SEMI- INVASIVE BONE EVALUATION TECHNIQUES AFTER GUIDED TISSUE REGENERATION IN PATIENTS WITH PERIODONTITIS. THE INFLUENCE OF ORTHODONTICS DURING NEW TISSUE DEVELOPMENT

PhD thesis book

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1. INTRODUCTION

The goal of periodontal regenerative surgical techniques is to restore the attaching apparatus with tissues of a function and morphology identical to that of the original, which were demolished due to periodontitis. In recent restorative techniques, the focus is being shifted from using materials with a regenerative purpose towards developing flap techniques and promoting the wound's inherent potential for wound healing. Thanks to research performed during the past 40 years, clear decision-making systems are available today in order to aid clinicians in choosing between evidence-based surgical techniques and the biomaterial to be used. In case of certain defects with unfavorable (wide, non-containing) morphology, using minimally invasive flaps is not possible, while a grafting material is also required to act as a tissue scaffold and promote space maintenance. The use of scraped particulate bone, which is still considered the gold standard, requires the extension of the flap and increases post-surgical morbidity, therefore the most commonly used graft material in Europe today is the deproteinized bovine bone mineral (DBBM). Human histological studies confirmed the excellent osteoconductive properties of this substance; however, when it is applied in a periodontal infrabony lesion, incorporation of the graft particles could be detected in the coronal part of the defect. This can contribute to a future relapse, as any biomaterial that fails to integrate into the bone is highly prone to inflammation in case of deterioration of individual plaque control. Thus it would be most desirable to have xenografts that would be absorbed during regeneration, preventing possible late complications; however, these materials are considered non-absorbable or poorly absorbable materials.

Another challenge in the treatment of periodontal diseases is the therapy of teeth subject to pathological migration. In these cases, only combined periodontal and orthodontic treatment can achieve long-term functional and aesthetic results. Many clinical studies demonstrated the efficacy of such a multidisciplinary therapy. We know mainly from animal studies that in case of intraosseous lesions, regenerative surgery should come first, followed by orthodontic treatment, otherwise the bone remodeling induced by tooth movement will fail due to the lack of periodontal ligaments (PDL). Moving the teeth as a part of a combined therapy can not only increase the efficiency of

periodontal regeneration thanks to bone apposition and resorption, but also resolves the problem of the occlusal trauma. Orthodontic tooth movement (OTM) has also been studied in combination with guided tissue regeneration (GTR), which was associated with a significant improvement in periodontal clinical parameters. Araujo et al. showed in a preclinical study that OTM is feasible in areas previously augmented with DBBM. On the pressure side, partial resorption of the graft particles were observed, while in areas not subjected to orthodontic intervention, the particles showed osteo-obstructive characteristics.

Histology is considered to be the gold standard approach in the evaluation of the regenerated periodontal tissues. However the sampling is traumatic for the patient, while the histological processing is quite expensive and needs laboratory background. Due to these reasons clinicians are still waiting for alternative approaches utilizing less invasive techniques, which can serve similar data about the quality and composition of the tissues.

2. OBJECTIVES

The goal of our prospective randomized controlled clinical study is to compare the guided tissue regenerative technique alone and when used in combination with early orthodontic tooth movement. We are treating unfavorable (non-containing) periodontal intrabony defects, where a graft material is also required to ensure blood clot stability. The main focus is on using histological methods for the quantitative and qualitative analysis of the changes in the xenogeneic bone substitute occurring as a result of tooth movement. This is compared to "undisturbed" wound healing, when no orthodontic forces are applied on the regenerated area and tooth. Our further goal is to compare the various forms of healing with the help of widely used clinical and radiological parameters. In addition, we would like to determine whether early postoperative initiation of orthodontic treatment causes impaired results during the wound healing process.

The null hypothesis of our research is that there are no significant differences between the two types of healing processes regarding the potential improvement of clinical parameters. However, histological examinations might reveal significant differences. We suppose that the remodeling resulting from OTM might interfere with the DBBM particles as well, causing their complete or partial, inflammation-free resorption.

According to our hypothesis, the periodontal attaching bone of patients undergoing orthodontic treatment has an appearance more similar to an original, intact morphology, including graft material in a lower ratio.

In a second, case series study the Raman spectroscopy, as a non- invasive technique in bone quality evaluation, is introduced. It is hypothesized that this optical method can serve data not only about the chemical compounds of the bone, but also reveals the possible occurrence of a mature and crystalline phase of bone indicating a true regeneration process. The further objective of this case series is to detect any potential differences between the bone (both of an original and an augmented one) of patients with or without the history of periodontitis.

3. METHODS

Non-containing periodontal defects with an intrabony component at least 4 mm deep are included in the study, where the tooth is in a simultaneous malocclusion position (underwent pathological tooth migration). After an initial cause-related treatment, each included tooth is subjected to a regenerative surgery based on the principles of GTR. The coronally advanced flap described by Zucchelli and De Sanctis is utilized, which is a variety of a similar flap used to cover gingival recession, adapted for intraosseous lesions. Flap elevation is followed by degranulation of the bony defects and cleaning the decontaminated root surfaces. The defects are filled with DBBM particles and covered with absorbable native collagen membranes (Bio-Oss and Bio-Gide, Geistlich Pharma Ag, Wolhusen, Switzerland). Tension-free wound closure is performed in multiple layers.

We randomize patients into 2 groups directly after the surgery. Individuals in the test group receive a multibond type orthodontic appliance 7 days after the surgery, which provides continuous moving of the examined teeth through gentle forces. The test group is divided into 2 subgroups, based on the direction of tooth movement. In the first subgroup, teeth are being moved away from the defect, exerting a tensile force on the surgical area (subgroup T1), while in the second subgroup the direction of the movement is towards the bony lesion, meaning that it is subjected to the pressure side from the aspect of tooth movement (subgroup T2).

In the control group, a periodontal splint preventing further movement is used during the healing process of the examined teeth. The observation period is 9 months, after which a minimally invasive reentry intervention is carried out. During this, a bone microbiopsy is harvested from the previously regenerated defect using a trephine. The samples are processed in an independent laboratory (Schupbach Ltd, Service and Research Laboratory, Thalwil, Switzerland).

The primary outcome variable is the qualitative and quantitative analyses of the bone samples. The recorded secondary variables are the clinical attachment level gain (ΔCAL), the reduction of pocket probing depth (ΔPPD), the changes in the intrabony component of the defect (ΔIC) and the osseous filling calculated from this measure (in %), the changes in gingival recession (ΔGR), and the bone loss (changes in the crestal bone level: ΔCEJ-BC). The latter clinical parameters are registered during the regenerative (baseline) and the reentry (endpoint) surgeries using a calibrated periodontal probe (UNC-15, Stoma, Tuttlingen, Germany). A statistics software (SAS, SAS Institute, USA) is used to compare the changes in the average values of the groups between the baseline and the endpoint (Wilcoxon signed-rank test). The baseline homogeneity and the comparison of the parameter changes between the groups are evaluated by Mann-Whitney U test. The histomorphometric data is compared with the help of a paired T- test between the two groups. The level of significance is set to 5% ($P \leq 0.05$). The changes in the intraosseous component is also evaluated by standardized parallel X-ray radiographs, so we can compare it to the clinical values with the help of a regression analysis (Bland-Altman plot).

In the second, case series study a total of 8 individuals are selected (half of these subjects are affected by periodontitis, half of them are considered to be periodontally healthy) for a maxillary sinus floor augmentation (lateral window technique). A small sample is removed in each case from the bony wall of the sinus floor's prepared lateral window serving data for the initial (original) in vitro analyses of the bone. DBBM is utilized as a grafting agent during the procedures. After 8 months of healing bony cores were harvested from the former place of the lateral window from the augmented bone (final, regenerated bone). Bone specimens harvested before and after the bone augmentation procedure are examined in vitro with the following devices: Raman spectroscopy, Energy Dispersive X-ray Spectroscopy (EDS) and Scanning Electron

Microscopy (SEM). Reference calcium phosphates compounds (*octacalcium phosphate*-OCP, amorphous *hydroxyapatite*- HAP and crystalline HAP from Sigma- Aldrich Chemicals Company, US) together with the used DBBM grafting agent are analyzed with the Raman device in order to identify their specific spectra. Relevant Raman shifts determined by the previous chemical compounds are assigned to the following type of components: extensive mineral immature bone (amorphous phase of calcium phosphates), mineral mature bone (crystalline phase of HAP) and collagen (CO).

4. RESULTS

In our randomized, controlled clinical trial a total of 24 patients (13 in the test and 11 in the control group) had results suitable for statistical processing by the end of the observation period. Histological analysis was performed in 20 cases (11 test and 9 control) due to financial limitations, out of which 1 sample was unsuitable for evaluation, most likely due to damage occurring during the biopsy procedure. Post-surgical complications developed in 5 patients. All of these were early complications accompanied by interdental gingival dehiscence.

4.1. Clinical parameters

Statistically significant reduction in pocket depth, increase in clinical attachment level and in intrabony component can be observed between the baseline and the endpoint measurements in both the test and the control group (within-group changes). At the same time, parameters describing the levels of marginal gingiva and crestal bone (GR and CEJ-BC) failed to confirm a significant change during the observation period. Bone filling was 70% in the test group and 67.5% in the control group.

Baseline parameters show homogeneity between the groups, which allows for the comparison of their changes with the achievement of the endpoint. Regarding the changes in all recorded clinical parameters, we do not find any significant difference between the two groups. This finding supports our null hypothesis.

Regression analysis revealed an average difference of 1.03 mm between the two measurement methods used to evaluate the intraosseous components. Based on the above,

we can conclude that the radiological method underestimates the depth of the defect, assuming that we consider the intraoperative measurements more reliable.

4.2. Histology

Histological analysis in most control group cases revealed nice embedding of graft particles into newly formed bone, which are predominantly present in the central and apical third of the biopsy samples. In the coronal third, these particles are more often encapsulated in the connective tissue. After 9 months of healing, ongoing new bone formation can still be detected, well demonstrated by the presence of osteoblasts, the secreted osteoid and the woven type bone. Histomorphometry confirmed a composition of 17,3 (± 8,7)% newly formed bone, 33,2 (± 3,6)% DBBM and 49,5 (± 10,7)% soft tissue components.

The samples of subgroup T1 show excellent incorporation of the graft material in the de novo bone, and bone formation ongoing at the time of biopsy can be observed as well. The proportion of bone marrow appears appropriate in the sections, and it is densely filled with blood vessel cross-sections. In one patient, cutting cones can be observed on the surface of the DBBM particles, with the anabolic activity of osteoblasts confirmed inside, technically leaving a trace of remineralization. Regarding the distribution of graft material, we can conclude that it is less present in the coronal third than in the other two-thirds of the sample.

Apart from the presence of osteoid confirming the ongoing formation of new bone, remodeling can also be demonstrated in the histological sections of subgroup T2. The activity of osteoclast cells creates resorption lacunas (cutting cones) on the surface of the bone graft, within which replacement bone formation can be detected. In higher magnification, a multinuclear giant cell can be seen on the surface of the graft, which is a clear proof of DBBM resorption. Osteoid bone ingrowth can be observed on the surface of the bone graft previously affected by resorption. The remodeling of graft particles is also supported by the histomorphometry results, showing a distribution of 37,2 (± 14,6)% and 31,4 (± 8,8)% newly formed bone, 19,5 (± 3,2)% and 13,6 (± 10,3)% DBBM, and 43,2 (± 14,2)% and 55 (± 6,4)% soft tissue component in subgroups T1 and T2, respectively. Statistically significant differences are found between control and all test groups (overall and subgroups' results) in respect of the graft's and the newly formed

bone's percentage. There are significantly increased de novo bone formation and reduced graft amount in the test groups compared to the control group.

In 5 cases of all histological samples (3 control and 1 T1 and T2 subgroup each) we observed unfavorable healing, confirming connective tissue infiltration with minimal new bone formation in the whole sample or in significant portion of it. Impaired wound healing inhibiting ossification is in close correlation with gingival dehiscence occurring in the early postoperative period in these few cases.

4.3. Results of Raman spectroscopy, SEM and EDS

The initial bone of a periodontally healthy patient consists both phases (balanced amorphous and crystalline) of HAP compound, while in a patient with a history of a periodontal disease only the amorphous phase corresponding to immature bone is confirmed. The proof for a mature type of bone, where the calcium phosphate compound has a more ordered and higher crystalline nature, is also marked by a representative Raman shift in both patients after treatment. The intensity of the Raman shift correlates with the presence of the DBBM material. However, a better result of healing process is noticed for healthy patients, where bone tissue develops on a larger scale than for patients with a history of periodontitis. The EDS is backing the Raman results and highlights that the two phases (amorphous/ crystalline) must coexist in a more or less balanced ratio according to bone type (augmented or not) or health status.

In the original bone of patients with periodontitis more fibrous tissue and large amount of collagen (CO) are detected. After the augmentation procedure more CO is present in the bone of patients without periodontitis. This finding is explained by the SEM pictures, where the augmented bone of healthy patients shows not only a larger amount of crystallites, but a better organized collagen structure around graft particles as well.

5. CONCLUSION

1. If pathological tooth migration has to be corrected in the presence of intrabony defects, regenerative surgery is essential as a complementary and preceding treatment in addition to orthodontic therapy.

2. There is a substantial need for a literature recommendation determining the time when it is optimal to initiate orthodontic treatment after a regenerative surgery. Based on our clinical study it seems that an early (1 week postoperative) orthodontic tooth movement can be safe and it does not have a negative impact on the outcomes of periodontal wound healing.

3. The extent of periodontal regeneration is limited in humans. In case of an unfavorable defect morphology, it is almost impossible to achieve complete regeneration in relation to its depth. Our test and control groups show bone fill in approximately 2/3 of the original depth of the intraosseous lesions.

4. Coronally advanced flap design adapted to intrabony lesions is a good surgical option in cases of unfavorable defect morphology and during the use of a guided tissue regeneration technique. With the help of the mesio- distal extension and the coronal advancement of the flap, we are able not only to treat non- containing and multiple type of defects in adjacent interdental areas, but also to enlarge the space for a better coagulation. The enlarged coagulum is similarly and essentially supported by the flap design and by the GTR intervention, which is responsible for the favorable bony fill of the intrabony lesions.

5. Most samples of the control group confirmed the mixed nature of human periodontal wound healing (a combination of regenerative and reparative processes), as the coronal graft particles showed incorporation in the connective tissue. This can be one of the reasons underlying a rapid relapse due to inadequate plaque control. Xenograft resorption confirmed in participants of the test group is a unique phenomenon, possibly caused by bone remodeling induced by orthodontic tooth movement. This is beneficial from a clinical aspect by all means, because the proportion of newly formed bone and marrow space shows an increasing trend, while the amount of bone substitute material shows a decreasing trend when compared to the control group. According to our assumptions, this structure that is closer to the original composition of the bone results in a periodontal attaching bone more resilient to remission on the long term.

6. Early gingival dehiscence together with the consequential secondary wound healing significantly reduces the regenerative capability of the bone tissue, and results in the encapsulation of the biomaterial in the connective tissue.

7. It would be essential in the future to develop new less invasive techniques in order to gather relevant information regarding the healing of the periodontal tissues. Raman spectroscopy, as a highly sensitive method to characterize bone, can be a future alternative. In our study Raman spectroscopy is considered to be a promising approach for bone quality evaluation and for periodontal disease associated analyses of the bone's chemical composition. However, we still lack enough clinical data about the reliability of the tool and how it can be used for in vivo studies. In addition, it is further a challenge optically how to make access to periodontal tissues from a less invasive exploration. Until that time histology and histomorphometry remain the gold standard for micro architecture assessment and quantitative description of the structures in the tissues.

New findings:

1. There has not been previously published any human histological evaluation of the combined OTM + GTR techniques. The candidate and his coworkers presented the first human histological data on this comprehensive treatment.
2. It was one of the first human histology conducted with a less invasive trephine biopsy technique to evaluate the microscopic process of wound healing and bone regeneration after orthodontic tooth movement around periodontal defects.
3. It proved that both the pressure and tension sides showed marked new bone formation and remodeling.
4. It appears that an early (1 week postoperatively) initiation of tooth movement is safe, without influencing negatively periodontal wound healing.
5. The extent of periodontal regeneration of unfavorable defects were comparable both in the control and test groups.
6. Periodontal regeneration is successfully applicable combined with orthodontic tooth movement, resulting similar clinical endpoint parameters, than used alone without OTM.
7. It was one of the first attempt to investigate the feasibility of the RAMAN technique for the evaluation of the bone's composition and quality.

6. THE BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

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