Novel methods for evaluation of osseointegration, bone regeneration and oral flap management

PhD thesis booklet

Dr. Sándor Farkasdi

Doctoral School of Clinical Medicine
Semmelweis University

Supervisor: Gábor Varga, D.Sc

Official reviewers:
Kinga Laczkóné Turzó, Ph.D
József Tímár, MD, D.Sc

Head of the Final Examination Committee:
Tibor Fábián, DMD, Ph.D

Members of the Final Examination Committee:
Senior Zoltán Rakonczay, D.Sc
Árpád Joób-Fancsaly, DMD, Ph.D

Budapest, 2019

1. INTRODUCTION

The **intraosseous implant** is the gold standard for the replacement of the extracted tooth. Dental implant placement is one of the most common procedures in oral healthcare. The crucial element for intraosseous implant placement is its integration into the living tissues, a process called ***osseointegration***. Since PI Branemark introduced osseointegration as a rigid fixation of an implant within bone tissue more than half-century ago, numerous ***in vitro, in vivo preclinical*** and ***clinical studies*** have been carried out to investigate this process.

Based on the new bone formation around the titanium implants, the osseointegration process consists of two elements: the ***contact*** and ***distance osteogenesis***. Then bone ***regeneration*** and ***remodeling*** proceed continuously, which finally leads to a rigid and stable fixation of the implant into the surrounding bone tissue.

Osseointegration can be assessed by invasive and non-invasive ways. The invasivity in this classification is distinguished based on the destruction of the bone to implant connection during the analysis or saving it for further analysis. ***Non-invasive methods*** include radiological analysis/diagnostics, resonance frequency analysis (RFA), "damping characteristics", and also the perception of surgeons. RFA is an easy-to-use and standardly reproducible clinical method. RFA is considered to determine the stability of the implants and the time of their ability to be loaded. ***Invasive techniques*** include pull-out/push-in tests, reverse/removal torque measurement, cutting torque resistance, tensional test or micromotion testing and histology/histomorphometry. The pull-out test is the most reliable biomechanical test for the evaluation of implant stability. This is because the main loading on oral implants and teeth arrives vertically. Accordingly, using pull-out tests with ***non-threaded implants***, we can precisely evaluate the bone anchorage strength to the implant surface. The histomorphology is the gold standard and the most descriptive method for bone to implant contact (ISO/TS_22911:2016). For preclinical testing, the

combination of both non-invasive and invasive methods could offer a good outcome providing a safe basis for clinical application. Accordingly, in the studies of this thesis we have chosen RFA and micro-CT X-ray diagnostics from non-invasive methods being the most thoroughly described, reliable non-invasive methods. From invasive methodology, the pull-out test and histomorphometry were selected.

When the available bone volume is not enough for implant placement because of a ***bone defect*** (BD) in a toothless area, bone regeneration should be performed before implant placement. There are different bone regenerative techniques. For most of them, ***bone grafting materials*** are used. These materials are usually classified according to their origin: autografts when harvested from the same individual receiving the graft, allografts when harvested from the same species, namely from other humans, xenografts when harvested from a species other than human, synthetic when produced in laboratory conditions, and, finally, the combination of grafting materials listed above. In dental clinical practice guided bone regeneration (GBR) procedure is considered to be one of the most frequent surgical procedures for rebuilding and regenerating bone defects.

In the field of regeneration and replacement, a huge variety of ***animal models*** have been used. The preclinical experimental setups have to have reasonableness and evidence from human clinical practice. Unfortunately, none of the experimental animal models combine minimal morbidity to the experimental animal, easy ***reproducibility***, ***similarity*** to the human jaws (histologically and anatomically), multifactorial analysis of the healing during osseointegration and bone regeneration of BD. Even the ***present guideline*** requires ***only*** morphological, ***radiographical and histopathological assessments*** but does not include any functional investigations of osseointegration (ISO/TS_22911:2016). This deficiency is clearly due to the lack of reliable, well-developed biomechanical tests for experimental animals.

Most animal models for investigating osseointegration were developed without considering the similarity of bone microstructure in animals to that of the human jaw bones. In 2009 J. Blazsek et al. first found that ***caudal tail vertebrae*** were constituted by abundant spongiosa with a cortical layer, which presented a similar alveolar structure as the human mandible, which is suitable to support titanium implants. Furthermore, J. Blazsek found, that the bone marrow parenchyma was also absent in the tail vertebrae, thus having higher similarity to the human jaw bone than the hematopoietic femur of the rat, a commonly used experimental model site. Based on these findings, J. Blazsek et al. developed a novel experimental model for the evaluation of new bone formation, bone regeneration, osseointegration and bone remodeling around longitudinally placed titanium implants in tail vertebrae and proposed to name it "OSSI" (OSSeoIntegration) model. The original methodology for osseointegration analysis using the rat tail vertebrae applied only rough evaluation procedures, which were not very sensitive. Thus, it was important to further develop the ***"OSSI" model for the quantitative and qualitative characterization of osseointegration***.

Vertical ridge augmentation (type of GBR) in the posterior mandible is a technique-sensitive procedure that requires adequate anatomical knowledge and precise surgical skills to minimize the risk of complications. One of the most important, but also challenging aspects of the ***surgical technique*** is proper flap management to allow passive flap closure and reduce the chances of postoperative complications affecting deep anatomical spaces. Some of the current ***flap management techniques*** present limitations associated to serious postoperative complications and have limited evidence. It is important to determine the effectiveness of different ***flap designs*** for oral and periodontal surgeries for the extent of lingual flap release, for the augmentation of the vertical ridge in the posterior mandible.

2. OBJECTIVES

1. The primary aim of the present work was to ***refine the original***, previously developed ***preclinical in vivo rat tail implant model*** to make it suitable ***for quantitative and qualitative monitoring of osseointegration*** of implants ***by*** the combination of ***biomechanical*** and ***structural evaluations***:

1.a: to adapt the resonance frequency analysis, originally developed for humans, to the rat tail model for more precise evaluation of osseointegration,

1.b: to develop an ***implant design*** that is suitable in the future ***for the investigation of*** the effect of surface modifications to osseointegration excluding the influence of macro-design on the ***bone-bonding strength*** to the implant surface,

1.b: to develop a ***complex*** biomechanical ***evaluation*** by the combination of ***resonance frequency analysis and pull-out techniques***,

1.c: to ***combine*** the ***biomechanical*** evaluations ***with structural*** tests in order to reliably monitor the osseointegration process in a small animal model that is suitable for preclinical screening,

1.d: to ***improve*** surgical conditions and postsurgical care.

2. We also aimed to develop an ***experimental model for monitoring the regeneration of multiple bone defects*** and ***integration of implants*** placed ***multiply,*** simultaneously in perpendicular direction into the tail by modifying the rat's original tail model.

3. Finally, we attempted to ***determine*** the ***effectiveness*** of ***two different flap designs*** for oral and periodontal surgeries in order to compare the extent of lingual flap release, applying fresh human cadaver heads. We compared the outcomes of the "non-detaching" and "detaching" techniques for the mylohyoid muscle.

3. MATERIALS AND METHODS

3.1 The refinement of original in vivo rat tail implant model for quantitative and qualitative monitoring of osseointegration

The original "OSSI" model, developed by Blazsek, was fundamentally new and innovative, it did not allow quantitative evaluations of implant osseointegration. Thus, it was necessary to further elaborate the surgical procedure, implant design, postsurgical care and also the complex detection of the integration process.

3.1.1 Development of an implant design that is suitable for the investigation of the effect of surface modifications to osseointegration

For RFA, a specially-designed direct connection was fabricated between an implant and a SmartPeg type 62 (magnetic transducer of the Osstell device). The boundaries of our developments were determined by the bone structure volume of rat tail vertebrae. The limits were the height/length of the vertebrae (approximately 9.8mm) and the width (approximately 3.8mm) of caudal vertebrae of the adult Wistar rats (from 380 to 500 grams). There were three major different macro designs developed considering rat caudal vertebrae sizes: fully-threaded, half-threaded and non-threaded. For all of them, the implants' core was cylindrically shaped with 1.3 mm diameter at the body part and 2.9 mm in diameter at the neck part. The total length of the implant was 9.5 mm, and only 7.5 mm were planned to be placed into the bone (surface treated part), and 2 mm were planned to be above the bone (non-surface treated part). The head part for all implant designs was the same and served as a connection point to the SmartPeg with the implant.

3.1.2 Development of complex biomechanical evaluation by the combination of resonance frequency analysis and pull-out techniques

The head geometry of our customized implants was specially-designed and fabricated to let a direct fixation of SmartPeg type 62 into the implant head by

screwing. We followed a similar strategy for developing the SmartPeg connection to our custom implants for the RFA method as described in a previous publication. The thread design inside the implant head allowed the direct screwing of a hook for further biomechanical evaluations by pull-out tests.

3.1.2.1 Validation of the individually developed connection between SmartPeg and customized implant using RFA

The validation of the newly-formed connection between SmartPeg and implant was done by RFA on implants after fixing them into the plaster. RFA measurements for each implant were performed from four different directions. Implants were inserted into amputated rat tail vertebrae during the second set of evaluations of newly-formed connection. Before implant insertion, the implant bed preparation protocol was developed ex vivo. Each step of the drilling was performed according to standard bone preparation. The surgical guide was jointly developed in collaboration with Full-Tech Ltd. (Hungary) for positioning the drills in the middle of the vertebrae irrespective of the exact diameter of the tail. The implants were installed up to the surface-treated part.

3.1.2.2 Evaluation of implant-hook connection during pull-out test

The evaluation of pull-out testing was performed with fully-threaded implants after their RFA in the vertebrae. A special hook was used for the pull-out test. The peak force was registered by the tensional test machine Instron® 5965 (Instron®, USA). Based on the efficient evaluation of RFA and pull-out test measuring methods it was decided to evaluate the primary stability with the mentioned methods by using three different implant geometries (fully-threaded, half-threaded and non-threaded) in polyurethane foam (PUF) artificial bone blocks.

3.1.2.3 Evaluation of three implant geometries with RFA

3.1.2.3.1 Artificial bone blocks

For in vitro implant stability evaluations there are standard D1, D2, D3, D4, D5 (according to Misch classifications) density PUF bone blocks (Sawbones Ltd., USA) (model 1522–05; Pacific Research Laboratories, Vashon Island, WA).

3.1.2.3.2 Implant bed preparation and implant insertion

The location of each implant bed in PUF blocks was performed on standard distances to each other. For achieving primary stability, the three types of implants were simply inserted into the prepared implant bed up to the surface-treated part.

3.1.2.3.3. Implant stability measurements using RFA

After implant insertion into the PUF blocks, RFA measurements were performed. For each implant, the RFA was repeated 4 times from 4 different directions.

3.1.3 Combination of biomechanical evaluations with structural tests for reliable, complex monitoring of the osseointegration process using rat tail vertebrae

In these experiments, we used the above-described non-threaded implant design, which was entirely suitable for further in vivo osseointegration analysis. The developed drilling sequence provided the possibility to place the non-threaded implant by press-fitting without any gap between the implant and the bone. Thus, the inserted non-threaded implant had a direct contact with the bone. Accordingly, we called the experimental in vivo setup (where non-threaded implant was placed in rat tail vertebrae longitudinally) “Direct OSSI” model.

3.1.3.1 Experimental animals for "Direct OSSI" model

A total of 63 male Wistar rats (450-550 g) were used (ethical permit: PEI/001/2894-11/2014). The rats were operated on and terminated under general anesthesia with sodium pentobarbital (Nembutal, CEVA, France, 40 mg/kg body weight, intraperitoneally (i.p.)).

3.1.3.2 Mini-implant design

The newly-designed, fabricated and tested non-threaded implants with a cylindrical shape were used during in vivo experiments. With such a design, we aimed to evaluate the pure biological integration without any additional influence of the geometrical design (threads, holes, self-tapping). By using a non-threaded implant design, we decreased the metal artifacts during the planned x-ray diagnostics by minimizing the geometrical complexity.

3.1.3.3 Surgical procedure in "Direct OSSI" experimental model

The surgical procedure is based on our previously-published model with a number of important modifications. After the amputation of the distal part of the tail, an axial cavity was made in the opened surface of the C4 vertebra to host the implants using specially selected and fabricated drills (pilot, twist drill and neck drill) (Full-Tech Ltd., Hungary) and developed the drilling protocol. We used a surgical guide to facilitate repeatable, reliable and independent placement of implants. After implantation, the soft tissues were repositioned and the wound was closed by suturing.

3.1.3.4 Postsurgical treatment

A special postsurgical care was developed and used.

3.1.3.5 Sample harvesting and evaluations for "Direct OSSI" model

We sacrificed 21 animals after 4 weeks, 21 animals after 8 weeks, 7 animals after 12 weeks, 14 animals after 16 weeks. The samples were used for either biomechanical (RFA and pull-out test) or for structural (micro-CT and histomorphometry) analysis.

3.1.3.5.1 Biomechanical evaluations

The two biomechanical tests were completed on the day of harvesting. We first performed the RFA, and then the pull-out test. 14-14 animals were tested at weeks 4 and 8, while 7-7 animals were evaluated at weeks 12 and 16.

3.1.3.5.1.1 RFA

First the RFA measurements were performed. For each integrated implant, the RFA was repeated 4 times.

3.1.3.5.1.2 Pull-out test

After the non-invasive evaluation by RFA, the axial extraction force was used to evaluate secondary implant stability. For the pull-out testing, we used the tensional test machine Instron® 5965 (Instron®, USA). The pull-out test was applied in accordance with the ASTM F543 - 17 and peak extraction value was detected to each implant.

3.1.3.5.2 Structural analyses

Twenty-one specimens (n=7 animals per group) were used for structural analysis and micro-CT and histomorphometric analyses. The evaluation endpoints for structural analysis were at weeks 4, 8 and 16.

3.1.3.5.2.1 Micro-CT analysis

We performed a 3D radiographic data acquisition to detect the structural basis of implant stability in the reconstructed 3D images (1172 SkyScan micro-CT, Bruker, USA). The micro-CT scanning and reconstruction algorithm protocol for scanning was designed and optimized to our experimental conditions in order to overcome the x-ray scattering on the metal surface. The calculated intersection surface/tissue surface ratio (i.S/TS) was used in a 2D analysis for characterizing the bone to implant contact. For bone volume assessment, a 38-voxel (0.461 μm) thick cylindrical volume of interest was selected around the titanium implant. For determining the percentage of bone volume value, bone volume/tissue volume ratio was calculated (BV/TS).

3.1.3.5.2.2 Histology and histomorphometry

After micro-CT measurements, the samples were embedded and prepared for histomorphometric analysis. During histomorphometric analysis, bone-growth around implants was then visualized under a light microscope.

3.2 Development of a preclinical model for monitoring multiple bone defects regeneration and integration of simultaneously placed multiple implants

Based on the principles, developed by J. Blazsek et al., we further elaborated the original "OSSI" model to enable multiple placements of implants in perpendicular position to the tail and to make multiple bone defects in collaboration with French colleagues in the frame of a joint Hungarian-French Science and Technology project. We first focused on the development of an experimental model based on rat tail vertebrae for monitoring the regeneration of bone defects. This model is called "BD OSSI" model. Second, we worked for modifying the original "OSSI" suitable for placements of multiple implants in a perpendicular direction of the tail. We called this model as "Gap OSSI" experimental model.

3.2.1 Ex vivo developments for rat caudal vertebrae bone drilling for creating transversal defects

Knowing the skeletal limits of rat tail vertebrae, we established the drilling protocol and drilling location of the bony bed transversally. During drill selection, we aimed to create the largest possible transversal bone defect, which still reproducibly permit the integrity of the remaining bone of the given vertebra.

3.2.2 Experimental setups for "BD OSSI" and "Gap OSSI" models

"BD OSSI" and "Gap OSSI" experimental work was performed at the Montpellier University, France. The "BD OSSI" experimental model focused on the evaluation of self-healing capacities of bone defects created in the bony

structure of rat tail vertebrae. The "Gap OSSI" was developed to evaluate the osseointegration process of implants, which were placed transversally in the axis of the tail. In both experimental setups, male Wistar rats were used (weighing 380 to 450 grams) for the evaluation of the "BD OSSI" model and then for the "Gap OSSI" model (ethical permit from Montpellier University, referral number 1083 16/06/2014).

3.2.2.1 Experimental setup for "BD OSSI" model

In this experimental model, the animals were divided into two groups based on the healing time after bone defect creation. In the first group, healing was evaluated after 4 weeks and in the second group it was checked after 8 weeks. Three rats were used per group. For each animal four transversal defects were created from C2 to C5 rat tail vertebrae. In both groups, randomly two vertebrae were left empty after bone defect creation. Two other bone defects in two different vertebrae were grafted with xenograft for the evaluation of graft incorporation. Micro-CT was used for the evaluation of the self-healing capacity and graft stability in bone defects.

3.2.2.2 Experimental setup for "Gap OSSI" model

Three rats were used for osseointegration evaluation of the multiple transversally placed implants in rat tail vertebrae. In each caudal vertebra (from C2 to C5) customized titanium implants were press-fitted into the bony bed. Implant shape was designed to allow "distance osteogenesis" (new bone growth from the bone walls towards the implant body). The gap between the body-part was left empty. Histology and micro-CT were used to assess "distance osteogenesis" around titanium implants after 12 weeks of healing.

3.3 Evaluation of the effectiveness of the two types of oral flap release designs for the extension of the lingual flap, applying fresh human cadaver heads

This study was performed at the Institute of Anatomy of the Medical University of Vienna (Austria) according to local ethical approval. Twelve

fresh human cadaver heads missing all posterior mandibular teeth bilaterally and with comparable extent of alveolar ridge resorption were selected. In this split-mouth study, the surgical technique corresponding to each side was randomly assigned. All surgical interventions were performed under the same environmental conditions and by the same surgeon, Dr. István Urbán-assisted by the PhD candidate of the present thesis – in order to control technical consistency.

3.3.1 Flap management technique

The control technique consisted on the mylohyoid muscle "detaching" release, as described elsewhere. The test side received the mylohyoid preservation technique ("non-detaching" technique), which considers the flap preparation in three key anatomical zones. Tunnelling and lifting of the retromolar pad (RP) was performed in Zone I. Flap separation with mylohyoid muscle preservation was performed in Zone II. Anterior, semi-blunt periosteal release was carried out in Zone III.

3.3.2 Outcome measurements

The amount of vertical flap release was measured bilaterally at Zones I, II and III from the alveolar crest to the margin of the lingual flap. The measurements were both sides. The lingual flap was stretched until it reached its maximum passive stretch by using a high-precision force gauge connected to a straight mosquito forceps. The forces were applied in a vertical direction, following a perpendicular vector respective to the floor of the mouth.

3.4 Statistical analysis of in vitro and in vivo evaluations

For all studies, the data were given in the mean ± standard error of the mean (SEM) form. Each statistical evaluation was performed according to appropriate test using Statistica 12 software (TIBCO Software Inc., USA).

4. RESULTS

4.1 Quantitative, qualitative monitoring of osseointegration using "Direct OSSI" model for refinement of the original in vivo rat tail implant model

4.1.1 Validation measurements of an implant design that is suitable for the investigation of the effect of surface modifications to osseointegration in rat tail

The primary validation measurements of the newly-formed connection between SmartPeg and fully-threaded implants were successful. The average ISQ of 20 stoned implants into the plaster was 58.82±1.59 ISQ. This observation verified the applicability of the newly-developed connection between the SmartPeg type 62 and the customized implant.

4.1.2 Development of complex biomechanical evaluation by the combination of resonance frequency analysis and pull-out techniques

The evaluation of the RFA and pull-out testing from the vertebrae ex vivo was also successful. The average RFA primary stability in vertebrae ex vivo of 5 fully-threaded implants was 33.81±4.17 ISQ. The average extraction force necessary for the removal of the inserted implants from the rat vertebra bony bed was 89.60±6.05 N, showing uniform, repeatable outcomes.

The ISQ values of non-threaded implants showed significant differences when compared to their respective PUF densities of the two types of threaded implants. Most importantly, ISQ values for the non-threaded implant showed a stepwise, continuous linear decrease by the decrease of bone density, a feature that does not apply to fully-threaded and half-threaded implants. These data show that the non-threaded implants are the most suitable for the evaluation of the difference around the implant, such as density of the hosting tissue and, potentially, the level of osseointegration.

4.1.3 Complex monitoring of osseointegration with biomechanical and structural tests; assessment of improved surgical conditions and postsurgical care

4.1.3.1 Biomechanical evaluation of implant osseointegration

In our rat tail model, the ***ISQ values*** moderately changed in the initial healing time. A significant increase (1.6 folds) in ISQ values occurred from week 4 (32.84±8.86 ISQ) to week 16 (58.58±1.32 ISQ). However, no significant difference was observed between values corresponding to healing periods at week 4 (32.84±8.86 ISQ), week 8 (34.67±2.08 ISQ) and week 12 (32.2±2.08 ISQ). The ***pull-out force*** significantly increased with time and reached a plateau at week 12 postoperatively. The high sensitivity of this test was demonstrated by the fact that the pull-out force increased by approximately 500% between week 4 and week 12. There was no further significant change in this parameter between weeks 12 and 16. The correlation analysis of ISQ values to the corresponding pull-out forces showed only a weak relationship ($r=0.203$). This was primarily due to the relatively low sensitivity of resonance frequency test over the pull-out test.

4.1.3.2 Structural evaluation of osseointegration

The 2D analysis results of micro-CT scans showed that the ***i.S/TS*** values were 58%±5.78, 48%±5.05, and 61%±4.49 at weeks 4, 8 and 16, respectively. Statistically significant difference ($p<0.05$) was observed between weeks 8 and 16. In the 3D evaluation, ***BV/TV*** values were 58%±6.64, 56%±4.48 and 61%±4.93 at weeks 4, 8 and 16, respectively. No significant differences were found between the groups in BV/TV results. A positive correlation was found between BV/TV and i.S/TS data ($r=0.544$) on bone micromorphometric results. This correlation indicated a relationship between intersection surface coverage of the bone and bone volume/tissue volume values in individual specimens.

At week 4, a low level of real ***BIC*** was detected corresponding to 28.55±3.54% coverage of the interface by histomorphometry. In comparison with week 4, BIC values (61.66±3.31%) increased significantly at week 8 ($p<0.05$). At week

16, BIC values further increased to 73.85±2.12% ($p<0.05$ vs week 8). The bone around the implant had a regular a trabecular structure. These data indicated that BIC sensitively reflected the progress of osseointegration with time during a 16 weeks' experimental period.

There was no correlation between BV/TV and the histomorphometric BIC results ($r=0.014$). However, a very weak positive correlation was detected between i.S/TS and BIC ($r=0.096$).

4.2 Development of "BD OSSI" and "GAP OSSI" models for monitoring bone defect regeneration, and integration of multiply placed implants

We found that the maximal size of the ***transversal defect*** was ***2.9x3 mm***. It was achieved by the in vitro development of transversal drilling protocol for the vertebae. This defect was reproduced in "BD OSSI" and "Gap OSSI" models. We successfully established two novel surgical procedures, one for the analysis of bone regeneration of ***multiple transversal bone defects*** of the rat tail ("BD OSSI"), while the other one for the simultaneous ***implantation of multiple implants*** transversal to the rat tail vertebra ("Gap OSSI").

In the case of "BD OSSI" model, the morphological results gained from micro-CT analyses did not show bone formation when the defect (2.9x3 mm) was left empty after either week 4 or 8. When we used a xenograft bone-grafting biomaterial to fill the cavity, we obtained a ***good retention of the material*** in the defect after 4 and 8 weeks.

The micro-CT and histology results of "Gap OSSI" model 12 weeks after implantation demonstrates the stable implant position through the vertebra. Because of the special narrowing shape of the implant and the wider implant bed preparation, we could preserve a gap between the bone walls and the implant in its apical part, where "distance osteogenesis" took place successfully.

4.3 Ex vivo evaluation of oral-surgical flap mobility following mylohyoid muscle "non-detaching" and "detaching" preparations

Only one of the measurement results were not included into the analysis, because in this case (specimen 3) the sample suffered a flap tear on the control side at the time of establishing the baseline standard force, which prevented a fair comparison with the test side. Therefore, the data from this specimen were excluded from the analyses, resulting in a final sample of 11 heads and 22 surgical sites (i.e. 11 test and 11 control).

All measurement results passed the normality test (Shaphiro-Wilk) ($p>0.05$). The difference between the test and control group in Zone I (retromolar pad area), Zone II (middle area) and Zone III (premolar area) was 8.3±0.54 mm, 10.1±0.89 mm and 10.3±0.89 mm, respectively, reaching very strong statistical significance ($p<0.0001$) in all of them. In proportional terms, relative to the control, the test technique allowed for 8.2, 2.5 and 5.3 times more flap release in Zones I, II and III, respectively.

6. CONCLUSION

1. In the present work, we ***successfully adapted*** the method of ***resonance frequency analysis*** - originally developed for humans - ***to the rat tail model***. For that, we ***designed*** a ***special connection*** in the implant head, which allowed us to screw in the transductor (SmartPeg) ***for resonance frequency analysis***. For the established connection, we also designed a special hook for performing the ***pull-out test***.

2. With the newly-established connection, we ***developed three implant designs*** (full-, half-, non-threaded) which are suitable ***for studying implant osseointegration into the rat tail vertebrae*** longitudinally. When testing these implant designs, we selected the ***non-threaded*** one for further applications. This implant shape proved to be the ***most sensitive for detecting the density differences*** of the hosting tissues in vitro. Thus, we ***excluded*** the ***influence of macro design on*** the ***bone-bonding strength*** to the implant surface caused by the threads. The non-threaded implant design was the ***most suitable for*** the assessment of ***biomechanical implant stability in a vertical direction*** by the pull-out test.

3. We ***established*** a ***drilling protocol*** of the vertebrae ***for positioning the implant bed standardly*** in the longitudinal middle of the vertebrae by using a specially-designed surgical guide. Accordingly, we successfully ***improved the surgical conditions*** and the ***postsurgical care*** of the rat tail after implantation.

4. We ***developed*** a ***complex biomechanical*** evaluation setup ***by the combination of resonance frequency analysis and pull-out techniques*** for the evaluation of implant stability in rat tail vertebrae. Also, we ***were the first*** to successfully perform the ***non-decalcified tissue-sectioning*** of the rat vertebrae ***with titanium implant***. In addition, we ***designed and optimized a protocol to overcome the X-ray scattering on the metal surface*** for micro-CT scanning and reconstruction of rat tail vertebrae with an implant. This was achieved by the removal of threads, minimizing the implant's geometrical complexity.

5. Our ***methodological developments*** resulted in a successful combination of the biomechanical evaluations with structural tests in order to ***reliably and multidisciplinary monitor the osseointegration*** process in a caudal vertebra in vivo. We called this experimental setup "Direct OSSI" model. This experimental model is suitable ***for*** the quantitative ***preclinical screening*** of the osseointegration of various intraosseous implants after ***different surface treatments*** under different local and general conditions.

6. We established a ***new drilling protocol for*** the creation of ***multiple bone defects*** and ***multiple implant placement in*** the ***rat tail*** vertebrae transversally.

7. The ***transversally created bone defects*** in the rat tail demonstrated ***no self-healing*** under our experimental conditions unless bone graft material was used. That model is called ***"BD OSSI"*** experimental model. Moreover, we ***successfully modified*** the original "OSSI" model and made the rat tail suitable for the placement of ***multiple implants*** in a perpendicular direction. We named this model ***"Gap OSSI"***.

8. We were the first to successfully ***determine the effectiveness of two lingual flap preparation techniques*** ("non-detaching" and "muscle-detaching" techniques) for oral and periodontal surgeries for the extension of lingual flap mobility in a standardized preclinical setup. We found that the ***"non-detaching" approach*** to lingual flap release in the posterior mandible is superior over the "muscle-detaching" technique. The "non-detaching" technique ***significantly increased lingual flap mobility***.

LIST OF OWN PUBLICATIONS RELATED TO THE PHD THESIS

1. **Farkasdi S**, Pammer D, Rácz R, Hriczó-Koperdák G, Szabó BT, Dobó-Nagy Cs, Kerémi B, Blazsek J, Cuisinier F, Wu G, Varga G *„Development of a quantitative preclinical screening model for implant osseointegration in rat tail vertebra”*, Clin Oral Investig. 2018 Oct 29. doi: 10.1007/s00784-018-2661-1. [Epub ahead of print] PubMed PMID: 30374828.

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2. Urban I, Traxler H, Romero-Bustillos M, **Farkasdi S**, Bartee B, Baksa G, Avila-Ortiz G. *„Effectiveness of Two Different Lingual Flap Advancing Techniques for Vertical Bone Augmentation in the Posterior Mandible: A Comparative, Split-Mouth Cadaver Study.”* Int J Periodontics Restorative Dent. 2018 Jan/Feb;38(1):35-40. doi: 10.11607/prd.3227. PubMed PMID: 29240202.

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3. Renaud M, **Farkasdi S**, Pons C, Panayotov I, Collart-Dutilleul PY, Taillades H, Desoutter A, Bousquet P, Varga G, Cuisinier F, Yachouh J. *“New Rat Model for Translational Research in Bone Regeneration.”* Tissue Eng Part C Methods. 2015 Dec 31. [Epub ahead of print] PubMed PMID: 26472155.

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