The Merit of Histological Evaluation of Clinically Successful Reconstructive Therapies in Periodontology and Related Fields

PhD Thesis Outline

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“The more I learn,

the more I learn how little I know.”

Socrates

(469-399 BC)

INTRODUCTION

Over the past decades, tremendous efforts have been made by scientists and clinicians to develop novel techniques and materials in order to improve the outcome of regenerative procedures in periodontology, implantology and related fields. Therefore, the market of such materials offers an overwhelming number of products, making the right choice for the right clinical scenario extremely difficult. Although most of these 'regenerative' methods and materials were claimed successful, they did not appear to routinely promote the formation of new bone, periodontal tissue or functional attachment. In addition, some of these materials may even hinder tissue regeneration. In fact, merely a fraction of these materials and methods seems to be subjected to meticulous and comprehensive scientific evaluations.

According to the current guidelines of the American Academy of Periodontology, a periodontal procedure could only be considered as 'regenerative', if (i) controlled animal histological studies demonstrate formation of new cementum, periodontal ligament (PDL) and bone (in absence of human histological data retrieved from controlled trials); (ii) human histological specimens demonstrate formation of new cementum, PDL and bone coronal to the former defect base and (iii) controlled human clinical trials demonstrate improved clinical probing attachment and bone levels.

Consequently, the improvement of radiographic or clinical parameters per se, such as pocket depth (PPD) reduction, clinical attachment (CAL) gain or reduced tooth mobility, without histological evidence do not verify true regeneration. Based on above, guided tissue and bone regeneration (GTR/GBR) and enamel matrix derivative (EMD) have indeed demonstrated their ability to regenerate intraosseous defects to a certain extent. Nevertheless, the treatment of intrabony defects with missing buccal/lingual wall or supra-alveolar ridge defects, as well as peri-implant dehiscence defects have not yet been fully resolved. Although the need of peri-implant bone augmentation, with its inherent cost, discomfort and morbidity, might be limited by alveolar ridge preservation (ARP) procedures that may follow tooth extraction and precede implantation, there is scarce scientific evidence behind the success of these treatments.

For such augmentations the use of 'bone filler matrix' was suggested in combination with GTR/GBR. An ideal matrix (i) functions as a space-providing buttress for the flap; (ii) fosters blood clot stabilization; (iii) induces proliferation, differentiation and maturation/mineralization of osteoblast/periodontal cells and (iv) resorbs totally, in harmony with the formation of the new tissue. Ideally, this material is synthetic, does not inflict allergic reaction, disease transmission, religious or animal right concerns, inexpensive and originates from an unlimited source.

Several grafts, bone substitutes and biomaterials were introduced to meet the above demand. Nevertheless, the additional benefit of the application of most of these materials has not been thoroughly investigated and confirmed histologically.

OBJECTIVES

The aims of the studies included the present thesis were to investigate the histological appearances and properties of some representative materials and methods in the field of regenerative periodontology and implant dentistry, which had shown favourable clinical outcomes already. In other words, are the treatments and employed materials that were claimed clinically successful in tissue regeneration really effective in the light of histology? In order to answer the above question three scenarios were set up.

Scenario A: Regeneration of periodontal defects

We aimed at examine the clinical and histological healing characteristics of EMD combined with a new biphasic alloplastic defect filler (BCP; Straumann BoneCeramic®) for the surgical treatment of human intrabony periodontal defects. (**Study 1**)

Furthermore, we aimed at examine the clinical and histological healing characteristics of a novel synthetic nanocrystalline hydroxyapatite bone substitute (nano-HA; Ostim®) that was claimed to promote healing without the need of barrier membrane for the surgical treatment of human periodontal intrabony defects. (**Study 2**)

Scenario B: Regeneration of peri-implant dehiscence defects

We aimed at assess the regenerative potential of the same novel biphasic bone substitute (BCP; Straumann BoneCeramic®) with a new resorbable, synthetic polyethylene glycol-based hydrogel barrier membrane (PEG; Straumann MembraGel®) in the field of reconstructive implant dentistry. Due to obvious ethical considerations it is not feasible to conduct a human histological study to investigate the performance of these materials on human peri-implant dehiscence defect. Therefore, a preclinical protocol was established to examine the effect of these materials on critical size porcine peri-implant dehiscence defects. (**Study 3**)

Scenario C: Regeneration of post-extraction alveolar sockets

We wanted to investigate whether or not the bone loss, associated with tooth extraction, could be prevented with any materials or methods, hence to limit the need of peri-implant bone augmentation with its inherent cost, discomfort and morbidity. The pilot search in the literature resulted in overwhelming number of various studies, however, a meticulous analysis of these data with structured methodology, especially the assessment the risks of bias and the histological healing characteristics was lacking. Therefore, we performed a systematic review in order to evaluate clinically and histologically the effect of ARP on human extraction sockets. (**Study 4**)

METHODS AND MATERIALS

Scenario A: Regeneration of periodontal defects (Study 1, 2)

Experimental design and subject population: Both trials were designed as prospective, single arm, human clinical and histological case series in accordance with the latest amendment of Declaration of Helsinki and approved by the Regional Ethical Committee of Semmelweis University. Patients were recruited and treated in the Department of Periodontology, Semmelweis University. Ten (Study 1) and six (Study 2) patients with advanced chronic periodontitis were enrolled in the trials in 2005 (Study 1) and in 2007 (Study 2). Subjects presented with advanced intrabony defect around the teeth scheduled for extraction due to advanced destruction of the periodontal attachment apparatus and further prosthetic considerations in conjunction with the overall treatment plan. Further major inclusion criteria were set: (i) 20-70 years of age; (ii) completed initial phase of periodontal therapy; (iii) good level of oral hygiene. Exclusion criteria were: (i) general medical history and medication that contraindicate elective surgery and may affect treatment outcome; (ii) systemic antibiotic treatment within three months prior to the current study; (iii) pregnancy during experimental period; (iv) heavy smoking (v) previous periodontal surgery at the selected site; (vi) presence of untreated endodontic lesion, hypermobility and occlusal overload.

Clinical parameters and outcome assessment: The primary outcome of the studies was the histological evaluation of the healing. Secondary outcomes were the change in PPD, CAL and gingival recession (REC) measured at baseline and before biopsy. In addition, standardized long cone radiographs were taken for radiological evaluation.

Reconstructive periodontal surgery: In local anaesthesia, full thickness mucoperiosteal flaps were reflected, granulation tissue was removed and the roots were meticulously debrided. A notch was placed on the root to mark the bottom of the defect. In Study 1, following root conditioning with ethylene diamine tetraacetic acid (EDTA), EMD was applied on the root surfaces and the defects were filled with a mixture of EMD + BCP. No root conditioning was employed in Study 2. The intrabony defect was filled with the nano-HA paste and adjusted to the alveolar crest, according to the manufacturer's instruction.

Re-entry, biopsy and histological procedure: After a healing period of nine (Study 1) or seven months (Study 2) the teeth were removed together with some of their surrounding periodontal tissues and subsequently placed in 10% buffered formalin for fixation. The extraction sites were then augmented with the use of various types of bone substitutes and barrier membranes. After the healing, subjects received either implant-based crowns or fixed partial dentures as part of the prosthetic rehabilitation.

The block biopsies were decalcified in EDTA, dehydrated in graded series of ethanol and embedded in paraffin. 40 sections per specimen (5 to 8 μm) were subsequently stained with hematoxylin–eosin and further with the oxone-aldehyde-fuchsin-Halmi staining method in Study 2. Height of newly formed cementum, periodontal ligament (PDL) and bone regeneration were measured by means of a computer-assisted toolbox.

Scenario B: Regeneration of peri-implant dehiscence defects **(Study 3)**

Experimental model and management: The present experiment was carried out on twelve female Göttingen minipigs under general and local anaesthesia and approved by the local Ethical Committee of the University of Lund. The treatment sequences in the study were:

Extraction and creation of chronic defect – Day 0/Baseline: The premolars and the first molar were extracted in both hemi-mandibles. Bone defect was created on both sides of the mandible by removal of the buccal plate with a chisel, on a length of 40 mm and a height of 6 mm. Flap closure was achieved.

Implantation with/without creation of acute defect, with/without the use of test materials (first stage surgery) – 3 months: In total, 48 bone level implants with a modified hydrophilic surface *(Bone Level, SLActive®, 4.1x8 mm, Straumann)* were placed with sufficient primary stability in 12 minipigs (four implants/animal; two in each hemi-mandible). In order to mimic real clinical circumstances of a partially edentulous ridge, combined chronic and surgically created standardised, pyramid shaped 'acute' buccal dehiscence-type defects were created. Consequently, the coronal 6 mm of the implants were exposed on the buccal aspect. According to a computer-generated randomization scheme, four groups were created:

P *(12 implants)* – In the positive (pristine) control group the implants were inserted into the chronic defects (i.e. without acute defect preparation).

N *(12 implants)* – In the negative control group the chronic and acute dehiscence defects were left untreated.

T1 *(12 implants)* – The exposed implant surface in the dehiscence defects was treated with BCP mixed with autologous blood.

T2 *(12 implants)* – BCP and the PEG membrane were used for covering the dehiscence defects and the implants.

Implants received closure screws and were covered by mucoperiosteal flaps for submerged healing.

Implant uncover and abutment connection according to two different loading protocols – 3 months after implant placement: At 3 months after implant placement (6 months after extraction) a second stage surgery was performed to uncover all implants. Either a long abutment (loaded side) or a short transmucosal closure screw (non-loadad side) was inserted in a randomised split-mouth design.

Termination of the study – 5 months after implant placement: At 5 months after implant placement (8 months following extraction and after 2 months of functional loading) histological evaluation was carried out.

Histological procedure and outcome variables: The biopsies were fixed in buffered formalin, dehydrated in ascending concentrations of ethanol, embedded in polymethylmetacrylate, cut in a bucco-lingual direction and stained with toluidine blue. Histomorphometrical outcome variables were the bone-to-implant contact percentage (BIC%), residual dehiscence defect depth from the implant shoulder to the most coronal bone-to-implant contact on the buccal side (S-BIC) and the area of regenerated bone (BS). Clinically, the distance from the implant shoulder to the most coronal peri-implant hard tissue level was also measured.

Scenario C: Regeneration of post-extraction alveolar sockets **(Study 4)**

Focused question

Following tooth/root extraction in humans, what is the effect of ridge preservation on the residual alveolar ridge (AR) dimension and on histological characteristics, compared to unassisted socket healing?

Types of studies

Only longitudinal prospective studies, such as randomised controlled trials (RCT), controlled clinical trials (CCT) and cohort studies with control group were included.

Populations of studies

Healthy individuals were included without age limit, who underwent any type of ARP following permanent tooth extraction. Smokers and patients with history of periodontal disease were not excluded. The minimum number of subjects per group was five. However, no limit was set for study follow-up period.

Interventions

Test groups

Studies reporting on any of the following types of interventions were included: socket grafting (autograft, allograft, xenograft, alloplastic materials); socket sealing (soft tissue grafts); GBR (resorbable/non-resorbable barriers); biological active materials (growth factors) and combinations of the above techniques/materials. Dimensional changes on two-dimensional radiographs or soft and hard tissue casts, as well as removal of third molars were excluded.

Control groups

The control groups of the included studies had to comprise empty sockets, i.e. unassisted socket healing.

Outcome variables

Primary outcome was the change in oro-facial (horizontal) and apico-coronal (vertical) ridge dimensions. *Secondary outcomes* were (i) change in buccal plate thickness; (ii) bone volume alteration following extraction; (iii) complications; (iv) histological healing characteristics; (v) site eligibility for placement of an adequate size dental implant with or without further augmentation; (vi) patient centred outcomes, such as quality of life and (vii) health economics.

Risk of bias and methodological quality assessment

In order to evaluate the methodological quality and risk of bias of individual studies, we took the following combination of parameters into consideration at the final analysis: sample size calculation, statement of eligibility criteria, ethics approval, informed consent, baseline homogeneity, randomisation method, allocation concealment, masking, calibration, follow-up, protocol violation, method of statistics, unit of analysis, implementation of Consolidated Standards of Reporting Trials (CONSORT), International Standard Randomised Controlled Trial Number Register (ISRCTN) and funding disclosure.

Search strategy

A sensitive electronic search was conducted by the sequence of specific key words and MeSH terms on the following databases: MEDLINE, EMBASE, CENTRAL, LILACS. In addition, a hand search was performed on the ten most relevant peer reviewed journals within a decade. No language restrictions were implemented.

RESULTS

Scenario A: Regeneration of periodontal defects (Study 1, 2)

All subjects completed the studies. Postoperative healing period was uneventful in all cases. No complications, such as allergic reaction, abscess, ulceration or infection were observed throughout the duration of the studies.

Study 1

The clinical measurements demonstrated a reduction in mean PPD from 8.6 ± 1.9 to 5.3 ± 2.0 mm at baseline and at nine months, respectively, resulting in a mean PPD reduction of 3.3 ± 1.4 mm. The mean CAL changed from 10.8 ± 2.0 to 7.8 ± 1.7 mm resulting in a mean CAL gain of 3.0 ± 1.6 mm. In one case the clinical and radiographical evaluation demonstrated excellent improvements. Furthermore, the re-entry revealed that the former intrabony defect presented with almost complete hard tissue fill, therefore, the tooth was not removed for the benefit of the patient.

In six out of nine (6/9) biopsies the histological findings indicated formation of new cementum to a varying extent. The newly formed cementum was a mixed acellular and cellular type in all specimens. Collagen fibres were inserting into the newly formed cementum in 6/9 specimens showing new attachment. In 3/9 specimens the healing resulted in long junctional epithelium (LJE) extending to the bottom of the defect. New connective tissue attachment (i.e. new cementum with inserting collagen fibres) varied from 0.0 to 2.1 mm (mean 0.7 ± 0.7 mm). The amount of newly formed bone was limited and varied from 0.0 to 0.7 mm (mean 0.2 ± 0.2 mm). The resorption of the bone substitute has not been completed. In most specimens, the remaining BCP particles were encapsulated in connective tissue, whereas formation of a bone-like tissue around the graft particles was observed only occasionally. Direct contact between the graft particles and the root surface (cementum or dentin) was not observed in any of the analysed specimens.

Study 2

Signs of accelerated early wound healing were observed clinically. At 7 months following surgery the mean PPD reduction was 4.0 ± 0.8 mm (8.7 ± 1.8 to 4.7 ± 1.7 mm) and the mean CAL gain was 2.5 ± 0.8 mm (12.2 ± 1.5 to 9.7 ± 0.7 mm). Mineralization of the newly formed tissue in the intrabony defect was observable on the radiographs. Moreover, the phenomenon of increased radiopacity was already visible at three months.

On the other hand, histological analysis revealed that in 3/6 biopsies, healing occurred through formation of LJE along the debrided root surfaces extending until the most apical part of the defects. In the remaining three specimens limited formation of new cementum with inserting connective tissue fibres and new bone were observed with a magnitude varying from 0.53 to 0.86 mm (mean 0.4 ± 0.4) and from 0.86 to 1.33 mm (mean 0.5 ± 0.6), respectively. Neither ankylosis, nor root resorption were observed in any of the biopsies. The nano-HA particles were resorbed apart from two biopsies, where some remnants were visible. The bone substitute was predominantly surrounded by connective tissue, without signs indicating a potential to promote periodontal or bone regeneration.

Scenario B: Regeneration of peri-implant dehiscence defects **(Study 3)**

Generally, uneventful healing was observed in most of the implant sites. In 4/12 cases of the T2 group rupture of the PEG membrane was noticed and reconstructed subsequently. One implant was lost due to lack of osseointegration in this group. Clinically, the distance from the implant shoulder to the most coronal peri-implant hard tissue level was measured as 3.35; 2.55 and 2.1 mm in the N; T1 and T2 groups, respectively, resulting in the least remaining dehiscence in T2 group.

Histomorphology:

N: Considerable amount of new coronal bone formation was observed alongside the hydrophilic implant surface. Nevertheless, the former defect was never filled, indicating that our defect model was in fact 'critical size'.
T1: New bone formation was observed in contact with the BCP particles in the apical portion of the defect, while in the coronal part, BCP particles were typically encapsulated by soft tissue.
T2: PEG was completely resorbed. Voluminous new bone was formed almost up to the implant shoulder. However, connective tissue interposed between the newly formed bone and the implant surface in most of the cases.

Histomorphometry:

BS varied between 8.81 ± 4.48, 9.69 ± 4.11, and 13.18 ± 5.85 mm^2 in the N, T1 and T2 groups, respectively. BIC% was measured as 52.3 ± 20.9, 54.3 ± 27.1 and 33.9 ± 26.5 in the N, T1 and T2 groups, respectively. S-BIC varied between 2.50 ± 2.17, 2.67 ± 1.40, 2.43 ± 1.74 and 3.71 ± 1.83 mm in the P, N, T1 and T2 groups, respectively, resulting in the least favourable implant-bone interface in the T2 group.

No statistical significant differences were detected in any of the investigated parameters with regard to the loading.

Scenario C: Regeneration of post-extraction alveolar sockets **(Study 4)**

Search sequence
The electronic search yielded 6,216 relevant hits after removal of duplicates. Subsequently, 157 titles were selected for the abstract stage. Following investigation of the abstracts, 42 articles qualified for full text evaluation. Four extra papers were then added as a result of the hand search. Assessment of these articles resulted in 14 publications eligible for the review. In these studies ARP was performed in 137 sockets of 119 patients, compared to 120 sockets that left to heal without any treatment in a total of 92 patients.

Quality assessment

Among the 14 included studies eight RCTs and six CCTs were identified. The randomisation technique was not stated in four RCTs and none of them described the method of allocation concealment. Masking of the examiner was reported at the clinical level in 2/8, at radiological level in 1/2 and at histological level in 4/11 studies. Examiner calibration was declared in three articles. Sample size calculation was reported only in three studies, although with insufficient data to evaluate the validity of the calculations. Statistical analysis was appropriately carried out and described in one study only. Appropriate statistics were either not carried out, or the reported data were insufficient to determine the validity in the rest of the studies. In addition, no RCTs were either registered with ISRCTN or reported using the CONSORT guidelines. Consequently, no studies met the low risk of bias category, four studies were classified as moderate and ten as high risk of bias.

In addition, the studies were characterised by considerable heterogeneity in terms of healing time, site location, defect morphology, reasons of extractions and treatment protocols. Two studies employed GBR technique; bone substitute was placed in eight experiments; combination of these was used in two trials**;** finally, biological active materials were employed in two further studies.

Clinical outcome

Average change in clinical AR width varied between −1.0 and −3.5±2.7 mm in ARP groups and between −2.5 and −4.6±0.3 mm in the controls, resulting in statistically significantly smaller reduction in the ARP groups in 5/7 studies. Mean change in clinical AR height varied between +1.3±2.0 and −0.7±1.4 mm in the ARP groups and between −0.8±1.6 and −3.6±1.5 mm in the controls. Height reduction in the ARP groups was statistically significantly less in 6/8 studies.

Histological outcome

Histological analysis indicated various degrees of new bone formation in both groups. Some graft interfered with the healing. Merely two studies reported statistically significantly more trabecular bone formation in the ARP group and more connective tissue in the control group. On the contrary, one study reported more vital bone in the controls compared to the ARP group. None of the differences of the investigated histomorphometric parameters reached statistical significance in other studies

No superiority of one technique for ARP could be identified; however, in certain cases, GBR was most effective. Successful implantations were carried out both at the untreated as well as at the ARP sites in 9/9 cases. Bone augmentation at implant placement was less frequently required in three trials, however the difference between ARP and control groups reached statistical significance in one study only. None of the studies reported the success or survival rate of the inserted implants, the role of buccal plate, patient centred outcomes and health economics.

CONCLUSIONS

Within the limitations of each study the following conclusions could be drawn:

1. The combination of EMD and BCP for the surgical treatment of human periodontal intrabony defects is safe and well tolerated; could lead to improvement of clinical periodontal parameters; could result in meagre formation of new cementum with associated PDL and in none or minimal new bone formation. Therefore, this combination does not seem to possess additional benefit for the regenerative periodontal treatment. (Study 1)
2. The use of nano-HA for the surgical treatment of human periodontal intrabony defects is safe and well tolerated; could lead to improvement of clinical periodontal parameters; could result in the formation of minuscule amount of new cementum with associated PDL and little amount of new bone. Therefore, the use of nano-HA seems to have limited potential to promote periodontal regeneration. (Study 2)
3. For simultaneous augmentation of critical size, porcine, peri-implant dehiscence defects, PEG membrane is safe and well tolerated; shows appropriate occlusive property, hence may be effective in bone formation. However, the fragile property of the polymerised material could lead to early rupture of the membrane, which could have a negative impact on the whole healing process, particularly on the bone-to-implant contact. (Study 3)
4. BCP alone does not result in predictable new bone formation in such defects. (Study 3)
5. The hydrophilic implant surface (SLActive) per se could support peri-implant new bone formation in these defects. (Study 3)
6. The short-term loading of SLActive implants inserted in augmented dehiscence defects may not have a negative influence on osseointegration and new bone formation. (Study 3)
7. The postextracion resorption of the AR cannot be totally prevented by ARP, but some of the ARP techniques can limit dimensional changes of the AR. However, ARP could be associated with increased incidence of adverse events. (Study 4)
8. Conflicting evidence exists on the benefit of ARP at the histological level, since ARP does not appear to promote de novo hard tissue formation routinely, in addition, some graft materials could interfere with the healing. (Study 4)
9. The strength of evidence of the included trials ranges from weak to moderate, therefore the results should be interpreted with caution. (Study 4)
10. Due to the broad variety of employed materials, techniques, defect morphologies, healing periods and small sample sizes, meta-analysis or comparative assessment of ARP could not be made. Consequently, no material or method can be claimed to serve superior to another, however, in certain cases, GBR appears to be most effective. (Study 4)
11. Only limited evidence supports the ultimate clinical benefit of ARP, namely the reduction of necessity of further augmentation in conjunction with implant placement. (Study 4)

Generally, the results of the present thesis strongly indicate, that meticulous (human) histological assessment should also be carried out prior to addressing ‘regenerative’ properties to a new material or method.

LIST OF PUBLICATIONS (IF: 20.193)

Thesis-related publications

1) **Horváth A**, Stavropoulos A, Windisch P, Lukács L, Gera I, Sculean A. Histological evaluation of human intrabony periodontal defects treated with an unsintered nanocrystalline hydroxyapatite paste. Clin Oral Invest, 2013;2:423-30. **IF: 2.200**

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2) Donos N, **Horvath A**, Dedi KD, Mezzomo L, Mardas N. The role of immediate provisional restorations on bone level implants: A randomised, controlled clinical trial. J Clin Periodontol 2012;s13:50.

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1) Gresz V, **Horvath A**, Gera I, Nielsen S, Zelles T. Immunolocalization of AQP5 in resting and stimulated normal labial glands and in Sjögren's syndrome. Oral Diseases 2014; doi: 10.1111/odi.12239. **IF: 2.377****

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5) Gera I, Kiss D, Tihanyi D, **Horváth A**, Rosta P. Clinical comparison of the plaque removal effect of two different Oral-B toothbrush. (Két különböző típusú Oral-B fogkefe interdentális plakkeltávolító hatásának klinikai vizsgálata) Magyar Fogorvos 2004; 13 (5):248-252.

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1) **Horváth A**, Papp Zs, Dobó-Nagy Cs, Gera I. Clinical effect of three glass ionomer cement restorative materials used in Class V cavities on the gingival tissue. J Clin Periodontol 2012;s13:200.

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