Effect of combined therapeutical methods on healing of intra-bony defects in regenerative periodontal surgery

Ph.D. Thesis

Dr. Ferenc Dőri D.M.D.

Semmelweis University
Doctoral School of Clinical Medical Sciences

Consultant: Dr. István Gera Ph.D.
Opponents: Dr. Katalin Nagy Ph.D.
Dr. Mihály Orosz Ph.D.

Chairman of the Examination Committee: Dr. Gábor Varga Ph.D.

Members of the Examination Committee: Dr. Márta Ujpál Ph.D.
Dr. Vilmos Tóth Ph.D.

Budapest
2008

Effect of combined therapeutical methods on healing of intra-bony defects in regenerative periodontal surgery

Ph.D. Thesis

Dr. Ferenc Dőri D.M.D.

Semmelweis University
Doctoral School of Clinical Medical Sciences

Consultant: Dr. István Gera Ph.D.

Opponents: Dr. Katalin Nagy Ph.D.
Dr. Mihály Orosz Ph.D.

Chairman of the Examination Committee: Dr. Gábor Varga Ph.D.

Members of the Examination Committee: Dr. Márta Ujpál Ph.D.
Dr. Vilmos Tóth Ph.D.

Budapest
2008

Introduction

True periodontal regeneration means healing after periodontal treatment that results in the regain of lost supporting tissues including new acellular cementum attached to the underlying dentin surface, a new periodontal ligament with functionally oriented collagen fibres inserting into the new cementum and new alveolar bone attached to the periodontal ligament.

The exclusion of epithelial and gingival connective tissue cells following periodontal surgery from the healing area by the use of a physical barrier may allow and guide periodontal ligament cells to repopulate the detached root surface. This observation provided the basis for the clinical application of the treatment principle termed „guided tissue regeneration" (GTR).

Another way to address periodontal regeneration is to mimic the processes that take place during the development of the nascent root and the periodontal tissues. The discovery of the enamel matrix layer between the peripheral dentin and the developing cementum and its function provided the fundamental concept for enamel matrix derivative-supported tissue engineering in regenerative periodontal therapy.

The conception behind the use of bone substitutes and grafts is the assumption that both the regrowth of alveolar bone and the formation of new attachment would be stimulated because these materials may either contain bone forming cells, or serve as a scaffold for bone formation, or because the matrix of the bone grafts contains bone-inducting substances.

The expression of various growth and differentiation factors (GDFs) following bone and soft tissue injury during periodontal disease may regulate the repair and regenerative process. Therefore it is conceivable that growth factors may represent a potential aid in attempts to regenerate the periodontium.

Objectives and studies

General and main objectives of the present clinical investigations are the evaluation of the effect on healing of new combination techniques using different materials and adjuvants in regenerative surgery of intrabony periodontal defects.

Combination of several types of membranes, bone substitutes, growth factors and other modalities like enamel matrix derivatives, root conditioning materials used in various combinations has been studied within the confines of regenerative periodontal surgery with diverse and dissimilar results and conclusions.

The questions examined in the present studies are the following:

1. a. Whether a synthetic graft material (a ß-TCP) with positive maxillo-facial reference is applicable in periodontal regenerative surgery;
   b. Are the clinical outcomes more favourable as in the same method with a natural bone mineral (NBM); (Study I.)
2. If important growth factors-containing autologous platelet-rich plasma enhances the clinical regenerative effect of natural bone mineral and guided tissue regeneration with a non-resorbable membrane (e-PTFE); (Study II.)
3. If platelet-rich plasma enhances the regenerative effect of natural bone bone mineral (NBM) and guided tissue regeneration with a resorbable membrane (collagen); (Study III.)
4. Whether the platelet-rich plasma's growth factors may influence positively the effect on periodontal healing of the synthetic bone graft and a non-resorbable polytetrafluoroethylene membrane; (Study IV.)
5. Is able the platelet-rich plasma (PRP) as a growth factors containing adjuvant to promote the effect of an another protein-mediated regenerative material (EMD) in regenerative surgery of intrabony periodontal defects; (Study V.)
6. Is the platelet-rich plasma capable to enhance the regenerative effect of natural bone mineral as a simple bone substitute material with regenerative potential in periodontal surgery; (Study VI.)

The thesis is based on the following six controled clinical trials:

Study I.: The purpose of this study was to compare the healing of deep intrabony defects following treatment with an enamel matrix protein derivative (EMD) combined with either a natural bone mineral (NBM) or ß–tricalcium phosphate (ß-TCP).

Study II.: The aim of this study was to clinically evaluate the effect of PRP (platelet-rich plasma) on the healing of deep intrabony defects treated with NBM and

GTR by means of a non-resorbable e-PTFE membrane.

Study III.: The aim of this study was to clinically compare treatment outcomes of deep intrabony defects treated either with PRP + NBM + GTR or NBM + GTR using a bioresorbable collagen membrane.

Study IV.: The purpose of this study was to clinically evaluate the effect of PRP on the healing of intrabony defects treated with ß-tricalcium phosphate (ß-TCP) and GTR using a non-bioresorbable e-PTFE membrane.

Study V.: The goal of this study was to compare clinically the treatment of deep intrabony defects with either EMD + NBM + PRP or EMD + NBM.

Study VI.: This study explores the capacity of PRP to enhance the regenerative effect of a natural bone mineral.

	Test	Control
Study I.	EMD + NBM	EMD + ß-TCP
Study II.	PRP + NBM + GTR	NBM + GTR
Study III.	PRP + NBM + GTRres.	NBM + GTRres.
Study IV.	PRP + ß-TCP + GTR	ß-TCP + GTR
Study V.	EMD + NBM + PRP	EMD + NBM
Study VI.	PRP + NBM	NBM

Materials and methods

Synthetic, xenogeneic and autologous materials were used in this randomized clinical studies. Mechanical barriers (polytetrafluoroethylene and collagen membranes) for GTR, biological barriers/enamel matrix proteins (EMD), synthetic (ß-TCP) and xenogeneic (NBM) bone grafts and autologous platelet-rich plasma (PRP) were combined in the test and control groups of the trials. For GTR we used non-resorbable membranes: e-PTFE Gore Tex Periodontal Material® (W. L. Gore & Associates, Inc., Flagstaff, AZ, USA) and resorbable barriers: Bio-Gide Perio® (Geistlich, Wolhusen, Switzerland), for enamel matrix proteins-mediated tissue engineering was used the Emdogain® (Straumann, Waldenburg, Switzerland, before BIORA, Malmö, Sweden). As xenogeneic bone graft the Bio-Oss® (Geistlich, Wolhusen,

Switzerland), as synthetic bone substitute the Cerasorb® (Curasan, Kleinostheim, Germany) were used. The PRP preparation was performed by using a standardized kit immediately prior to operation (Curasan PRP kit, Curasan, Kleinostheim, Germany).

Main clinical variable was the clinical attachment level (CAL) and subsidiary the probing pocket depth (PPD), estimated at baseline and after one year.

The steps of the surgical procedure were as follows:

- local anaesthesia, full flap preparation, curettage of the pocket and the defect, scaling, root planing
- preparation of the platelet-rich plasma during the surgery (Studies II.-VI.)
- root conditioning and application of EMD and EMD + graft or graft with or without PRP on root surface, respectively in defect (Study I. and V.)
- application of bone substitutes or of the combined materials in the defect and their coverage with barrier membrane (Study II., III. and IV.), respectively the application of graft with or without PRP in defect (Study VI.)
- sutures
- pre- and postoperative use of chlorhexidine 0.2% and postoperative antibiotics per os
- removal of the non-resorbable membranes after 6-8 weeks (Study II. and IV.)

Results

Eleven periodontal regenerative methods were evaluated in six randomized controled clinical trials. 162 cases were treated in parallel studies which compared two methods within the frameworks of one trial.

The summation of the results after the statistical analysis takes cognizance of the followings: a) each of the eleven regenerative methods evaluated (ten combined procedures) leads to significant CAL gain and PPD decrease; b) using ß-TCP or NBM with EMD or with PRP + GTR and GTR's, the difference between the parameters of the test and control groups were not statistically significant; c) in four studies was confirmed that the addition of PRP to graft materials has not increased significantly the positive outcomes independent from type of barrier or graft; d) adding platelet-rich plasma to natural bone mineral no benefit was observed from point of view of the clinical variables; e) the polypeptide proteins of the platelet-rich plasma does not enhance the clinical regenerative effect of enamel matrix proteins.

The difference between the average CAL gain and PPD reduction of the test and control groups were statistically not significant in each study. (See the tables below).

Study I.

	Baseline	1 Year	Difference	Significance
PPD				
EMD + NBM	7.9 ± 1.0	3.2 ± 0.6	**4.8 ± 0.9**	p<0.001
EMD + ß-TCP	7.8 ± 1.2	3.2 ± 0.9	**4.6 ± 0.8**	p<0.001
			NS	
CAL				
EMD + NBM	8.8 ± 1.1	4.5 ± 0.6	**4.3 ± 0.8**	p<0.001
EMD + ß-TCP	8.8 ± 1.2	4.7 ± 1.2	**4.1 ± 0.8**	p<0.001
			NS	

Study II.

	Baseline	1 Year	Difference	Significance
PPD				
PRP + NBM + GTR	8.6 ± 1.7	3.1 ± 1.7	**5.5 ± 1.2**	p<0.001
NBM + GTR	8.8 ± 1.7	3.1 ± 1.0	**5.7 ± 1.2**	p<0.001
			NS	
CAL				

PRP + NBM + GTR	10.3 ± 1.4	5.7 ± 1.6	**4.7 ± 1.1**	p<0.001
NBM + GTR	10.4 ± 2.6	5.9 ± 1.8	**4.6 ± 0.8**	p<0.001
			NS	

Study III.

	Baseline	1 Year	Difference	Significance
PPD				
PRP + NBM + GTRres	8.9 ± 2.3	3.4 ± 2.0	**5.5 ± 1.3**	p<0.001
NBM + GTRres	8.9 ± 2.5	3.4 ± 1.0	**5.5 ± 1.7**	p<0.001
			NS	
CAL				
PRP + NBM + GTRres	10.9 ± 2.2	6.4 ± 1.8	**4.5 ± 1.1**	p<0.001
NBM + GTRres	11.1 ± 2.5	6.5 ± 2.3	**4.6 ± 1.1**	p<0.001
			NS	

Study IV.

	Baseline	1 Year	Difference	Significance
PPD				
PRP + ß-TCP + GTR	9.1 ± 0.6	3.3 ± 0.5	**5.8 ± 0.6**	p<0.001
ß-TCP + GTR	9.0 ± 0.8	3.6 ± 0.9	**5.4 ± 0.7**	p<0.001
			NS	
CAL				
PRP + ß-TCP + GTR	10.1 ± 1.3	5.7 ± 1.1	**4.1 ± 0.7**	p<0.001
ß-TCP + GTR	9.9 ± 1.0	5.9 ± 1.2	**3.9 ± 0.9**	p<0.001
			NS	

Study V.

	Baseline	1 Year	Difference	Significance
PPD				
EMD + NBM + PRP	8.8 ± 1.9	3.1 ± 0.9	**5.8 ± 1.8**	p<0.001
EMD + NBM	8.8 ± 2.0	2.8 ± 1.6	**5.9 ± 1.3**	p<0.001
			NS	
CAL				
EMD + NBM + PRP	10.8 ± 2.0	6.0 ± 1.5	**4.8 ± 1.3**	p<0.001
EMD + NBM	10.5 ± 1.6	5.5 ± 1.4	**5.0 ± 0.9**	p<0.001
			NS	

Study VI.

	Baseline	1 Year	Difference	Significance
PPD				
PRP + NBM	8.6 ± 1.8	3.4 ± 1.4	**5.2 ± 1.8**	p<0.001
NBM	8.5 ± 2.0	3.1 ± 1.3	**5.3 ± 2.0**	p<0.001
			NS	
CAL				
PRP + NBM	10.2 ± 2.0	5.5 ± 1.9	**4.7 ± 1.9**	p<0.001
NBM	9.3 ± 2.7	4.9 ± 2.0	**4.4 ± 1.7**	p<0.001
			NS	

Conclusions

Analysing the results of each study and synthesizing the clinical outcomes of eleven regenerative (including ten combined modalities of periodontal tissue engineering) methods, it may be concluded the following inferences:

1. a. A synthetic graft material, a beta-tricalcium phosphate used with positive references in maxillo-facial surgery is successfull applicable in periodontal combined regenerative techniques; (Study I. and IV.)
   b. The clinical parameters obtained are not favourable as in case of using the same technique with a natural bone mineral; (Sudy I.)
2. Important growth factors-containing autologous platelet-rich plasma does not enhance the regenerative effect of a natural bone mineral and a non-resorbable membrane; (Study II.)
3. The platelet-rich plasma does not enhance the regenerative potential of a natu-ral bone mineral and guided tissue regeneration by means of a bioresorbable membrane; (Study III.)
4. Platelet-rich plasma's growth factors have not a positive influence on perio-dontal healing after regenerative surgery with a synthetic bone graft and a non-resorbable membrane; (Study IV.)
5. A growth factors-containing adjuvant, the autologous platelet-rich plasma has not even had the minimal promotional regenerative effect on an another protein-mediated regenerative material, on enamel matrix derivatives; (Study V.)
6. Clinical regenerative effect of a natural bone mineral was not increased by

addition of platelet-rich plasma; (Study VI.)

Statistical evaluation of the main clinical variables bears record to significant improvement in each examination. This outcomes indicates that all of the analysed procedures allows periodontal regeneration and are capable to promote the natural regenerative capacity of this tissues. In variant combined methods the effects of the used natural bone mineral and beta-tricalcium phosphate are comparable.

In five studies was clinically proven that in periodontal applications the platelet-rich plasma has not additional benefit to healing. This is especially to apply to it's combined use with enamel matrix derivatives.

The option of the periodontal surgeon between this methods depends on mainly from the defect morphology, the medical concept of the physician, the technical possibilities and the clinical experience of the periodontal specialist.

Acknowledgements

The thesis is result of a harmonious and coherent teamwork of three Universities: the Semmelweis University, Budapest, Hungary, the Radboud University, Nijmegen, the Netherlands and the Albert-Ludwigs University, Freiburg, Germany.

I wish to express my acknowledgements to the following persons and establishments:

- Prof. Dr. István Gera, head of the Department of Periodontology, Semmelweis University, Budapest, Hungary, for his support and professional subsidization;
- Prof. Dr. György Szabó, previous head of the Clinic for Maxillo-Facial Surgery, Semmelweis University, Budapest, for his encouragement;
- Prof. Dr. Anton Sculean, head of Department of Periodontology, Radboud University, Nijmegen, the Netherlands, for his unlimited professional assistance;
- Prof. Dr. Nicole B. Arweiler, Department of Operative Dentistry and Periodontology, University of Freiburg, Germany, for her direct assistance in the statistical processing and evaluation;
- Dr. Tamás Huszár, Department of Maxillo-Facial Surgery, Semmelweis University, Budapest, for his assistansce and advices in the studies involving the preparation of the platelet-rich plasma;
- Dr. Erika Benedek, reader of the Department of Periodontology, Semmelweis University, for her scientific stimulation;
- Dr. Dóra Tihanyi, Dr. Viola Kovács and Dr. Attila Horváth, my colleagues from our Department of Periodontology, for their technical and surgical assistance;
- Ágnes Szokodi, hungarian representative of W. L. Gore and Associates, Flagstaff, AZ, USA, for her co-operation and collection of datas;
- Beáta Bruckner, hungarian representative of Geistlich A. G., Wolhusen, Switzerland and Zoltán Szakács, hungarian representative of Curasan A. G., Kleinostheim, Germany for their co-operation;
- Department of Radiology of the Clinic for Maxillo-Facial Surgery, Semmelweis University;
- Erzsébet Szegedi and Zoltán Barabás from Semmelweis University for their technical and digital support;
- and last but not least for my parents, for their tolerance and encouragement.

Publications

Thesis-related publications

1. **F. Dőri**, N. Arweiler, I. Gera, A. Sculean: Clinical evaluation of an enamel matrix protein derivative combined with either a natural bone mineral or beta-tricalcium phosphate.
   J. Periodontol. 76: 2236-43; 2005.
2. **F. Dőri,** T. Huszár, D. Nikolidakis, NB. Arweiler, I. Gera, A. Sculean: Effect of platelet rich plasma on the healing of intrabony defects treated with a natural bone mineral and a collagen membrane.
   J. Clin. Periodontol. 34(3): 254-61; 2007.
3. **F. Dőri,** T. Huszár, D. Nikolidakis, NB. Arweiler, I. Gera, A. Sculean: Effect of platelet rich plasma on the healing of intrabony defects treated with an anorganic bovine bone mineral and expanded polytetrafluoroethylene membranes.
   J. Periodontol. 78(6): 983-90; 2007.
4. **F. Dőri,** D. Nikolidakis, T. Huszár, NB. Arweiler, I. Gera, A. Sculean: Effect of platelet-rich plasma on the healing of intrabony defects treated with an enamel matrix protein derivative and a natural bone mineral.
   J. Clin. Periodontol. 35(1): 44-50; 2008.
5. **F. Dőri,** T. Huszár, D. Nikolidakis, D. Tihanyi, A. Horvath, NB. Arweiler, I. Gera, A. Sculean: Effect of Platelet Rich Plasma on the Healing of Intrabony Defects Treated with a ß-Tricalcium Phosphate and Expanded Polytetrafluoroethylene Membranes.
   J. Periodontol. 2007; JOP-07-0473.R1 (accepted for publication)

Other publications

1. Szabó Gy., Németh Gy., Le van Tach, Kovács Á., Fodor A., **Dőri F.:** Az alsó állcsont helyreállításának szempontjai sugárkezelés után.
   Magyar Radiológia 58: 9-17; 1984.
2. **Dőri F.,** Kövesi Gy., Suba Zs.: Papillon-Lefévre szindróma.
   Orvosi Hetilap 128: 1360-1362; 1987.

3. **Dőri F.,** Gera I., Benedek E., Őrfi L., Szabó Gy.: A chlorhexidines készítmények rutinszerű parodontológiai és szájsebészeti alkalmazásának feltételei.
Fogorvosi Szemle 81: 73-78; 1988.
4. Sallay K., Kövesi Gy., **Dőri F.:** Circulating immune complex studies on patients with oral lichen planus.
Oral Surg., Oral Med., Oral Path. 68: 567-570; 1989.
5. G. Szabó, **F. Dőri**: Invited Commentary to: "Benigne und maligne neoplastische Veränderungen im Bereich des Kiefergelenkes".
Acta Chirurgica Austriaca 29**.** 1: 35-36; 1997.
6. Windisch P., Kövesi Gy., Keglevich T., **Dőri F.,** Gera I.: Az irányított szövetregenerációs technikákkal nyert három éves klinikai tapasztalatok
Fogorvosi Szemle 91: 295-304; 1998.
7. **Dőri F.,** Gera I., Szabó Gy., Keglevich T., Nagy E.: Multifunkcionális fogkrémek laboratóriumi és klinikai vizsgálata. Egy új Triclosan-tartalmú fogkrém klinikai kipróbálása.
Fogorvosi Szemle 92**:** 67-78; 1999.
8. Szűcs A., Suba Zs., Martonffy K., Hrabák K., Gyulai-Gaál Sz., **Dőri F.,** Szabó Gy.: A ß-Tricalcium foszfát (CERASORB) jelentősége a preprotetikai sebészetben.
Fogorvosi Szemle 93: 45-52; 2000.
9. Keglevich T., Ratkóczi L., **Dőri F.,** Gera I.: A konzerváló és protetikai munkák minőségének hatása krónikus destruktív parodontitisben szenvedő betegek alveoláris csontnívójára.
Fogorvosi Szemle 93: 225-232; 2000.
10. Gera I., **Dőri F.,** Keglevich T., Sculean A., Szilágyi E., Windisch P: A ß tricalcium phosphate (Cerasorb®) klinikai alkamazásával nyert tapasztalatok humán parodontális csonthiányok pótlásában.
Fogorvosi Szemle 95: 143-147; 2002.
11. A. Sculean, P. Windisch, M.T. Auschill, **F. Dőri**: Treatment of peri-implantitis with EDTA decontamination and application of an enamel matrix protein derivative: a report of 3 cases.
PERIO (Periodontal Practice Today) 1: 237-245; 2004.
12. Sculean A., Windisch P., **Dőri F.,** Keglevich T., Molnár B., Gera I.: Emdogain in regenerative periodontal therapy. A review of the literature.
Fogorvosi Szemle 100: 220-232; 211-219; 2007. (English, Hungarian).

Abstracts

F. Dőri., T. Keglevich: A five year follow-up clinical study on patients treated with different barrier membrane techniques.
Europerio 3. Geneva, Switzerland.
Journal of Clinical Periodontology 27: 56. 2000.

T. Keglevich., L. Ratkoczi., **F. Dőri.,** I. Gera.: The effect of the quality of restorations on the alveolar bone loss in patients with chronic destructive periodontitis.
Europerio 3. Geneva, Switzerland.
Journal of Clinical Periodontology 27: 78. 2000.

F. Dőri., A. Sculean., N. B. Arweiler., I. Gera: Treatment of intrabony defects with Emdogain® and two different bone substitutes.
80th General Session of the IADR, 31st Annual Meeting of the AADR, 26th Annual Meeting of the CADR 2002 March. 6-9. San Diego, California, USA.
Journal of Dental Research 81. Special Issue. 2002.

T. M. Auschill., P. Windisch., **F. Dőri:** Treatment of periimplantitis with an enamel matrix protein derivative.
80th General Session of the IADR, 31stAnnual Meeting of the AADR, 26th Annual Meeting of the CADR 2002 March. 6-9. San Diego, California, USA.
Journal of Dental Research 81. Special Issue. 2002.

I. Gera., **F. Dőri.,** A. Sculean., P. Windisch et al: Evaluation of ß-tricalcium phosphate grafts.
Europerio 4. Berlin, Germany.
Journal of Clinical Periodontology 30: 12. 2003.

T. Keglevich., **F. Dőri.,** I. Gera: The surgical correction of gingival recession.
Europerio 4. Berlin, Germany.
Journal of Clinical Periodontology 30: 77. 2003.

F. Dőri: Treatment of peri-implant defects with enamel matrix derivatives.
Europerio 4. Berlin, Germany.
Journal of Clinical Periodontology 30: 88. 2003.

F. Dőri., T. Huszár., I. Gera., N. B. Arweiler., A. Sculean: Regenerative periodontal surgery with PRP, Natural Bone Mineral and GTR.
82nd General Session and Exhibition of the IADR/AADR/CADR March 10-13. 2004. Honolulu, USA.
Journal of Dental Research 83. 2004.

F. Dőri., T. Huszár., I. Gera., N. B. Arweiler., A. Sculean: Regenerative periodontal surgery with PRP, beta-tricalcium phosphate and GTR.
83rd General Session and Exhibition of the IADR/AADR/CADR March 9-12 2005. Baltimore, USA.
Journal of Dental Research 84. 2005.

F. Dőri, Z. Barna, M. Füzi, I. Gera, A. Sculean: Bacterial contamination of e-PTFE membranes following regenerative periodontal therapy.
Joint Meeting of the Continental European and Scandinavian Division of the IADR. 2005. Amsterdam. The Netherlands. www.iadr2005amsterdam.org

F. Dőri, N. B. Arweiler, T. Huszár, A. Horváth, D. Tihanyi, I. Gera, A. Sculean: Regenerative treatment with a natural bone mineral, PRP and GTR.
84nd General Session & Exhibition of the IADR, Brisbane, 2006. June 28.- July 1. Australia.
Journal of Dental Research 85. 2006.

F. Dőri, Z. Barna, M. Füzi, I. Gera, A. Sculean: Bacterial contamination of e-PTFE membranes following regenerative periodontal therapy with a synthetic bone-graft and PRP.
Europerio 5. Madrid, Spain.
Journal of Clinical Periodontology 33: supp.7. 2006.

F. Dőri, N. B. Arweiler, T. Huszár, I. Gera, A. Sculean: Does PRP Enhance Healing after Surgery with Emdogain + Bio-Oss?
85th General Session & Exhibition of the IADR, 2007. New Orleans, USA.
Journal of Dental Research 86. Special Issue A. 2007.