts of MMP-8 with the use of titanium granules (or other materials able to make a TiO<sub>2</sub>- layer) in the treatment of osseous defects. The reason why we did not find any such reduction, may be due to that the TiO<sub>2</sub> effect initially was similar for both groups (implants of titanium), and that the use of titanium granules may only provide a negligible additional effect beyond this.

The level of MMP-8 in saliva is higher than the level in blood. Contamination of saliva in our samples might be one of the reasons for some of the high values and the great variation between individual samples in table 1, and one reason for lack of significant differences between the two surgical treatments as the statistical power ended up to be lower than desired. We chose to use all the samples, despite that several of them were outside the values of the standard curve, and some very much above (table 1). This was chosen in order not to reduce the sample size further, although statistical power is very adversely affected by small effect size.

## 5 Acknowledgments

Thanks to my supervisors professor Janne E. Reseland for all her help with laboratory work and the writing process, and Johan Caspar Wohlfahrt (Specialist in Peridontics) for collecting the samples used in this paper.

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<sup>33</sup> [Sahlin, H. et al, Journal of biomedical materials research 2006; 77 \(1\); 43-49](http://www.ncbi.nlm.nih.gov/pubmed/16345099)  
(<http://www.ncbi.nlm.nih.gov/pubmed/16345099>) (last visited 13.5.2010)

<sup>34</sup> [Contreras, R. et al, Journal of biomedical materials research 2007; 80 \(2\); 480-485](http://www.ncbi.nlm.nih.gov/pubmed/17013866)  
(<http://www.ncbi.nlm.nih.gov/pubmed/17013866>) (last visited 13.5.2010)