Doctoral Thesis

Time-lapsed in vivo bone response to implantation in a mouse model of disease and treatment

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Time-lapsed *in vivo* bone response to implantation in a mouse model of disease and treatment

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presented by

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# Table of Contents

Acknowledgements iii

Summary v

Zusammenfassung ix

摘要 xiii

1 Introduction 1
  1.1 Thesis motivation ................................................................. 3
  1.2 Specific aims ........................................................................... 5
  1.3 Outline of the thesis ................................................................. 6

2 Background 11
  2.1 Bone response following implantation ...................................... 13

3 Development of a novel method to investigate bone response following implantation 43
  3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging........................................................................... 45

4 Bone response following implantation in disease and treatment 75
  4.1 Effect of osteoporosis on bone response following implantation............... 77
  4.2 Effect of loading on osteoporotic bone response following implantation .. 103

5 Synthesis 133
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Zihui
Summary

Osteoporosis is a serious public health issue which is associated with high fracture risk. These fractures usually require fixation with implants. The first stage of a successful long-term fixation is a good bone-implant integration, which greatly depends on the peri-implant bone response to implantation. A critical step in the peri-implant bone response is the bone remodeling process following implant placement which shapes the peri-implant bone architecture and therefore is eventually responsible for the mechanical stability of the bone-implant system. In osteoporosis, however, the low quality of the host bone and the impaired bone remodeling process jeopardize the bone-implant integration and hence the long-term stability of the fracture fixation. In order to develop advanced fracture fixation treatments, it is essential to better understand the influence of osteoporosis on the peri-implant bone response, including time-lapsed changes in bone architecture and in bone remodeling (including both formation and resorption) following implantation. Moreover, bone remodeling is believed to be regulated by local mechanical cues with bone formation occurring more likely at locations of high mechanical strains and bone resorption at regions less strained. The anabolic effect of mechanical stimuli on bone remodeling is also apparent in the presence of the implant. Therefore, controlled external mechanical loading has been suggested as an effective medication-free treatment for promoting peri-implant bone formation and enhancing bone-implant integration. However, the precise role of mechanical loading on peri-implant bone regeneration, especially in osteoporosis, remains to be determined. To explore the aforementioned aspects, this doctoral thesis is divided into the following three aims: i) to develop a suitable in vivo monitoring technique for investigating bone response following implantation; ii) to assess the effects of osteoporosis on bone structure and remodeling changes after implant insertion; iii) to evaluate the role of mechanical loading on peri-implant as well as whole bone
behavior in osteoporotic conditions and to assess the specific nature of the mechanical regulation of bone remodeling in the presence of the implant.

In the first step, an *in vivo* monitoring technique for investigating bone response following implantation in a mouse model was developed: an implant made of composite material with low X-ray attenuation but high stiffness was manufactured and coated with a thin titanium layer to enhance biocompatibility; an implantation procedure was adapted to directly insert the implant into the mouse caudal vertebra; a non-destructive imaging and image processing method was developed to allow time-lapsed measurement on the same animal using *in vivo* micro-computed tomography (micro-CT). A detailed reproducibility study showed that both bone architecture and bone remodeling assessed by the proposed framework were highly reproducible. By using this framework we were able: i) to eliminate the image artifacts which were normally induced by metal implants; ii) to monitor the spatio-temporal changes of bone architecture; iii) to assess the interplay between bone resorption and bone formation following implantation in both cortical and trabecular bone.

Furthermore, the developed *in vivo* monitoring technique was applied to assess the bone response following implant placement in both healthy and osteoporotic bone. Ovariectomized (OVX) mice were used as a model for low quality bone associated with osteoporosis, while sham-ovariectomized (SHM) mice were used as control. Following implantation, transient accelerated bone formation was observed in both OVX and SHM animals especially at the endocortical surface, resulting in a rapid increase in the cortical thickness in the peri-implant region. This increase was limited in osteoporotic bone, leading to a reduced bone-implant contact compared to healthy bone. Trabecular bone, however, showed a decrease in the entire area with the reduction in SHM animals being higher than in OVX animals.

The last part of the thesis investigated the effect of mechanical loading on bone response following implantation in osteoporotic bone: the load-induced changes in bone architecture and bone remodeling were assessed using the newly developed *in vivo* monitoring technique; the time evolution of the strength of the implanted bone and the mechanical regulation of bone remodeling in the loading scenario were investigated using image-based micro-finite element (micro-FE) analysis. In the presence of the implant, mechanical loading increased bone formation rate and decreased bone resorption rate by modulating the bone surface under formation.
(mineralizing surface) and resorption (eroded surface) in both trabecular and cortical bone. However, the effect of loading on the remodeling parameters in the peri-implant region was less evident as no statistically significant difference was detected between loaded and control animals. Nevertheless, the strength of the whole implanted bone was enhanced under mechanical loading both by increasing trabecular bone volume and cortical thickness, which suggested a corresponding enhancement in the fixation-strength of the implant.

In conclusion, we have developed a novel in vivo monitoring technique for investigating bone response, including both bone architecture and bone remodeling, following implantation in a mouse model and we have demonstrated its usefulness for investigating the processes in diseased (osteoporosis) and treated (administration of mechanical loading) bone in vivo. The knowledge gained from these findings may improve the current understanding of peri-implant bone regeneration in osteoporosis and facilitate the development of new and adequate loading protocols to enhance peri-implant bone regeneration, especially in an osteoporotic scenario.
Zusammenfassung

Im ersten Schritt wurde eine in vivo Überwachungstechnik entwickelt, um die Antwort des Knochens auf die Implantation in einem Mausmodell zu untersuchen: Ein Implantat wurde aus einem Verbundmaterial mit einer geringen Röntgendichte und hohen Steifigkeit hergestellt und einer dünnen Titanschicht überzogen um die Biokompatibilität zu verbessern; das Vorgehen bei der Implantation wurde angepasst um das Implantat direkt in den Schwanzwirbel der Maus einsetzen zu können; ein zerstörungsfreies Bildgebungs- und verarbeitungsverfahren wurde entwickelt, das wiederholte Messungen des selben Tieres mittels in vivo mikro-Computertomographie (mikro-CT) ermöglicht. Eine ausführliche Reproduzierbarkeitsstudie zeigte, dass die Knochenarchitektur und der Knochenumbau, die mit dem vorgestellten Protokoll bestimmt wurden, hochgradig reproduzierbar waren. Indem wir dieses Protokoll benutzten, konnten wir: i) Bild-Artefakte, die normalerweise durch Metallimplantate verursacht werden, eliminieren, ii) räumliche und zeitliche Veränderungen der Knochenarchitektur beobachten, iii) das auf die Implantation folgende Zusammenspiel von Knochenresorption und –neubildung sowohl im kortikalen als auch trabekulären Knochen bewerten.

Weiterhin wurde die entwickelte in vivo Überwachungsmethode angewendet, um die Reaktion des Knochens auf die Implantation sowohl in gesundem als auch in osteoporotischem Knochen zu bestimmen. Ovarektomierte (OVX) Mäuse wurden als Model für die schlechte Knochenqualität, mit der Osteoporose einhergeht, verwendet, Schein-operierte Tiere (SHM) als Kontrolle. Nach der Implantation wurde in OVX und SHM Tieren eine vorübergehende Beschleunigung der Knochenbildung beobachtet, die vor allem an der endokortikalen Oberfläche stattfand und in einer schnellen Zunahme der kortikalen Dicke um das Implantat
herum resultierte. Diese Zunahme war im osteoporotischen Knochen eingeschränkt, was zu einem im Vergleich zu gesundem Knochen verringerten Knochen-Implantat-Kontakt führte. Im Gegensatz dazu zeigte trabekulärer Knochen im gesamten Gebiet eine Abnahme, die in SHM Tieren stärker als in OVX Tieren war.

Im letzten Teil dieser Arbeit wurde der Einfluss von mechanischer Belastung auf die Reaktion des Knochens auf die Implantation in osteoporotischen Knochen untersucht: Mittels der neu entwickelten \textit{in vivo} Überwachungstechnik wurden durch Belastung verursachte Veränderungen von Knochenarchitektur und -umbau bestimmt; die zeitliche Entwicklung von Stärke des Implantattragenden Knochens und die mechanische Regulation des Knochenumbaus im Belastungs-Szenario wurden mittels mikro-Finite-Elemente (mikro-FE) – Analyse untersucht. In Gegenwart des Implantates vergrößerte die mechanische Belastung die Knochenbildungsrate und verringerte die Knochenresorptionrate sowohl im kortikalen als auch trabekulären Knochen indem die Oberflächen an denen Knochenbildung (mineralisierende Oberfläche) und Resorption (erodierte Oberfläche) stattfanden, moduliert wurden. Jedoch war der Einfluss der Belastung auf die Umbauparameter in der Region um das Implantat weniger offensichtlich, es konnte kein Unterschied zwischen belasteten und Kontrolltieren festgestellt werden. Dessen ungeachtet war die Stärke des ganzen implantattragenden Knochens mit mechanischer Belastung größer, was sowohl durch eine Vergrößerung des trabekulären Knochenvolumens als auch der kortikalen Dicke verursacht wurde, was eine damit einhergehende Steigerung der Fixations-Stärke des Implantats nahelegt.

Als Fazit haben wir eine neue \textit{in vivo} Beobachtungstechnik entwickelt, um die Reaktion des Knochens, inklusive seiner Architektur und Umbauvorgänge, auf Implantation in einem Mausmodell zu untersuchen und konnten ihre Brauchbarkeit bei der Untersuchung der Prozesse im kranken (Osteoporose) und behandelten (Applikation von mechanischer Belastung) lebenden Knochen zeigen. Das daraus gewonnen Wissen könnte das gegenwärtige Verständnis der Knochenregeneration um ein Implantat herum bei Osteoporose verbessern und die Entwicklung neuer und angemessener Belastungsprotokolle um die Knochenregeneration um das Implantat insbesondere in einem Osteoporose-Szenario zu verstärken, ermöglichen.
摘要

骨质疏松，以及由此引发的骨折发生率的升高，是严重的社会公共健康问题。治疗骨质疏松性骨折通常需要人工种植体的植入和固定。种植体长期稳定性的前提是其与骨形成骨整合，这种整合取决于种植体周围骨组织再生过程。骨重建是种植体周围骨组织再生的关键步骤，它重塑种植体周围骨组织的形态，并最终决定种植体和骨整体结构的机械稳定性。然而，由骨质疏松引起的骨质量下降和骨重建过程受损，严重危害种植体的骨整合及其长期稳定性。深入理解骨质疏松对种植体周围骨组织再生能力的影响，包括骨形态和骨重建过程（包括骨形成和骨吸收）在植入后随时间的变化，将有助于制定合理有效的种植体治疗方案。此外，骨重建受骨组织局部力学环境的控制：在机械应力较高的区域，骨生成的频率高；而在机械应力较低的区域，骨更容易被吸收。种植体植入后，机械应力刺激也能促进骨重建过程中的骨生成。因此，适度的外部机械载荷被建议作为促进种植体周围骨生成和提高种植体骨整合的有效非药物治疗方法。但是，机械载荷对种植体周围骨再生过程的确切影响机制还有待研究，尤其是在骨质疏松状态下。综合以上，本论文目的共有三个层面：一、建立一个合适的活体监测方案以研究种植体植入后周围的骨组织再生过程；二、评估骨质疏松对种植体植入后周围骨形态和骨重建变化过程的影响；三、评估骨质疏松状态下机械载荷对种植体周围以及整体骨区域骨组织再生的影响，探索机械应力刺激对种植体植入后的骨重建过程的影响机制。

研究一建立了一个活体监测方案以研究种植体植入小鼠后周围的骨组织再生过程：该方案中采用的种植体由高硬度低X射线衰减系数的复合材料制成，并镀以钛表面涂层以提高生物相容性；采用一种改进型植入技术，将
摘要

种植体直接插入小鼠尾椎骨；采用X射线活体计算机断层扫描（micro-CT）进行无损图像扫描和处理，以记录和分析相同小鼠在不同时间的状态。此外，本论文通过详细的再现性研究，证实了应用该方案定量的骨形态和骨重建能够达到很高的再现性。应用该方案，我们能够：1）去除CT图像中经常由金属种植体产生的伪影；2）监测种植体周围骨形态随时间和空间的变化；3）定量评估种植体周围密质骨和松质骨内的骨生成和骨吸收的相互关联。

研究二旨在应用研究一中建立的活体监测方案，定量评估在健康和骨质疏松状态下种植体植入后周围骨组织再生过程。卵巢切除小鼠被用以模拟骨质疏松状态下的骨质量下降，而假手术组小鼠被作为健康对照。种植体植入后，卵巢切除小鼠和假手术组小鼠都出现了一个短暂而快速的骨生成阶段，该阶段在骨内膜区域尤其显著，引发种植体周围密质骨厚度的快速增长。而该增长阶段在骨质疏松模型小鼠中被抑制，导致种植体-骨接触面积相对健康小鼠减少。另一方面，松质骨体积分数在种植体植入后呈整体下降趋势，且相对于骨质疏松模型小鼠，健康小鼠松质骨体积分数的下降程度更显著。

研究三旨在探索骨质疏松状态下机械载荷对种植体植入后周围骨组织再生的影响及其机制：应用研究一中建立的活体监测方案，定量评估种植体周围骨形态和骨重建在机械载荷刺激下的变化；应用基于断层扫描图像的微有限元（micro-FE）方法，评估骨的整体强度随时间的变化，并分析机械载荷对骨重建的影响机制。种植体植入后，在整体密质骨和松质骨区域内，机械载荷通过影响骨生成（矿化表面）和骨吸收（侵蚀表面）的发生表面，提高骨生成率并降低骨吸收率。然而，在靠近种植体的区域，加载机械载荷的小鼠和对照组小鼠的骨重建参数没有显著性差异，说明在该区域机械载荷对骨重建的影响并不显著。尽管如此，机械载荷促进了整体松质骨体积分数和密质骨厚度的提高，从而增大了种植体植入后骨的整体强度，并增加了种植体的固有强度。

综上所述，本论文建立了一个新型的活体监测方案以研究种植体植入小鼠后周围的骨组织再生过程，包括骨形态和骨重建随时间和空间的变化，并成功地应用该方案探索了在疾病（骨质疏松）和治疗（加载机械载荷）状
态下种植体周围的骨再生过程。这些知识的获得将有利于进一步理解骨质疏松状态下种植体周围的骨组织再生过程，并将促进新型合理的机械载荷治疗方法的建立，以增强尤其是骨质疏松状态下的种植体周围的骨组织再生能力。
Chapter 1

Introduction
1.1 Thesis motivation

Osteoporosis is one of the most common musculoskeletal diseases among the elderly population. It is characterized by a reduction in bone strength resulting in an increased risk of fracture [1]. Such fractures usually require fixation and stabilization with implants. The long-term success of fracture fixation is based on a good early biomechanical anchorage of the implant in the bone stock as well as on proper skeletal regeneration around the implant. However, due to poor bone quality and compromised systemic conditions, both implant anchorage and integration may be greatly reduced in osteoporotic bone [2]. Although strong clinical evidence correlating the osteoporotic condition with implant failure is still missing [3, 4], numerous biomechanical experiments [5, 6] and in vivo animal studies [7] suggest that osteoporosis jeopardizes the outcome of fracture fixation. The underlying mechanisms are not fully understood yet; however, a key factor for the maintenance of the mechanical integrity of the bone-implant system is the process of bone remodeling, where old or damaged bone is continuously replaced with new bone [8]. Bone remodeling is mechanically controlled [9, 10] and the lack of appropriate mechanical stimulation as well as the changes in the mechanical environment induced by the implant can lead to bone loss and impair long-term implant anchorage [11].

Over the past years, implant research has mainly been concentrating on improving implant osteoinduction (i.e., stimulation of osteoprogenitor cells to differentiate into bone forming cells) and osteoconduction (i.e., stimulation of bone growth on the implant surface) which are the two fundamental mechanisms leading to osseointegration, defined by the direct formation of bony tissue in contact with the implant [12]. Common approaches to increase osseointegration comprise the manipulation of implant surface topography both at the micro- and nano-level [13], the application of biomimetic surface coatings such as calcium phosphate [14], extracellular matrix components [15] and growth factors [16] and the local delivery of medication promoting bone formation [17] or inhibiting bone resorption [18]. Surprisingly, despite the fundamental importance of bone remodeling for both early and late bone response to implant insertion [2], a detailed characterization of the spatial and temporal patterns of bone formation and resorption close to the implant,
in the peri-implant bone and especially at the whole bone level (i.e. organ level) is still missing. Such information is obviously essential to better understand the modifications in bone architecture taking place after implantation which are ultimately responsible for anchoring the implant into the peri-implant bone bed. Moreover, although mechanical loading is unanimously considered a potential natural stimulator for bone formation, its effect on implant osseointegration and peri-implant bone regeneration is not well characterized; a particularly poorly understood aspect is the role of mechanical loading on bone formation and bone resorption at the local tissue level in the presence of the implant [19].

Numerous animal models have been proposed to investigate bone behavior following implant insertion ranging from small animals such as mice [20] to larger animals like sheep [21]. Common to all animal models are the strategy to characterize bone remodeling and bone architecture. Bone remodeling is mostly investigated with dynamic histomorphometry, which is a technique based on the analysis of histological sections of bone tissue after the administration of proper fluorescent labels [22]. This procedure provides detailed information on bone formation but not on bone resorption [23]; additionally, it can capture bone changes only within one time interval, typically of two or three weeks. Similar limitations exist in the most common ways to measure bone architecture, which are based on static histomorphometry [24] or ex vivo micro-computed tomography (micro-CT) [25]: both techniques are destructive and limited to a single time point. The recent introduction of in vivo micro-CT has provided a new option to perform bone research in living animals as it allows monitoring changes in bone remodeling (formation and resorption) as well as in bone architecture on the same animal for several weeks and in three dimensions. Indeed in vivo micro-CT is increasingly used in studies involving small animals such as mice and rats to characterize the time evolution of bone structure [26], bone remodeling [27] and bone mineralization [28]. Furthermore, the judicious combination of in vivo micro-CT with image-based high resolution micro-finite element (micro-FE) analysis has enabled to unravel the details of the local mechanical control of bone remodeling at the tissue level in mice subjected to external mechanical loading [9]. This is a particularly appealing framework to be used in implant research to clearly answer the question whether mechanical loading is still able to control and to direct local bone formation and resorption in the peri-implant bone even if the presence of the implant may interact with the mechanical environment.
The Institute for Biomechanics at ETH Zurich has played a pivotal role in the characterization of the biological process of bone remodeling in living mice using *in vivo* micro-CT. To date, the use of that approach to characterize the bone response following implant insertion is very limited and hampered by the well-known metal artifacts caused by the implant [26], which jeopardize the possibility to extract quantitative information on the remodeling process around as well as close to the implant. The main hypothesis of this thesis is that the combination of *in vivo* micro-CT imaging and image-based micro-FE analysis will facilitate the understanding of the precise role of bone remodeling and mechanical loading in the bone regeneration process around the implant, especially in osteoporosis where bone remodeling is already imbalanced before implantation. Such knowledge would provide opportunities to develop new treatments based on novel “loading procedures” for improving implant integration and anchorage. The specific aims of the thesis are described in the next section.

### 1.2 Specific aims

The main goal of the thesis is to develop a combined experimental-computational framework for the quantification of bone remodeling following implant placement and for the characterization of the mechanical control of peri-implant bone regeneration. In short, bone formation and resorption close to the implant and in the entire bone will be assessed in mouse caudal vertebra *in vivo*, in three-dimensions and in a time-lapsed fashion using micro-CT together with a novel implant and implantation procedure. High resolution micro-FE models will be developed to assess the local mechanical environment in peri-implant bone as a function of the applied loads. The link between local mechanical stimuli and the subsequent remodeling behavior around the implant will be unraveled. This approach will then be used to investigate the effects of osteoporosis on bone response following implantation and to prove the beneficial effect of mechanical loading as a “therapy” to improve peri-implant bone regeneration in an osteoporotic scenario.

Specifically, the following three aims are defined:

**Aim 1**: To design an implant and implantation procedure which enables the use of *in vivo* micro-CT to monitor in a time-lapsed fashion the changes in bone remodeling, including both formation and resorption, and the corresponding
modifications in bone architecture following the placement of the implant in mouse caudal vertebra.

**Aim 2**: To assess the main effect of bone deterioration following estrogen depletion on the biological processes of bone remodeling (including both formation and resorption) and the consequential modifications in bone architecture, within three different bone compartments including trabecular bone, endocortical and periosteal surface both close to and far from the implant by using the framework established in Aim 1.

**Aim 3**: To administer controlled external mechanical loading to the implanted vertebra and to monitor its possible beneficial effect on bone regeneration in an osteoporotic scenario by combining the technique developed in Aim 1, the knowledge acquired in Aim 2 with high resolution micro-FE for the detailed characterization of the local mechanical environment.

### 1.3 Outline of the thesis

The thesis consists of 5 chapters. In addition to the current chapter where thesis motivation and specific aims are presented, the content of the subsequent chapters is the following:

- **Chapter 2** provides the required background knowledge to understand the multifaceted bone response to implantation and the main factors influencing this complex process. First of all, the biological behavior of bone shortly after implantation was described. Then the main animal models for investigating the influence of osteoporosis on bone-implant integration were reviewed. Thirdly, the various applications of mechanical loading as an anabolic treatment to enhance peri-implant bone formation in animal models were evaluated.

- **Chapter 3** describes the development of the *in vivo* monitoring technique for investigating bone response following implantation. The choice of the implant material for eliminating the metal artifact and the corresponding implantation procedure were addressed. The framework based on a non-destructive method to allow time-lapsed measurements on the same animal using *in vivo* micro-CT was described. A detailed reproducibility study showing the precision of the novel technique was reported. Finally, the chapter comprises a case study where
the newly developed approach was used to monitor the spatio-temporal changes of bone architecture and to assess the interplay between bone resorption and bone formation following implantation in both cortical and trabecular bone in mouse caudal vertebrae.

- **Chapter 4** contains the application of the *in vivo* monitoring technique for the investigation of the post-implantation bone behavior in two clinically relevant settings including an estrogen depleted scenario which simulates the typical bone deterioration occurring in osteoporosis and its treatment via the judicious administration of controlled external mechanical loading. Specifically, the first section of the chapter describes the assessment of peri-implant bone behavior in osteoporosis using the developed *in vivo* monitoring technique described in Chapter 3, with a particular emphasis on the differences in bone formation and resorption occurring at three different locations within the same bone: trabecular bone, endocortical surface and peristeal surface of cortical bone. The second section presents a detailed study on the main effects of mechanical loading on bone formation and resorption in the implanted vertebra: the load-induced changes in bone remodeling and bone architecture were evaluated using the developed *in vivo* monitoring technique; furthermore, the basic mechanical control of bone remodeling with the presence of the implant was investigated by the detailed characterization of the local mechanical environment using high resolution image-based micro-FE analysis.

- **Chapter 5** is the synthesis of this thesis including the major findings, the limitations of the presented work, and an outlook for future research.

**References**


1.3 Outline of the thesis


Chapter 1 Introduction


Chapter 2

Background
2.1 Bone response following implantation

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in preparation
as “Bone regeneration following implantation in animal models: the influence of osteoporosis and mechanical loading”

Abstract:
The long-term success of orthopaedic implant fixation depends on a good integration of the implant into the host bone. In osteoporotic bone where the risk of fracture is higher than healthy bone, bone-implant integration is believed to be jeopardized by the low bone quality. However, the influence of osteoporosis on peri-implant bone regeneration is not well understood. Mechanical loading has the ability to stimulate bone formation and to decrease bone resorption. Enhanced bone formation has also been observed at the bone-implant interfaces; therefore mechanical loading is considered a possible anabolic treatment to promote implant integration. To date, less is known on the effect of mechanical loading on peri-implant bone regeneration. In order to develop effective loading protocols to improve implant integration especially in osteoporotic bone, a greater understanding of the in vivo load-induced bone regeneration around the implant must be achieved. This review aims firstly to provide an overview of the in vivo studies investigating the possible influence of osteoporosis on implant integration and, secondly, to report the recent achievements on the use of external mechanical loading to improve the quality of the peri-implant bone which is ultimately related to implant integration.

Keywords:
Bone regeneration, implant, peri-implant bone, animal models, osteoporosis,
mechanical loading, anabolic treatment

### 2.1.1 Introduction

Orthopaedic implants are widely used to restore or replace function of diseased and damaged bone. In order to fulfill their desired functions in the body, it is important for the implants to achieve long-term stability, which requires a good integration of the implant into the host bone. The need for orthopaedic implants and the associated treatments for fracture fixations is growing due to the aging of our society and the corresponding increase in the incidence of osteoporosis, one of the most prevalent skeletal disorder, which is continuously increasing the amount of bone fractures [1]. In osteoporosis, the low bone density and weakened bone structure are believed to jeopardize the outcome of fracture fixation with orthopaedic implants [2]. The underlying mechanisms are not fully understood, but evidences have shown that the interplays between impairments in bone remodeling and the complex bone regeneration process around the implant play a key role in the poor implant integration in osteoporotic bone [3]. Therefore, in order to develop novel strategies for improving the long-term stability of fracture fixation in osteoporotic bone, it is imperative to better understand the *in vivo* bone regeneration process after implant insertion.

In the complex biological process of bone regeneration following implantation, a critical stage is bone remodeling, in which old or damaged bone is replaced with new bone to attain and maintain the integrity of the bone-implant system. It is well accepted that the remodeling process is mechanically regulated: bone is most likely formed at sites with high strains and is removed from sites with low strains [4]. The general features of the mechanical control are believed to be present during bone repair and regeneration after injury or following implantation surgery. In fact, controlled external mechanical loading has been shown to stimulate bone formation at the bone-implant interface and to promote implant integration, although the precise role of mechanical stimulation in the process of peri-implant bone regeneration, especially under *in vivo* conditions, remains poorly understood [5, 6]. Nevertheless, several *in vitro* studies have shown that mechanical stimuli play an important role in the stimulation of osteogenic cells differentiation [7-10] and in downregulation of osteocyte expression of sclerostin, the potent inhibitor of bone formation [11]. Greater understanding of load-induced bone regeneration under *in
2.1 Bone response following implantation

In order to analyze the possible influence of osteoporosis on implant integration and to evaluate the potential benefit of controlled external mechanical loading on enhancing implant integration, animal models are an essential research tool for *in vivo* mechanobiological experiments which, obviously, cannot be performed on human individuals. The specific aims of this paper are: *i*) to summarize the biology of early bone regeneration following implantation; *ii*) to review the *in vivo* animal studies investigating the influence of osteoporosis on bone-implant integration; *iii*) to report the recent achievements on the *in vivo* effect of mechanical loading on peri-implant bone regeneration.

### 2.1.2 Biology of bone regeneration following implantation

A series of cellular and extracellular events take place at the bone-implant interface following implantation until the implant is fully integrated with the surrounding bone. Soon after an orthopaedic implant has been inserted into the bone, non-specific protein adsorption takes at the implant interface [12]. Afterwards the implant is detected by neutrophils and macrophages, followed by the release of cytokines which attracts fibroblasts and drives the foreign body encapsulation process [13]. The integration of implant into the bone stock begins when osteoprogenitor cells migrate to the implant site and differentiate into osteoblasts which are responsible for bone formation (Figure 2.1a). A critical process for the long-term stability of an orthopaedic implant [14] is a proper osseointegration, defined as a direct structural and functional connection at the bone-implant interface without interposition of non-bone tissue (Figure 2.1b) [15, 16]. Over the next several weeks following the placement of the implant, peri-implant bone keeps continuously remodeling to maintain the integrity of the bone-implant system.

Bone remodeling occurs in the so-called basic multicellular units comprising osteoclasts, the bone resorbing cells, and osteoblasts, the bone forming cells. Bone remodeling is initiated by osteocytes, the mechanosensor in bone, and their secretion of signaling molecules. Osteocytes sense the stimuli, recruit and activate osteoclasts, which start to form the resorption cavities. Then osteoblasts are differentiated and assembled within resorption cavities to secret organic matrix,
which is later on mineralized and formed new bone. During new bone formation some osteoblasts are embedded within the matrix and become osteocytes. In remodeling homeostasis, resorption and formation are balanced with equal amounts of resorbed and formed bone, resulting in steady bone mass. In a changing mechanical environment, bone is able to adapt its mass and structure according to the mechanical needs, a process which requires spatially decoupled bone formation and resorption. Peri-implant bone remodeling is obviously critical for attaining and maintaining the mechanical integrity of the bone-implant system [17]. Similar to normal bone remodeling in intact bone, peri-implant bone is constantly remodeled in order to repair damage and adapt to the mechanical environment, particularly to the changes in the loading pattern induced by the implant. The response of bone remodeling to implantation has been described as a “regional acceleratory phenomenon”, i.e., a complex reaction which transiently and locally accelerates the biological processes in bones and soft tissues due to external invasive stimuli (e.g., surgery). The regional acceleratory phenomenon is characterized by an accelerated
2.1 Bone response following implantation

Bone formation and resorption and it can persist for several weeks after the trauma [18]. In addition, peri-implant bone remodeling is also believed to be triggered by the presence of extensive microdamages produced by the implantation procedure even far away from the implant location. These microdamages need to be repaired by highly targeted osteoclastic resorption [19, 20].

2.1.3 Influence of osteoporosis on bone response around implants

In remodeling homeostasis, resorption and formation are balanced and therefore bone mass does not change. In osteoporosis, however, bone remodeling becomes unbalanced with increased bone resorption and impaired bone formation [21]. This imbalance leads to a decreased bone mass and a weakened bone structure. Typical effects on bone structure includes: cortical thinning, increased cortical porosity, decreased trabecular thickness and decreased trabecular connectivity density [1]. The poor bone quality together with the increasing tendency of fall suffered by the osteoporotic patients result in a high probability of fracture, which may require fixation with implants. It is presumed that osteoporosis not only causes a higher risk of fracture, but also a higher risk of implant failure. A clear correlation between osteoporotic conditions and implant failure could not be shown in clinical studies due to the lack of accurate osteoporosis assessment, missing complication definitions and heterogeneous inclusion criteria in clinical studies [22]. Therefore, animal studies have been performed to evaluate the influence of osteoporosis on peri-implant bone regeneration. This review summarizes the in vivo studies for investigating peri-implant bone structure and bone remodeling in osteoporosis (Table 2.1): firstly the animal models used in these studies are described; thereafter the findings of these studies are summarized.

Animal model

Mouse models

Mice show several advantages in pre-clinical research because of the existence of inbred strains which limits biological variation, the extended knowledge of the mouse genome and the possibility of introducing genetic modifications. However, mouse models are not widely used in studying bone-implant integration due to the
Table 2.1: Studies of bone response following implantation using osteoporotic animal models.

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal model</th>
<th>Implantation site</th>
<th>Method</th>
<th>Bone architecture</th>
<th>Bone remodeling</th>
<th>Study period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhang et al. (2015)</td>
<td>OVX, mice</td>
<td>Distal femur</td>
<td>Static histomorphometry</td>
<td>BIC, BV/TV</td>
<td>–</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Beppu et al. (2013)</td>
<td>Senile, mice</td>
<td>Tibial medullary canal</td>
<td>Light microscopy, ex vivo micro-QCT</td>
<td>BIC, BMD</td>
<td>–</td>
<td>1 - 4 weeks</td>
</tr>
<tr>
<td>Alghamdi et al. (2013)</td>
<td>OVX, rats</td>
<td>Femoral condyle</td>
<td>Ex vivo micro-CT, static histomorphometry</td>
<td>BIC, BA</td>
<td>–</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Kettenberger et al. (2014)</td>
<td>OVX, rats</td>
<td>Femoral condyle</td>
<td>In vivo micro-CT</td>
<td>BV/TV, Tb.Th, Tb.N, Tb.Sp</td>
<td>BFR, BRR</td>
<td>0 - 8 weeks</td>
</tr>
<tr>
<td>Virdi et al. (2015)</td>
<td>OVX, rats</td>
<td>Femoral medullary canal</td>
<td>Ex vivo micro-CT, dynamic histomorphometry</td>
<td>BIC, BV/TV, Ct.Th</td>
<td>BFR, ES</td>
<td>4 - 12 weeks</td>
</tr>
<tr>
<td>Irish et al. (2013)</td>
<td>OVX, rats</td>
<td>Femoral medullary canal</td>
<td>Ex vivo micro-CT, dynamic histomorphometry</td>
<td>BV/TV, Tb.Th, Tb.N, Tb.Sp, Ct.Ar</td>
<td>BFR, MS, MAR, ES</td>
<td>4 - 12 weeks</td>
</tr>
<tr>
<td>Du et al. (2009)</td>
<td>OVX, rats</td>
<td>Proximal tibia</td>
<td>Static histomorphometry</td>
<td>BIC, BA</td>
<td>–</td>
<td>4 - 12 weeks</td>
</tr>
<tr>
<td>Stadlinger et al. (2013)</td>
<td>OVX, rats</td>
<td>Proximal tibia</td>
<td>Ex vivo micro-CT, static histomorphometry</td>
<td>BIC, BA, BV/TV</td>
<td>–</td>
<td>2 - 4 weeks</td>
</tr>
<tr>
<td>Dudeck et al. (2014)</td>
<td>OVX, rats</td>
<td>Tibial medullary canal</td>
<td>Ex vivo micro-CT, static histomorphometry</td>
<td>BA</td>
<td>–</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Almagro et al. (2013)</td>
<td>OVX, rabbits</td>
<td>Proximal tibia</td>
<td>Dual X-ray absorptiometry, SEM</td>
<td>BMD, BIC, BA</td>
<td>–</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Mori et al. (1997)</td>
<td>OVX, rabbits</td>
<td>Distal tibia</td>
<td>Static histomorphometry, radiography</td>
<td>BIC</td>
<td>–</td>
<td>2 - 12 weeks</td>
</tr>
</tbody>
</table>

increased difficulty of implantation surgery caused by the small size of the animals and the implants. Nevertheless, mice show similar reactions to estrogen deficiency as humans and therefore are considered suitable for mimicking osteoporotic bone loss [23]. For instance, Zhang et al. [24] placed cylindrical titanium implants in the distal femur of ovariectomy (OVX) and sham-ovariectomy (sham) mice. Beppu et al. [25] used senescence-accelerated prone mice to mimic osteoporotic conditions, while senescence-accelerated resistant mice were used as control. The main outcomes of these studies will be presented in the next section.

**Rat models**

Rats are the most commonly used animal models in studying bone-implant integration in osteoporosis. OVX rats mimic postmenopausal trabecular bone loss in the distal femur, proximal tibia, and lumbar vertebrae, and are recommended as a preclinical animal model for the development of osteoporosis therapy by the U.S. Food and Drug Administration [26]. Osteoporotic conditions can also be introduced in male rats using orchiectomy (ORX) [27]. Most studies using rats as osteoporotic animal models focus on implant integration in trabecular bone by placing the implants in the distal femur [28], the femoral condyle [29, 30], the medullary canal of femur [31, 32], and the proximal tibia [27, 33]. This is due to the fact that trabecular bone loss is the major concern for osteoporotic patients: compared to cortical bone, trabecular bone having larger surface is exposed to more remodeling and thereby suffers from a faster bone loss. Nevertheless, implant integration in cortical bone is equally important as cortical bone allows higher contact surface with the implant than trabecular bone [34] and it plays a major role in the mechanical stability of the bone-implant system by confining the deformation of the trabecular compartment [35, 36]. However, peri-implant cortical parameters were reported in only a limited number of studies [32, 37, 38] and, in some case, cortical and trabecular bone were not separated [38].

There are some major disadvantages of using rodent models in the investigations of bone response following implantation: in general bones in rodents show lifelong longitudinal growth as the epiphyses do not close completely, while human bones stop growing in length in early adulthood; rodents also lack a well-developed Haversian remodeling system in the skeleton, which is important for the maintenance of cortical bone strength [39] and is the main cause of the increased cortical porosity in osteoporotic patients [40]; due to the small size of the animal,
only miniaturized implants could be used in these studies, which were different in shape and aspect ratio from implants used in clinics.

**Rabbit models**

Rabbit models have been introduced to overcome the disadvantages of rodent models: rabbits reach skeletal maturity after complete sexual development [39]; they have an active Haversian remodeling system [39]; there is adequate bone mass to permit the insertion of implants used in clinics, especially in dentistry [41]. However, rabbits are resistant to the conventional method to induce bone loss based on estrogen depletion. Therefore, for inducing osteoporotic conditions in rabbits, combined approaches are required. Mori et al. [41] introduced a rabbit model subjected to OVX and low-calcium diet [41]; titanium screws were inserted in the distal tibial diaphysis of the osteoporotic animal model and sham animals with normal diet. Almagro et al. [42] performed OVX combined with methylprednisolone administration to induce osteoporotic condition in rabbits; titanium screws were inserted in the proximal tibial metaphysis of the osteoporotic animal model and a healthy control group.

**Sheep models**

Among larger animals, sheep are extensively used in orthopaedic pre-clinical research. OVX sheep showed significant decrease in bone mineral density [43] and trabecular bone volume [44], and thereby have been used as models for investigating peri-implant bone healing in osteoporotic conditions at weight-bearing bone, such as femur [45] and tibia [46]. Moreover, sheep have been considered good models for studying implant fixation in the osteoporotic spine due to the similar size compared to human [47], although the loads applied to the ambulating spine are different [48].

**The influence of osteoporosis on bone regeneration around implants**

Bone-to-implant contact (BIC) has been considered a major outcome of osseointegration [49]. BIC indicates a direct formation of bony tissue at the bone-implant interface, which allows stable implant anchorage within the host bone. BIC has been measured using static histomorphometry [24, 31, 37, 41, 50], light microscopy [25], *ex vivo* micro-computed tomography (micro-CT) [33, 51], and scanning electron microscopy (SEM) [42]. It has been shown that BIC is significantly reduced in osteoporotic animal models than in sham or healthy control.
2.1 Bone response following implantation

Figure 2.2: Histological observations after 28 days of inserting titanium screw-shaped implants in the proximal tibia of rats [37]. Direct contact was observed between the implant surface and the newly formed trabecular bone in sham-operated group, with the newly formed trabeculae being connected with preexisting bone (a and d). The new bone formation around implant surface in ovariectomized group (b and e) was thin and discontinuous. (for a and b, bar = 2 mm; for c and d, bar = 0.5 mm). (Reproduced with permission from John Wiley and Sons).
group within a period ranging from 1 to 12 weeks following implantation (Figure 2.2) [24, 33, 37, 50]. However, there were also studies showing that the decrease of BIC was not significant in osteoporosis compared to control animals [25, 29, 31, 42].

Peri-implant bone area (BA) is a parameter measured in two-dimension and defined as the bone area per tissue area in a region adjacent to the implant. Higher BA indicates larger amount of bone mass in the peri-implant region, which stabilized the bone-implant construct. BA can be measured using the same techniques as measuring BIC. Significant less BA was observed in peri-implant trabecular bone in osteoporotic animal models than in sham animals 2 – 12 weeks following implantation [29, 33, 37]. Nevertheless, it should be taken into account that bone area per tissue area is already decreased in osteoporosis before implantation.

Bone mineral density (BMD) is the most widely used non-invasive assessment of bone strength to diagnose osteoporosis in routine clinical practice. It has also been considered as an important factor which influences implant stability [52]. BMD was investigated using *ex vivo* micro-quantitative computed tomography (micro-QCT) [25] and dual X-ray absorptiometry [42]. Lower BMD was reported in the peri-implant bone marrow region (2 weeks following implantation) [25] and trabecular bone (12 weeks following implantation) [42] in osteoporotic animal models compared to control groups.

Bone architecture in both trabecular and cortical compartments is critical for the biomechanical stability of bone-implant construct and thereby has become more and more important in analyzing bone quality in osteoporosis [1]. The gold standard of assessing bone architectural parameters is static histomorphometry, which measures the various parameters in two-dimensional sections with fairly high resolution. The introduction of micro-computed tomography (micro-CT) has provided a robust and non-invasive method for visualization and quantification of bone architecture in three dimensions [53]. Significant negative changes in architectural parameters are generally observed from 4 to 12 weeks after implant placement in the peri-implant region of osteoporotic animal models compared to control groups. The most affected parameters are: trabecular bone volume fraction (BV/TV) [24, 32, 50], trabecular thickness (Tb.Th) [32], trabecular number (Tb.N) [32], and cortical area (Ct.Ar) [44]. The influence of osteoporosis on peri-implant cortical thickness (Ct.Th) is more debated with some authors reporting no significant changes between OVX and control animals [31].
2.1 Bone response following implantation

Bone remodeling is important for attaining and maintaining the integrity of the bone-implant construct. It is a dynamic process and has been investigated using dynamic histomorphometry with multiple labels. The most often measured remodeling parameters included bone formation rate (BFR), mineralizing surface (MS), mineral apposition rate (MAR), and eroded surface (ES) [31, 32]. Recently, the introduction of in vivo micro-CT and image registration techniques enabled a detailed assessment not only of bone formation but also of bone resorption, the latter being normally not accessible using dynamic histomorphometry [30]. Transient accelerated bone remodeling has been shown 4 – 8 weeks following implantation especially in the regions closer to the implant in both OVX and sham animal groups; the accelerated parameters included BFR in trabecular [30-32] and cortical bone [32], MAR in trabecular and cortical bone [32], MS in cortical bone [32], and BRR in trabecular bone [30]. However, the difference in peri-implant bone remodeling between OVX and sham animals was not clearly addressed.

The aforementioned studies demonstrated the influence of osteoporosis on peri-implant bone regeneration in the lower BIC, BA and BMD, as well as the weakened structure of trabecular and cortical bone. It is believed that these influences increase the failure risk of the implant in osteoporotic fracture fixation [3]. However, most of the studies reported results at single time point and failed to monitor the bone regeneration (including both bone architecture and bone remodeling) at different time stages following implantation. A few studies reported the time course of the bone formation following implantation [30-32] and only one study monitored the time-lapsed changes in bone resorption around an implant [30]. Nevertheless, the reduced bone quality in osteoporosis is a result of imbalance in the bone remodeling process. Therefore, a better understanding in the remodeling process following implantation, including both bone formation and bone resorption, is mandatory for developing advanced treatment for osteoporotic fracture fixation.

The development of in vivo micro-CT and registration techniques enables the assessment of time-lapsed bone morphometric analysis including tissue level bone remodeling [54-56]. Recently, in vivo micro-CT has been used to monitor the time course of peri-implant bone regeneration around titanium implants within the same animals [57]. Undoubtedly, this technique significantly decreases the effect of biological variation between individual animals and reduces the number of animals needed in pre-clinical studies. A major drawback of using this technique to image
bone-implant systems is that, the image quality is usually compromised by the artifacts caused by the high X-ray absorption of metallic implants, which can be further amplified by the movements of the animal during scanning [57]. Because of the metallic artifacts, a fairly large region within the peri-implant bone usually has to be removed from the analysis [58]. However, this region, especially close to the implant, plays an important role in implant integration as it provides a direct fixation of the implant into the bone.

2.1.4 Influence of mechanical stimuli on bone response around implants

It is widely accepted that bone remodeling is mechanically regulated with bone formation occurring at sites with high strains and bone resorption at regions less strained. Osteocytes are believed to be the main mechanosensor cells in bone which coordinated the process of adaptive bone remodeling [59]. There are evidences that osteocytes have the ability to respond to different mechanical stimuli such as fluid flow, hydrostatic pressure, mechanical stretching and low-magnitude high-frequency (LMFH) vibrations [11, 60-63]. These mechanical stimuli have been proved to be anabolic (i.e., to enhance bone formation) at both cellular and tissue level. Specifically, at the cellular level, mechanical stimulation has the main following consequences: i) stimulation of osteogenic differentiation of various cells including osteocytes [11], osteoblasts [7], mesenchymal stem cells [8, 9] and bone marrow stromal cells [10]; ii) downregulation of the expression of Sost/Sclerostin of osteocyte, which is a potent inhibitor of bone formation [11]; iii) release of biological factors which regulate cells involved in bone remodeling (osteoblasts and osteoclasts) [60, 62, 63]. At tissue level, in vivo animal studies confirmed that mechanical stimuli can stimulate bone formation and inhibit bone resorption and thereby leading to an overall increase of bone mass [54, 55, 64-66].

Bone adaptation to a changing mechanical environment is also present during bone repair and regeneration after injury or following implantation surgery. Mechanical loading has been shown to stimulate peri-implant bone formation and to promote implant integration in the host bone [5, 6]. Therefore mechanical loading has a great potential as an anabolic treatment to improve peri-implant bone quality and to facilitate bone-implant integration particularly in osteoporotic bone [67]. However,
### Table 2: Studies of influence of mechanical loading on bone response following implantation using animal models.

<table>
<thead>
<tr>
<th>Study</th>
<th>Animal model</th>
<th>Implantation site</th>
<th>Type of load</th>
<th>Loading regime</th>
<th>Bone architecture</th>
<th>Bone remodeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucht et al. (2007)</td>
<td>Mice</td>
<td>Proximal tibia</td>
<td>Micromotion to implant</td>
<td>Immediate, daily, 3-25 d</td>
<td>Bone matrix deposition</td>
<td>–</td>
</tr>
<tr>
<td>Roshan-Ghias et al. (2011)</td>
<td>Rats</td>
<td>Distal femur</td>
<td>Cyclic loading through leg</td>
<td>Delay 2 wk, bi-daily, 10 d</td>
<td>BV/TV, Tb.Th, Tb.Sp, BFR, BRR, MAR, Tb.N, BMD</td>
<td>MRR, MS, ES</td>
</tr>
<tr>
<td>Wiskott et al. (2012)</td>
<td>Rats</td>
<td>Proximal tibia</td>
<td>Bending moment to implant</td>
<td>Delay 2/4 wk, daily, 4/8 wk</td>
<td>BIC, BV/TV, TbTh, Tb.N</td>
<td>–</td>
</tr>
<tr>
<td>Ogawa et al. (2011)</td>
<td>Rats</td>
<td>Proximal tibia</td>
<td>LMHF loading via whole-body vibration</td>
<td>Immediate, 5 d/wk, 3-25 d</td>
<td>BIC, BF</td>
<td>–</td>
</tr>
<tr>
<td>Chen et al. (2012)</td>
<td>OVX rats</td>
<td>Proximal tibia</td>
<td>LMHF loading via whole-body vibration</td>
<td>Delay 1 wk, 5 d/wk, 8 d/wk</td>
<td>BIC, BF</td>
<td>–</td>
</tr>
<tr>
<td>Zhang et al. (2012)</td>
<td>Rats</td>
<td>Proximal tibia</td>
<td>LMHF compressive load to implant</td>
<td>Immediate, daily, 3-25 d</td>
<td>Bone ingrowth</td>
<td>MAR</td>
</tr>
<tr>
<td>Vandamme et al. (2007)</td>
<td>Rabbits</td>
<td>Proximal tibia</td>
<td>Cyclic implant displacement</td>
<td>Immediate, daily, 3 d/wk, 12 d</td>
<td>Bone mineralization</td>
<td>–</td>
</tr>
<tr>
<td>Willie et al. (2010)</td>
<td>Rabbits</td>
<td>Femoral condyle</td>
<td>Compressive loads to implant</td>
<td>Immediate, daily, 4 wk</td>
<td>Bone ingrowth</td>
<td>MAR</td>
</tr>
<tr>
<td>De Smet et al. (2006)</td>
<td>Guinea pigs</td>
<td>Distal tibia</td>
<td>Bending moment to implant</td>
<td>Delay 1 wk, 5 d/wk, 4 d/wk</td>
<td>Bone mineralization</td>
<td>–</td>
</tr>
</tbody>
</table>

**Notes:**
the response of peri-implant bone to an external mechanical stimulation is still not well characterized. Nevertheless, this knowledge is essential for developing novel loading protocols to improve bone healing and implant stability in compromised systemic conditions and in bone of poor quality. This review summarizes the recent studies investigating peri-implant bone regeneration under controlled mechanical stimulation in vivo in animal models (Table 2.2).

**Mechanical loading applied directly to the implant**

Different loading devices have been developed to apply external load directly to the implant (Figure 2.3). The devices, used in combination with animal models, have been used to answer the question whether it is better to apply mechanical loading immediately after implant placement or rather after some recovery period.

Leucht et al. [68] applied micromotion to an implant inserted in the proximal tibia of mice (Figure 2.3a) immediately after implantation. After only 7 days of mechanical loading, enhanced osteoblastic differentiation and bone matrix deposition were observed at the peri-implant bone. Duyck et al. [69] have developed a special device called “bone chamber” containing two layers of chambers which were in close contact with each other and had matched perforations: the outer chamber protected the analyzed bone tissue from external environment and thereby enabled investigation of specific influence within the chamber; the inner chamber could be replaced after harvesting of the implant and the surrounding tissue, allowing new tissue regeneration within the chamber to realize repeated experiments within the same animal and at the same anatomical site. The bone chamber was implanted at the proximal tibia of rabbits and it included an external fixator anchored to cortical bone with a mobile implant located within the chamber. The implant could be loaded cyclically through an external loading device (Figure 2.3b) [70]. Vandamme et al. [71] used the bone chamber model to demonstrate that 12 weeks of well-controlled mechanical loading starting immediately after implantation accelerated tissue mineralization, and increased both BIC and BA within the peri-implant bone. Moreover, by using this method, the authors pointed out that immediately loaded implants might have the same outcome as delayed loaded implants, as long as the loads were well controlled. In the case of immediate loading, if unsuitable loading parameters are used, there could be micro-motion at the bone-implant interface which could impair implant osseointegration [72, 73]. Zhang et al.
2.1 Bone response following implantation

Figure 2.3: Illustrations of devices applying *in vivo* mechanical loading directly to the implant. a) A motion device positioned on the proximal tibia applying micromotion to the implant which was guided through the middle of the motion device [68]. b) A two-layer bone chamber device installed in the proximal tibia applying displacement to the implant positioned in the middle of the chamber [70]. c) Axial LMHF load applied directly to the implant placed in the proximal tibia [74]. d) Two implants (15 – 18 mm apart) placed in the proximal tibia were loaded normally to the implants’ long axes [75]. e) An automated system applying compressive load to the loading device containing the implant which was placed in the femoral condyle [76]. (a reproduced with permission from Elsevier; b and d reproduced with permission from John Wiley and Sons; c reproduced with permission from Professor Joke Duyck; e reproduced with permission from Mary Ann Liebert).
[74] applied vibrational loading (and specifically LMHF) loading directly to a titanium screw immediately after its insertion into the proximal tibia of rats using a miniaturized displacement-controlled device (Figure 2.3c). The main outcome of the study is that mechanical loading significantly increased cortical BIC, while medullar BIC as well as peri-implant bone fraction (BF) were not loading-responsive. Willie et al. [76] placed a porous titanium foam-like implant into the femoral condyle of rabbits and immediately applied a 4-week loading regime with compressive load to the implant (Figure 2.3c). Results showed that loading enhanced trabecular bone ingrowth into the porous structure of the titanium foam and thereby improved osseointegration of the implant; however, dynamic histomorphometric analysis indicated that in peri-implant trabecular bone load-induced changes were not present.

Mechanical loading applied directly to the implants not immediately following implantation but after a certain healing period has also been shown to be beneficial for peri-implant bone regeneration. De Smet et al. [77] applied a sinusoidally varying bending moment to titanium alloy screw (through a force perpendicular to the implant axis) one week after implantation in the distal tibia of guinea pigs. After 4 weeks of loading, significantly higher bone mass was observed for the loaded group than for the control group, especially in the marrow cavity around the implant. Bending load was also applied to titanium implants inserted in the proximal tibia of rats by Wiskott et al. [75, 78] (Figure 2.3d). This study tested loading protocols which differed in the starting point (2 or 4 weeks after implantation), in the loading duration (4 or 8 weeks), as well as in the loading magnitudes (corresponding to intratissular strains of 750, 1125, 1500 and 2250 ± 5% µε). Results showed that the starting point of the loading regime did not affect the load-induced outcomes. By optimizing the loading magnitude and duration, significant increases were obtained for BIC, BV/TV, Tb.N, and Tb.Th in the peri-implant bone.

**Mechanical loading applied to the bone with the implant (implanted bone)**

Mechanical loading via intact skeleton has been shown to be beneficial for bone formation at both cellular [7-10] and tissue level [54, 55, 64-66]. However, it has been shown that the reaction of fractured bone to loading is different than intact bone [79], and the effect of loading depended on type of bone and location of fracture [80]. In this context, the role of mechanical loading on implanted bone
2.1 Bone response following implantation

remains to be explored. A few animal models have been developed to investigate the effects of mechanical loading via the skeleton on bone regeneration around an implant (Figure 2.4).

Zhang et al. [81] implanted titanium screws into the proximal tibia of rats and applied LMHF compressive load through the axis of tibia (Figure 2.4a). By testing different loading parameters, they showed that although both high- (HF) and low-frequency (LF) loading contributed to peri-implant bone healing, higher magnitudes were required under LF loading to induce a positive bone response compared with HF loading. Moreover, a sustained period of loading at HF is needed to induce an overall enhanced osseointegration, in both cortical and medullar area. Roshan-Ghias et al. [84] placed a bone scaffold made of poly(L-lactic acid) (PLA)/ 5% β-tricalcium
phosphate (β-TCP) into the distal femoral epiphysis of rats and applied cyclic loading through the leg. Increase in bone formation rate and decrease in bone resorption rate were shown by registration of in vivo micro-CT images at different time points, resulting in higher bone volume fraction inside the loaded scaffold compared to the control group [85].

LMHF loading via whole-body vibration (WBV) has been shown to improve bone strength by increasing bone formation and decreasing bone resorption. The physiological effects of vibration are mediated by targeting cell types such as mesenchymal stem cells, osteoblasts, osteocytes, adipocytes, osteoclasts, myocytes, and neurons [83]. Recently, WBV has been used to improve implant osseointegration. Ogawa et al. [82] placed titanium screws in the proximal metaphysis of the tibiae of rats and immediately applied LMHF loading by means of WBV (Figure 2.4b). Results suggested that BIC and BF, especially close to the bone-implant interface, were significantly increased by loading. LMHF loading via WBV using a human vibration platform (Figure 2.4c) was also proved to be beneficial for bone-implant integration in osteoporotic bone by enhancing BIC and BF in OVX rats [86].

**Optimization of the loading parameter**

To develop effective loading protocols, studies have been designed to optimize the loading parameters. Some of the main parameters being considered are shortly summarized here.

*Starting point of treatment*

The choice of a suitable starting point is a critical aspect of the loading regime. There were debates on whether an immediate loading treatment has negative effect on the early stages of bone healing [87]. In fact, some authors showed that loading starting before the attainment of a good bone-implant integration may induce micromotion at the bone-implant interface which, in turns, may trigger bone resorption [88, 89]. On the other hand, the therapeutic effect of loading may be compromised if mechanical stimulation is unnecessarily delayed [90]. As peri-implant bone healing depends on multiple influencing factors including the implant itself, the host bone condition and the implantation site, the starting point of a specific loading protocol should be customized for each individual study based on prior knowledge of the healing time scales.
2.1 Bone response following implantation

**Location of mechanical stimulation**

Mechanical stimulation within the peri-implant bone can be attained by applying external loading directly to the implant or by loading administered to the local implanted bone or even at the level of the entire skeleton. Although loading therapy such as vibration has been used to promote bone and muscle strength in clinics [83], it has been shown that the reaction of fractured bone to loading is different than intact bone [79]. Nevertheless, load-induced higher BIC and BF were observed in osteoporotic animal model by using a commercial human vibration platform [86]. Mechanical loading directly to the implant, although has been proved to be beneficial for implant osseointegration [68, 73, 74, 76-78], has the possibility of initiating micromotion at the bone-implant interface, which jeopardized bone-implant integration, if not be applied properly [88, 89]. Further investigations are needed to explore the differences among the different loading application strategies.

**Magnitude of mechanical stimuli**

The effective range of mechanical stimuli has been investigated in a limited number of studies [78, 81, 82]. It is well accepted that overhigh loads may cause micro-motion which, in turn, inhibits implant osseointegration [72], while insufficient loads have no effect on bone healing [91]. However, it is quite challenging to compare absolute loading magnitudes among different studies, giving that outcomes are influenced by numerous variables including animal species, type of implant, implantation site, etc.

**2.1.5 Discussion and conclusion**

This paper reviews the main *in vivo* animal studies used for investigating peri-implant bone regeneration in osteoporosis and the effect of external mechanical loading on this process. In pre-clinical bone research, animal studies are essential for: *i*) a better understanding in the influencing mechanism of osteoporosis on the fracture fixation outcomes; *ii*) analyzing the effectiveness of a novel loading treatment under *in vivo* conditions. The reviewed animal studies have improved the current knowledge on the effects of osteoporosis and mechanical loading on peri-implant bone regeneration at the tissue level.

Animal studies for osteoporotic fracture fixation show the negative influence of osteoporosis on bone-implant integration. Lower bone-to-implant contact, peri-
implant bone area and bone mineral density, as well as weekend trabecular and cortical architecture close to the implant were observed in the implanted bone of osteoporotic animal models. It is believed that these influences contribute to the high failure risk of osteoporotic fracture fixation. Nevertheless, controversial results remained between studies and comparison between different studies is challenging due to the variance in the study settings including animal species, type of implant, implantation site, etc. Moreover, a less investigated aspect is the time course of the bone architecture and bone remodeling after implant placement in osteoporotic bone. Prospective animal studies are therefore required to better understand the process of bone-implant integration in osteoporosis.

Animal studies for loading applications have repeatedly demonstrated the anabolic effect of mechanical stimulation at the bone-implant interface. Mechanical loading has been shown to enhance bone-to-implant contact and peri-implant bone formation in both health and osteoporotic animal models, and therefore been consider beneficial for implant integration. Moreover, studies have been performed to analyze the most effective loading protocols in stimulating bone formation. However, less is known on the behavior of peri-implant bone and even of the response of the entire implanted bone at the organ scale. Further research is required to elucidate the biological mechanisms of load-induce peri-implant bone regeneration and to establish effective loading protocol for obtaining predictable clinical outcomes.

In conclusion, animal models allow investigation of bone regeneration around an implant under in vivo conditions. The knowledge obtained from animal studies has led to a greater understanding in the effects of osteoporosis and mechanical loading on peri-implant bone regeneration, which may facilitate the development of novel loading treatments for enhancing bone-implant integration in osteoporotic fracture fixation.

References


Chapter 2 Background


2.1 Bone response following implantation


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2.1 Bone response following implantation


Chapter 2 Background


2.1 Bone response following implantation


Chapter 3

Development of a novel method to investigate bone response following implantation
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

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Abstract:
The mechanical integrity of the bone-implant system is maintained by the process of bone remodeling. Specifically, the interplay between bone resorption and bone formation is of paramount importance to fully understand the net changes in bone structure occurring in the peri-implant bone, which are eventually responsible for the mechanical stability of the bone-implant system. Using time-lapsed in vivo micro-computed tomography combined with new composite material implants, we were able to characterize the spatio-temporal changes of bone architecture and bone remodeling following implantation in living mice. After insertion, implant stability was attained by a quick and substantial thickening of the cortical shell which
counteracted the observed loss of trabecular bone, probably due to the disruption of the trabecular network. Within the trabecular compartment, the rate of bone formation close to the implant was transiently higher than far from the implant mainly due to an increased mineral apposition rate which indicated a higher osteoblastic activity. Conversely, in cortical bone, the higher rate of bone formation close to the implant compared to far away was mostly related to the recruitment of new osteoblasts as indicated by a prevailing mineralizing surface. The behavior of bone resorption also showed dissimilarities between trabecular and cortical bone. In the former, the rate of bone resorption was higher in the peri-implant region and remained elevated during the entire monitoring period. In the latter, bone resorption rate had a bigger value away from the implant and decreased with time. Our approach may help to tune the development of smart implants that can attain a better long-term stability by a local and targeted manipulation of the remodeling process within the cortical and the trabecular compartments and, particularly, in bone of poor health.

**Keywords:**
Implant osseointegration, implant anchorage, bone remodeling, metal artifacts, *in vivo* micro-computed tomography

### 3.1.1 Introduction

The remodeling process around the implant plays a key role for attaining and maintaining the mechanical integrity of the bone-implant system. During bone remodeling, bone is locally resorbed by osteoclasts and new unmineralized bone is laid down by osteoblasts. The mineral content and hence the mechanical competence of newly deposited bone increases rapidly with time [1, 2]. Bone material can be renewed with virtually no changes in bone architecture if bone formation and bone resorption are spatially coupled; conversely, structural adaptation to modifications in the loading conditions requires bone formation and bone resorption to happen at different locations. This is feasible as the remodeling process is (partially) regulated by local mechanical cues with bone formation occurring more likely at locations of high mechanical strains and bone resorption at regions less strained [3-5].
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

After the initial inflammatory phase which follows implant insertion [6], the subsequent bone regeneration process is strongly influenced by the implant. The forces needed to insert the implant can cause a fairly high amount of microdamages up to 500 µm away from the implant surface [6] which, in turn, triggers a substantial but short-term increase in peri-implant bone resorption followed by formation [7]. The presence of the implant usually promotes bone formation directly onto the implant surface [8] and different strategies have been proposed to enhance this process including the manipulation of surface topography [9], the application of biomimetic coatings [10], and the local delivery of drugs increasing bone formation [11] or inhibiting bone resorption [12, 13]. Moreover, the implant produces a complex redistribution of the loading pattern with some bone resulting “under-used” and therefore being (most likely) resorbed. This is a particularly critical aspect which can undermine the long-term mechanical stability of the bone-implant system [14-16].

The response of bone to implantation is a highly dynamic process; in contrast, the main methodologies which are used to characterize it, such as histomorphometry and in vitro micro-computed tomography (micro-CT), are based on single time points and cannot capture the full time evolution of the structural and the remodeling changes after implant placement. Dynamic histomorphometry, for instance, allows characterizing the location and, to some extent, the time-changes of bone formation (i.e. formation rate) by using multiple labels [17]; however, similar dynamic information on bone resorption cannot be obtained [18, 19]. Nevertheless, the interplay between bone resorption and bone formation is of paramount importance to fully understand the net changes in bone structure occurring in the peri-implant bone, which are eventually responsible for the mechanical stability of the implant [6, 15, 16].

The introduction of in vivo micro-CT has provided a new option to perform bone research in living animals. This technique, not only gives direct access with high spatial resolution to the time evolution of bone structure [20] but also to the processes of bone remodeling [3, 18, 21] and mineralization [2]. Although micro-CT has been used to characterize the peri-implant bone response ex-vivo [22-24], the in vivo applications are to date very limited [13, 25] and hampered by the well-known metal artifacts caused by the implant [26], which jeopardize the possibility to extract quantitative information on the remodeling process around as well as close to the
Therefore, our first aim was to develop a suitable implant, implantation procedure and imaging protocol to assess the spatio-temporal changes in bone architecture and bone remodeling - both formation and resorption - after implant insertion using *in vivo* micro-CT. In view of the organ-scale adaptation of the entire vertebra, we hypothesized that there would be an interplay between the structural responses of cortical and trabecular bone. We also hypothesized that bone formation and bone resorption would have substantially different time courses after implant insertion reflecting the complex bone regeneration scenario found in the peri-implant bone and ultimately linked with the recruitment and activity of osteoblasts and osteoclasts. Specifically, with our approach we could answer the following questions: *i)* Are the structural changes observed in the peri-implant bone mainly due to modifications in bone formation or in bone resorption? *ii)* Is the remodeling behavior around the implant qualitatively similar in cortical and trabecular bone? To investigate these issues we monitored weekly for six weeks the response of murine caudal bone after implantation using *in vivo* micro-CT.

### 3.1.2 Materials and methods

**Implant, animal model and implantation**

Special implants made of an aluminum matrix composite (AMC) material, which combines high stiffness (130 GPa) with low density (3.4 g/cm³) to eliminate image artifacts [26], were developed (Composite Metal Technology Limited, Hampshire, United Kingdom). The smooth implants (without threads) have a cylindrical shape (diameter of 0.5 mm and length of 1.5 mm) with sharp ends. To enhance biocompatibility, implants were coated with a titaniferous layer [27] of about 30 nm by plasma enhanced chemical vapor deposition (pfm medical titanium gmbh, Nuremberg, Germany). Implants were sterilized and inserted in the sixth caudal vertebrae (CV6) of seven 13-week old female C57BL/6J (B6) mice (JANVIER, Saint Berthevin Cedex, France) using a well-established pinning procedure [28]. In short, the mouse tail was firmly fixed into a pinning device and the sharp needle-shaped implant was directly pushed (without pre-drilling) into CV6. Each animal received a total of three implants: one AMC implant in CV6 and two stainless steel
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

pins (Fine Science Tools, Heidelberg, Germany) placed into CV5 and CV7, respectively. The additional pins were used to hold in place a small transparent hollow tube made of poly(methyl methacrylate) (PMMA) and with a thickness of 1 mm. The main function of the tube was to prevent the animal from removing the implant (mainly by chewing on it) especially in the initial days after implant insertion. The tube did not provide mechanical support for the implant nor did it restrict the activities of the mice. Implantation sites were located with fluoroscopic imaging and implant surgery was performed under isoflurane anesthesia. To compare the behavior of the implanted vertebra against intact bones, we have also analyzed data of age-matched C57BL/6J mice (n=19) used in previous studies [29, 30] which were hosted in the same animal facility, subjected to a similar imaging protocol but without any implant/implantation surgery into the sixth caudal vertebra. All in vivo animal procedures were approved by the local authorities (Kantonales Veterinäramt Zürich, License No. 190/2010, Switzerland).

Imaging and image processing

The entire caudal vertebra containing the ACM implant (i.e., CV6) was scanned in vivo using micro-CT (vivaCT 40, Scanco Medical, Brüttisellen, Switzerland) right after implantation and weekly, for the following 6 weeks. The scans were performed at a nominal isotropic resolution of 10.5 µm, with an integration time of 350 ms, 500 projections and no frame averaging. The total scanning time for imaging the entire vertebra with the implant was about 15 minutes. The micro-CT peak voltage and current were set to 55 kVp and 145 µA, respectively. A beam hardening correction algorithm provided by the manufacturer was applied to all scans [31]. Images of the mouse vertebra taken at 7 consecutive time points (week 0 – week 6) were aligned using rigid registration [32]. The grey-level images were then Gaussian filtered (support 1, sigma 1.2) to reduce the noise and a global fixed threshold, corresponding to 22% of the maximum gray value, was used to separate the bone-implant system from the background. Regions of bone formation and bone resorption were obtained by superimposing the scans of the vertebra at two different time points [18] and a filter for removing single formed and resorbed voxels was implemented to decrease registration and partial volume errors [33]. The segmentation of implant and bone required a semi-automatic approach as they had similar grey values (Figure 3.1c and d). First, a mask was manually drawn around a
Figure 3.1: Gray-scale micro-CT reconstructions of the titanium implant (a and b) and the aluminum matrix composite implant (c and d, highlighted by the green line) inserted into a mouse caudal vertebra. In a and b strong metal artifacts are evident.
Figure 3.2: Masks of the peri-implant and distant regions within the cortical and the trabecular compartments.

cross-section of the implant (Figure 3.1d); the mask was then automatically adapted to match all the transverse cross-sections of the implant along the entire implant length (Figure 3.1c). Full bone, cortical and trabecular compartments were automatically identified as previously reported [30]. Micro-CT data were analyzed in the trabecular and cortical compartments considering two regions of interest (Figure 3.2): a peri-implant bone region (which included the detectable damaged zone due to implantation) and a so-called distant region containing bone not directly compromised by implantation. The peri-implant region was a cylindrical region around the implant starting two voxels away from the implant surface (identified by manual contouring) and extending radially, i.e. perpendicular to the implant surface, for 420 µm. The peri-implant region was then intersected with the trabecular and cortical masks to identify peri-implant trabecular and cortical bone, respectively. The distant region was obtained by subtracting the peri-implant region from the cortical or trabecular compartments (without the growth plates).

**Evaluation of bone architecture and bone remodeling**

At each individual time point we computed full tissue volume (TV) and full length (along the cranio-caudal axis) of the entire sixth caudal vertebra. Measures of cortical bone included cortical thickness (Ct.Th), cortical porosity (Ct.Po), total
cross-sectional area inside the periosteal envelope (Tt.Ar), cortical bone area (Ct.Ar) and cortical area fraction (Ct.Ar/Tt.Ar). Changes in trabecular microarchitecture were characterized in terms of bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp) and degree of anisotropy (DA). All morphometric parameters were calculated and reported according to standard guidelines [34]. Bone remodeling was evaluated based on formed and resorbed voxels [18] within a two-week time interval in both cortical and trabecular compartments as well as in the peri-implant and distant region. The calculated parameters were: bone formation rate (BFR), bone resorption rate (BRR), mineral apposition rate (MAR), mineral resorption rate (MRR), mineralizing surface (MS) and eroded surface (ES).

Reproducibility

The reproducibility of the scanning and image processing procedure was assessed for all architectural and remodeling parameters. Right after animal sacrifice at week 6, each CV6 was scanned ex vivo 5 times, with the same protocol used for the in vivo measurements, including repeated repositioning between the scans, resulting in a total of 35 repeated scans. The reproducibility of bone remodeling was assessed by overlaying the repeated scans to their respective previous measurements at week 5 (one-week time interval) or at week 4 (two-week time interval). The reproducibility was characterized by the precision error expressed both in absolute value (PE\(\text{SD}\)) and as coefficients of variation (PE\(\%\text{CV}\)) and by the intraclass correlation coefficient (ICC) [35].

Statistics

Statistics were performed using SigmaPlot (Systat Software, San Jose, CA) and R (Auckland, New Zealand). Architectural and remodeling parameters were tested for significance between peri-implant and distant region using Mann-Whitney Rank Sum Test since the two groups in each time point did not always follow a normal distribution and nor did they always have equal variance. To test the significance between the first and last time point within the same group a paired t-test was used. P-values smaller than 0.05 were considered significant. All data are shown as mean ± standard deviation.
### 3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

Table 3.1: Reproducibility of bone architectural parameters characterized by the precision error of the standard deviation (PE$_{SD}$), the precision error of the coefficient of variation (PE$_{%CV}$) and the intraclass correlation coefficient (ICC) [28]. The subscript “imp” denotes values measured in the peri-implant bone whereas the subscript “dis” indicates measurements in the distant region (see Figure 3.2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>PE$_{SD}$</th>
<th>PE$_{%CV}$ (%)</th>
<th>ICC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortical bone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ct.Th$_{imp}$ [mm]</td>
<td>0.263 ± 0.019</td>
<td>0.002</td>
<td>0.948</td>
<td>0.983</td>
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<tr>
<td>Ct.Th$_{dis}$ [mm]</td>
<td>0.182 ± 0.016</td>
<td>0.001</td>
<td>0.915</td>
<td>0.992</td>
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<tr>
<td>Ct.Ar$_{imp}$ [mm$^2$]</td>
<td>0.629 ± 0.039</td>
<td>0.003</td>
<td>0.421</td>
<td>0.996</td>
</tr>
<tr>
<td>Ct.Ar$_{dis}$ [mm$^2$]</td>
<td>0.684 ± 0.035</td>
<td>0.011</td>
<td>1.652</td>
<td>0.910</td>
</tr>
<tr>
<td>Ct.Ar/Tt.Ar$_{imp}$ [%]</td>
<td>54.347 ± 4.557</td>
<td>0.219</td>
<td>0.406</td>
<td>0.998</td>
</tr>
<tr>
<td>Ct.Ar/Tt.Ar$_{dis}$ [%]</td>
<td>48.418 ± 2.328</td>
<td>0.384</td>
<td>0.794</td>
<td>0.974</td>
</tr>
<tr>
<td>Ct.Po$_{imp}$ [%]</td>
<td>17.558 ± 1.629</td>
<td>0.095</td>
<td>0.544</td>
<td>0.997</td>
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<tr>
<td>Ct.Po$_{dis}$ [%]</td>
<td>21.437 ± 1.388</td>
<td>0.109</td>
<td>0.509</td>
<td>0.995</td>
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<tr>
<td><strong>Trabecular bone</strong></td>
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<td></td>
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<tr>
<td>BV/TV$_{imp}$ [%]</td>
<td>7.073 ± 6.412</td>
<td>0.163</td>
<td>4.243</td>
<td>0.999</td>
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<tr>
<td>BV/TV$_{dis}$ [%]</td>
<td>19.024 ± 2.800</td>
<td>0.255</td>
<td>1.401</td>
<td>0.992</td>
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<tr>
<td>Tb.Th$_{imp}$ [mm]</td>
<td>0.096 ± 0.022</td>
<td>0.002</td>
<td>1.884</td>
<td>0.994</td>
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<tr>
<td>Tb.Th$_{dis}$ [mm]</td>
<td>0.096 ± 0.010</td>
<td>0.001</td>
<td>0.641</td>
<td>0.996</td>
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<tr>
<td>Tb.Sp$_{imp}$ [mm]</td>
<td>0.481 ± 0.054</td>
<td>0.003</td>
<td>0.636</td>
<td>0.997</td>
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<tr>
<td>Tb.Sp$_{dis}$ [mm]</td>
<td>0.375 ± 0.026</td>
<td>0.003</td>
<td>0.789</td>
<td>0.988</td>
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<tr>
<td>Tb.N$_{imp}$ [1/mm]</td>
<td>1.822 ± 0.232</td>
<td>0.030</td>
<td>1.640</td>
<td>0.983</td>
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<tr>
<td>Tb.N$_{dis}$ [1/mm]</td>
<td>2.392 ± 0.140</td>
<td>0.023</td>
<td>0.969</td>
<td>0.975</td>
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<tr>
<td>DA$_{imp}$ [-]</td>
<td>1.475 ± 0.215</td>
<td>0.027</td>
<td>1.999</td>
<td>0.985</td>
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<tr>
<td>DA$_{dis}$ [-]</td>
<td>1.972 ± 0.146</td>
<td>0.010</td>
<td>0.513</td>
<td>0.996</td>
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</table>
Chapter 3 Development of a novel method to investigate bone response following implantation

Table 3.2: Reproducibility of bone remodeling parameters characterized by the precision error of the standard deviation (PESD), the precision error of the coefficient of variation (PE%CV) and the intraclass correlation coefficient (ICC) [28]. The subscript “imp” denotes values measured in the peri-implant bone whereas the subscript “dis” indicates measurements in the distant region (see Figure 3.2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>One-week time interval</th>
<th>Two-week time interval</th>
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</thead>
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<tr>
<td></td>
<td>Mean ± SD</td>
<td>PEsd</td>
</tr>
<tr>
<td>Reproducibility of cortical bone remodeling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BFRimp [%/d]</td>
<td>0.013 ± 0.009</td>
<td>0.001</td>
</tr>
<tr>
<td>BFRdis [%/d]</td>
<td>0.025 ± 0.014</td>
<td>0.009</td>
</tr>
<tr>
<td>BRRimp [%/d]</td>
<td>0.121 ± 0.085</td>
<td>0.010</td>
</tr>
<tr>
<td>BRRdis [%/d]</td>
<td>0.129 ± 0.039</td>
<td>0.013</td>
</tr>
<tr>
<td>MARimp [µm/d]</td>
<td>1.225 ± 0.109</td>
<td>0.034</td>
</tr>
<tr>
<td>MARdis [µm/d]</td>
<td>1.024 ± 0.044</td>
<td>0.038</td>
</tr>
<tr>
<td>MRRimp [µm/d]</td>
<td>3.211 ± 0.424</td>
<td>0.036</td>
</tr>
<tr>
<td>MRRdis [µm/d]</td>
<td>2.982 ± 0.098</td>
<td>0.038</td>
</tr>
<tr>
<td>MSimp [%]</td>
<td>15.204 ± 5.112</td>
<td>1.129</td>
</tr>
<tr>
<td>MSDis [%]</td>
<td>12.101 ± 2.581</td>
<td>1.441</td>
</tr>
<tr>
<td>ESimp [%]</td>
<td>26.120 ± 7.839</td>
<td>1.181</td>
</tr>
<tr>
<td>ESdis [%]</td>
<td>16.310 ± 4.550</td>
<td>1.089</td>
</tr>
<tr>
<td>Reproducibility of trabecular bone remodeling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BFRimp [%/d]</td>
<td>0.413 ± 0.230</td>
<td>0.090</td>
</tr>
<tr>
<td>BFRdis [%/d]</td>
<td>0.330 ± 0.096</td>
<td>0.059</td>
</tr>
<tr>
<td>BRRimp [%/d]</td>
<td>2.371 ± 1.365</td>
<td>0.130</td>
</tr>
<tr>
<td>BRRdis [%/d]</td>
<td>1.207 ± 0.343</td>
<td>0.085</td>
</tr>
<tr>
<td>MARimp [µm/d]</td>
<td>2.341 ± 0.218</td>
<td>0.083</td>
</tr>
<tr>
<td>MARdis [µm/d]</td>
<td>2.097 ± 0.135</td>
<td>0.086</td>
</tr>
<tr>
<td>MRRimp [µm/d]</td>
<td>3.313 ± 0.317</td>
<td>0.057</td>
</tr>
<tr>
<td>MRRdis [µm/d]</td>
<td>3.292 ± 0.341</td>
<td>0.067</td>
</tr>
<tr>
<td>MSimp [%]</td>
<td>13.724 ± 4.661</td>
<td>1.439</td>
</tr>
<tr>
<td>MSDis [%]</td>
<td>15.940 ± 3.598</td>
<td>1.312</td>
</tr>
<tr>
<td>ESimp [%]</td>
<td>29.098 ± 7.451</td>
<td>1.656</td>
</tr>
<tr>
<td>ESdis [%]</td>
<td>24.093 ± 4.387</td>
<td>1.184</td>
</tr>
</tbody>
</table>
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

3.1.3 Results

Elimination of image artifacts

The novel ACM implant effectively eliminated the strong artifacts which are a well-known problem when imaging metal implants \([25, 26]\) (Figure 3.1). This facilitated easy segmentation of the bone-implant system from bone marrow and soft tissues, which could be done by a simple global thresholding approach. Moreover, the coating on the implant surface with a titaniferous layer provided the required biocompatibility as signs of local swelling or inflammation were absent during the whole experiment.

Reproducibility

Despite the presence of the implant, the reproducibility of the architectural parameters was very high (Table 3.1): \(\text{PE}_{\%CV}\) ranged from 0.406\% (\(\text{Ct.Ar}/\text{Tt.Ar}_{\text{imp}}\)) to 4.243\% (\(\text{BV}/\text{TV}_{\text{imp}}\)). The reproducibility of the remodeling process depended on the time-interval considered as well as on the bone compartment analyzed. When computing the remodeling parameters in trabecular bone close to the implant within only one-week interval (Table 3.2), the reproducibility of BFR and MS was fairly low (e.g., \(\text{PE}_{\%CV}\) of 20.596\% for \(\text{BFR}_{\text{imp}}\) and 11.298\% for \(\text{MS}_{\text{imp}}\)) due to the interplay between measurement/registration errors and a low amount of newly formed bone. Considering a two-week time interval, the reproducibility of both trabecular and cortical remodeling improved substantially and \(\text{PE}_{\%CV}\) was always smaller or equal to 10\% (Table 3.2). Differences in the mean values of the formation parameters between one-week and two-week time interval were observed. Considering the very low number of formed voxels post implantation detected within one-week time interval (especially in cortical bone, Table 3.2) and assuming a physiological MAR of 1 \(\mu\text{m}\) per day for mice of similar age \([18]\), a precise measurement of bone formation following implant insertion seems to require a time interval longer than one week for an image resolution of 10.5 \(\mu\text{m}\). Based on these results, remodeling was measured within a two-week time interval.
Table 3.3: Architectural parameters in peri-implant and distant region of cortical and trabecular bone at first (week 0) and last (week 6) time point. p-Value < 0.05 denotes a significant difference between week 0 and 6 within the same region.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Peri-implant</th>
<th>Distant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 6</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Cortical bone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ct.Th [mm]</td>
<td>0.223 ± 0.017</td>
<td>0.266 ± 0.016</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ct.Ar [mm²]</td>
<td>0.577 ± 0.039</td>
<td>0.635 ± 0.037</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ct.Ar/Tt.Ar [%]</td>
<td>53.563 ± 4.007</td>
<td>54.840 ± 4.106</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ct.Po [%]</td>
<td>15.576 ± 1.271</td>
<td>17.473 ± 1.423</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Trabecular bone</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV/TV [%]</td>
<td>12.781 ± 6.132</td>
<td>7.186 ± 6.820</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tb.Th [mm]</td>
<td>0.094 ± 0.011</td>
<td>0.098 ± 0.024</td>
<td>0.271</td>
</tr>
<tr>
<td>Tb.Sp [mm]</td>
<td>0.353 ± 0.042</td>
<td>0.481 ± 0.055</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tb.N [1/mm]</td>
<td>2.499 ± 0.238</td>
<td>1.850 ± 0.273</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DA [-]</td>
<td>1.451 ± 0.160</td>
<td>1.463 ± 0.231</td>
<td>0.411</td>
</tr>
</tbody>
</table>
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

Architectural changes after implantation: the interplay between cortical and trabecular bone

Full bone: Full TV and full length of the vertebrae increased significantly with time (12.0% and 5.6%, respectively, week 0 – week 6, Table 3.3), indicating that implant placement and monitoring procedure did not prevent bone growth as expected in relatively young mice [19, 30] where, for instance, the trabecular bone volume fraction increased by about 1% per week and the cortical thickness by about 0.6% per week within a 4 week-interval from 15 to 19 weeks of age [30].

Cortical bone: A large increase in cortical thickness (Ct.Th) was observed mainly at the periosteal surface and particularly occurring in peri-implant bone (Figure 3.3a and Table 3.3). There, Ct.Th increased by about 19.3% within the first 3 weeks and stayed virtually constant in the remaining weeks. The increase of Ct.Th in the distant region was much smaller (about 2.4%, p > 0.05). The thicker cortex was also more porous (Figure 3.3c), with the total increase in porosity in the peri-implant region (12.2%) being a factor of two higher than in the distant region (6.1%, Table 3.3).

Trabecular bone: The large increase in Ct.Th was contrasted by a progressive and substantial bone loss in the trabecular compartment (Figure 3.4a), with the reduction of the bone volume fraction (BV/TV) in the peri-implant bone (-51.8% ± 27.9%, week 0 – week 6) being significantly higher than in the distant region (-19.5% ± 8.3%, week 0 – week 6). In the peri-implant region, a higher bone loss was observed when the implant was placed in the center of the vertebra (mice 4 – 7, Figure 3.4b and 3.4c) with respect to a more distal or proximal insertion (mice 1 – 3, Figure 3.4b and 3.4c). The trabecular network showed also a progressive coarsening, indicated by a pronounced decrease in Tb.N (-26.0%, week 0 – week 6) together with a slight increase in Tb.Th and Tb.Sp (Table 3.3).

Bone remodeling after implantation: cortical versus trabecular response

Cortical bone: After implantation, bone formation rate in peri-implant bone (BFR\textsubscript{imp}) was roughly a factor of five higher than bone formation rate in the distant region (BFR\textsubscript{dis}) (Figure 3.5a). This initial enhancement of BFR\textsubscript{imp} was due to a more than 90% larger (p < 0.05) mineralizing surface (MS\textsubscript{imp}) as well as to a 50% higher (p < 0.05) mineral apposition rate (MAR\textsubscript{imp}), with the former being dominant (Figure
Figure 3.3: a) Changes in cortical thickness (Ct.Th) in peri-implant and distant regions; b) representative cross-section close to the implant and c) its corresponding time evolution (in gray scale) showing the cortical thickening and the related increase in cortical porosity. A considerable amount of newly formed bone, less mineralized and thus with lower gray value, is evident starting from week 2. * indicates significant differences (p < 0.05) between peri-implant and distant bone for the time interval considered; # indicates significant differences (p < 0.05) between first and last time point.
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

Figure 3.4: a) Changes in bone volume fraction (BV/TV) in peri-implant and distant regions; b) changes in peri-implant BV/TV for each individual animal and c) corresponding overlaid images of the first and last scan showing the locations of bone formation and resorption. * indicates significant differences (p < 0.05) between peri-implant and distant bone for the time interval considered; # indicates significant differences (p < 0.05) between first and last time point.
Chapter 3 Development of a novel method to investigate bone response following implantation

3.5b and 3.5c). With time, BFR\textsubscript{imp} decreased (mainly due to a decrease in MS\textsubscript{imp}) and 3 weeks after implantation, the values close to and far from the implant were comparable. A total reduction of 91.5% (p < 0.05) in BFR\textsubscript{imp} was measured when comparing the first to the last time interval. Conversely, BFR\textsubscript{dis} stayed practically constant at about 0.1 %/d over the course of the experiment. Implantation had an opposite effect on bone resorption (Figure 3.5d): initially, a high bone resorption rate (BRR) was measured not close but far from the implant (BRR\textsubscript{dis} = 0.38%/d) while in the vicinity of the implant bone was removed at a considerably lower speed (BRR\textsubscript{imp} = 0.08 %/d). From the second week-interval, BRR\textsubscript{dis} decreased linearly with a slope of -0.011 %/d ($R^2 = 0.99$) whereas BRR\textsubscript{imp} displayed a slight increase with time. At the end of the experiment, BRR\textsubscript{imp} and BRR\textsubscript{dis} were no longer statistically different from each other. The initially high BRR\textsubscript{dis} was due to a larger eroded surface in distant region (ES\textsubscript{dis}) which was up to 40% higher than the eroded surface in peri-implant bone (ES\textsubscript{imp}) (Figure 3.5f). On the contrary, mineral resorption rate was still higher close to the implant (MRR\textsubscript{imp} = 1.25 MRR\textsubscript{dis}, Figure 3.5e) which may indicate a strong spatial coupling with mineral apposition rate. In the last time interval, MRR\textsubscript{dis} was almost equal to MRR\textsubscript{imp} whereas the behavior of ES was reversed with ES\textsubscript{imp} = 1.6 ES\textsubscript{dis} (Figure 3.5e and 3.5f). Furthermore the remodeling parameters measured in the implanted bones were compared against those relative to the same caudal vertebrae but without the implant of age-matched mice. Considering the distant region, BFR\textsubscript{dis}, MAR\textsubscript{dis}, MS\textsubscript{dis}, and ES\textsubscript{dis} laid either within or very close to the one-standard-deviation range (denoted as the grey area, Figure 3.5) of the corresponding parameters for the intact vertebrae, while BRR\textsubscript{dis}, and MRR\textsubscript{dis} tended to be higher.

Trabecular bone: Bone formation within the trabecular region behaved qualitatively similar to bone formation in the cortical compartment. Initially, after implant insertion BFR\textsubscript{imp} increased by roughly a factor of 2.3 with respect to BFR\textsubscript{dis} (Figure 3.6a) but two weeks later, BFR\textsubscript{imp} dropped to the values measured in the distant region. Such behavior was mainly dictated by mineral apposition rate as MAR\textsubscript{imp} was always above MAR\textsubscript{dis}, with the difference being higher in the first two time intervals (Figure 3.6b). Conversely, MS\textsubscript{imp} was – with the exception of the initial week – up to 40% smaller than MS\textsubscript{dis}, (Figure 3.6c). Interestingly, close to the implant bone was resorbed, for the entire duration of the experiment, at a roughly 70% faster rate than in the distant region, where BRR\textsubscript{dis} did not change substantially over time (Figure
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

3.6d). Such elevated BRR_{imp} was initially due to high MRR_{imp} (Figure 3.6e) and, at later stages, also due to increased ES_{imp} (Figure 3.6f). As for cortical bone, bone formation parameters were closer than bone resorption parameters to the values measured in implant-free vertebrae of age-matched animals.

3.1.4 Discussion

The purpose of this study was to investigate architectural and remodeling changes after implant insertion in mouse caudal bone using time-lapsed in vivo micro-CT. With a novel low-density implant we were able to remove the strong metal artifacts which have been, up to now, limiting the use of in vivo micro-CT to characterize bone response to implants [26]. A previous work monitoring architectural changes around metal implants with in vivo micro-CT [25] showed the potential of this technique to assess the time evolution of bone architecture as well as of the bone-implant fixation strength. However, as pointed out by the authors, due to metal artifacts, which were even amplified by the movements of the animal, several scans (up to 15%) were discarded. In general, the bone voxels close to the implant have an artificially higher grey level than far away and special threshold methods which depend on the implant dimensions and shape have to be implemented and validated [22, 23, 25]. Even if we did not observe metal artifacts (Figure 3.1), the removal of a two-voxel layer around the implant was done for two purposes. Firstly, the implant surface was identified by manually drawing an ellipse around the implant cross-section (Figure 3.1d) and there could be a small degree of ambiguity right at the bone-implant interface coming from the jagging effect of the micro-CT resolution as well as from small irregularities of the implant shape. Secondly, due to the interplay between beam-hardening and partial volume effects, the voxels close to the implant surface have a grey level which is artificially high and, hence, they could be wrongly identified as bone. Furthermore, surface voxels can be a source of inaccuracy when measuring bone remodeling or mineralization parameters on registered time-lapsed micro-CT scans [2, 19, 33]. Several authors have investigated the minimum distance from the surface of small metal implants that gives reliable measures of the local bone volume with ex-vivo micro-CT. Depending on implant dimensions, scanning parameters (mostly voxel size, energy and scanning time) and implant orientation relative to the X-ray beam, this distance ranges from about 50
Chapter 3 Development of a novel method to investigate bone response following implantation

Figure 3.5: Remodeling behavior of cortical bone measured in the peri-implant and in the distant region: a) bone formation rate (BFR); b) mineral apposition rate (MAR); c) mineralizing surface (MS); d) bone resorption rate (BRR); e) mineral resorption rate (MRR); and f) eroded surface (ES). * indicates significant differences (p < 0.05) between peri-implant and distant bone for the time interval considered; # indicates significant differences (p < 0.05) between first and last time interval. The gray area denotes the one standard deviation range of the corresponding parameters in the intact vertebrae of age-matched mice.
Figure 3.6: Remodeling behavior of trabecular bone measured in the peri-implant and in the distant region for the time interval considered. * indicates significant differences (p < 0.05) between peri-implant and distant bone for the time interval considered; # indicates significant differences (p < 0.05) between first and last time interval. The gray area denotes the one standard deviation range of the corresponding parameters in the intact vertebrae of age-matched mice.
µm to 200 µm [36, 37]. The distance is obviously higher (up to 1500 µm) for in vivo micro-CT [26] as the radiation dose has to be limited and the implant position (with respect to the X-ray beam) cannot be optimized. In our work, the removal of two voxels close to the implant could lead to an overestimation of the newly formed bone within the peri-implant region, especially in the initial weeks. An upper bound for this overestimation could be calculated by assuming bone formation taking place on the entire implant surface engaged in the bone (i.e., roughly 1.3 mm) and assuming also that the newly formed bone packets have at least a thickness of 2 voxels. The resulting volume of newly formed bone would then be around 0.002 mm$^3$ which is less than 3% of the peri-implant bone volume at the beginning of the monitoring time.

We found an interplay between the structural modifications of cortical and trabecular bone: the insertion of the implant caused a thickening of the cortical shell which, plausibly, contributed to restore the mechanical stability of the bone-implant system. The increase in Ct.Th was accompanied with a considerable loss of trabecular bone. Such loss was not observed in the mice of similar age without implantation [30], indicating that it may be due to the disruption of the trabecular network by implant insertion with a consequent unloading of the remaining intact trabeculae. The fact that both cortical thickness and cortical porosity increased rapidly (i.e. within 2 weeks) together with the possible damage of the periosteum due to implant insertion, may suggest the formation of a localized bony callus, as normally seen in fracture models [38].

Following implant insertion, the rate of bone formation in the peri-implant bone increased substantially in comparison with the values measured far from the implant; however, within one week (in the trabecular compartment) or two weeks (in the cortical compartment) it returned to the values calculated in the distant region, i.e. far from the implantation site. In the cortical shell, the initially high BFR was mainly a result of an extended surface undergoing bone formation which covered more than 60% of the available bone surface. Instead, in the trabecular compartment, the increase in BFR was mostly due to a high MAR. By relating MS to the recruitment and MAR to the activity of osteoblasts [19, 33] the response of peri-implant trabecular bone reflected a longer and faster deposition of new bone (with newly formed bone packets thicker than far away) whereas the behavior of peri-implant cortical bone indicated an initial dominating phase of recruitment rather than a
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

higher activity of bone forming cells. The transient increase in the rate of bone formation close to the implant is in agreement with previous experiments: for instance, Irish and colleagues [39] found elevated bone formation rates up to 8 weeks after the insertion of the implant into a rat femur. Obviously, values among different studies should be compared only qualitatively, as the studies involved different animals, implants and implantation sites.

Here, we could also measure the rate of bone resorption, normally not accessible with dynamic histomorphometry. After implant placement, BRR was higher far from the implantation site than close to the implant in the cortical compartment: although around the implant the resorbed bone packets were in average thicker, the relative surface undergoing bone resorption was initially quite limited (i.e., < 20% of the bone surface) due to prevailing bone formation. In the trabecular compartment, BRR increased and remained elevated during the 7-week monitoring period. The fact that BRR

imp was always higher than BFR

imp is responsible of the observed loss of trabecular bone. Moreover, BRR close to the implant was almost two-times the value far away; this was due to deeper excavation pits in the early time intervals (as expressed by MRR) and, at later stages, to the increase in the relative portion of the surface undergoing bone resorption within the peri-implant bone region. Again, relating ES to the recruitment and MRR to the activity of osteoclasts, one possible biological interpretation is that, at the implantation site, cytokines (released for instance from macrophages) can enhance the resorptive capacity of the existing osteoclasts and, later, they can stimulate the differentiation of progenitor cells to form more osteoclasts [40]. An opposite behavior was observed within the cortical compartment with BFR

imp higher than BRR

imp which, together with the fact that BRR

imp was much smaller than BRR

dis explained the substantial gain in cortical thickness. When interpreting the remodeling behavior, it is important to notice that the animals used in this study had an age of 13 weeks at the beginning and of 19 weeks at the end of the experiment. Although B6 mice within this age-range show a quite limited bone growth at the caudal vertebrae [30], they were preferred over older animals as the latter have highly mineralized and hence more brittle bones [41], which may be more prone to microdamage formation or even fracture during implant insertion as also indicated by previous testing on 76-week old mice [42].

We would like to emphasize that with our work we could not directly assess differences in bone formation/resorption before and after implant insertion as we
neither included a control group without the implant nor did we measure the remodeling rates before implantation. However, we could compare the time-course of bone formation and bone resorption after implant insertion with the remodeling behavior measured in previous studies of our group on age-matched mice [29, 30]. Such “qualitative” comparison indicates that bone formation away from the implant could represent fairly well bone formation in mice of the same age but without the implant; conversely, bone resorption – even far away from the implant – was always higher for the implanted bone than for the intact vertebra. This suggests that the effect of the implant on bone formation is mainly localized to the peri-implant bone whereas on bone resorption it could extend over the entire vertebra.

This study has some limitations that should be considered. First of all, the accuracy of the bone formation changes in the peri-implant bone was not compared against standard histomorphometry. However, several previous studies on similar mice and on the same skeletal location as well as on different anatomical regions have shown that bone morphometry, bone remodeling and bone mineralization can be quantified accurately by registration of in vivo micro-CT scans at different time points [2, 18, 19, 30]. In our study, the presence of the implant did not induce metal artifacts (Figure 3.1c and 3.1d) and thus did not require new thresholding methods to distinguish mineralized tissue from background noise. Furthermore, we analyzed only bones with implants and all the implants were identical in terms of material composition, shape and dimensions; hence, the presence of the implant, which could have a minor influence on the micro-CT images due to the unavoidable beam hardening effect, is expected to affect all the scans in a similar way and, therefore, should not play a role in the relative comparison among bones with similar implants inside. Considering the implant material, it is not clear whether it induces the same peri-implant bone response as steel or titanium implants widely used in clinics. Nevertheless, the elastic modulus of the implant material (130 GPa) is quite similar to that of titanium (110 GPa) and the coating of the implant surface with a thin titaniferous layer ensured that the bone cells were not in direct contact with the ACM implant but with a biocompatible titanium based layer [27]. No treatments were done to increase the surface roughness of the implant, which may not be ideal for enhancing osseointegration. Considering the implantation site, the most used bones in small rodents are the tibia or the femur and it is quite uncommon to implant into the caudal vertebra, probably due to the small dimensions and the
3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

related difficulties with the surgery. Nevertheless, the tiny size of the caudal vertebra enabled us to image the full bone including both cortical and trabecular compartments within a reasonable scanning time, keeping the radiation exposure within a safe level as we did not observe any signs of poor health condition. With the imaging setting used here, the radiation dose estimated by the manufacturer corresponded to ca. 480 mGy per scanning session and, based on previous works [30, 43, 44], such dose, most likely, does not play a role in the structural and remodeling changes reported here. The high elastic modulus of the implant material together with the sharp needle-like shape facilitated the implantation process: in view of the small dimensions of the host bone (approximately 4 mm in length and 2 mm in diameter) and of the implant (0.5 mm in diameter) a direct insertion was preferred over pre-drilling. However, even with this procedure, it was difficult to achieve high consistency of the implant location in the caudal vertebra. We were able to avoid inserting the implant in the growth plates but we could not control well the positioning along the cranio-caudal direction (Figure 3.4c). This had the evident disadvantage of increasing the variability of the observed response, especially of trabecular BV/TV and MS. One additional limitation is that the implant, even if not completely unloaded, was not subjected to controlled mechanical stimulation, which has been shown to be an important regulator of bone regeneration [45].

The use of in vivo monitoring approaches has the key advantage of reducing the number of animals needed in pre-clinical studies (according to the 3R philosophy: Replace, Reduce, Refine): in fact, in vivo micro-CT allows obtaining information at each time point without the need to sacrifice the animal. This obviously leads to a reduction in the total number of animals required in a longitudinal study compared to a cross-sectional approach. The gained knowledge on architectural and especially remodeling changes after implant insertion may be used to tune the development of smart implants which are able to manipulate the remodeling process to accelerate osseointegration and to favor long-term anchorage. Indeed, a recent work of Kettenberger and colleagues used a similar monitoring approach based on in vivo micro-CT to characterize the effect of bisphosphonates, introduced in the peri-implant bone bed, on the structural and remodeling changes around the implant [13]. Our methodology can be used in future work in combination with controlled mechanical loading [3] to predict the interplay between mechanical stimulation and
medications affecting bone remodeling on implant integration and stability.

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3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging

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Chapter 3 Development of a novel method to investigate bone response following implantation


3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging


Chapter 3 Development of a novel method to investigate bone response following implantation


3.1 Bone architecture and remodeling after implant insertion from in vivo time-lapsed imaging


Chapter 4

Bone response following implantation in disease and treatment
4.1 Effect of osteoporosis on bone response following implantation

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\textit{in preparation}

as “Spatio-temporal changes in bone remodeling and architecture following implantation in a mouse model for osteoporosis”

\textbf{Abstract:}
Bone remodeling is a process in which old or damaged bone is resorbed by osteoclasts and new bone is locally laid down by osteoblasts, leading to an adaptation in mass and structure of the bone tissue. In osteoporosis, the imbalance in bone remodeling induces bone fragility and increases the probability of fracture in need of implant fixation. Good implant anchorage – the first stage of a successful fracture fixation – greatly depends on the bone remodeling process and the consequential changes in bone architecture following implantation, which, although being an essential knowledge in fracture fixation, still requires exploration. In this study, the spatio-temporal changes in bone remodeling and bone architecture following implantation were investigated by using \textit{in vivo} micro-computed tomography in ovariectomized mice mimicking estrogen-deficient bone loss, at three different locations within the same bone: the trabecular bone, the endocortical surface and the periosteal surface of cortical bone. A transient accelerating bone forming phase following implantation was observed especially at the endocortical surface, resulting in an increase in cortical thickness in the peri-implant bone in both estrogen depleted and healthy control mice. Contrarily, bone loss was observed in the entire trabecular compartment with the reduction in healthy control mice being higher than in estrogen depleted mice. These results indicated that the accelerating
bone forming phase and the consequential increase in the thickness of peri-implant cortical bone were responsible for the implant integration and the stability of the bone-implant system. However, this integration process was impaired in osteoporotic bone with both the bone formation and the increase in cortical thickness being limited, leading to a reduced bone-implant contact compared to healthy bone. These findings may provide useful guidelines in the development of advanced treatments for remodeling modulation and regulation to enhance implant integration and stability in osteoporotic fracture fixation.

Keywords:
Osteoporosis, peri-implant bone remodeling, implant anchorage, in vivo micro-computed tomography

4.1.1 Introduction

The remarkable ability of healthy bone to avoid minimal trauma fractures for the entire lifespan is based on a constant renewal of bone tissue through the biological process of bone remodeling. Unfortunately, impairments in the remodeling process can lead to a rapid loss of bone mass together with a deterioration of bone structure and material properties [1], causing a generalized weakening of the bone. This is a typical scenario occurring in osteoporotic individuals, which often leads to an increased likelihood for a bone fracture to happen under small loading forces (referred to as fragility fracture) [2]. Often fragility fractures need to be repaired with orthopaedic devices and the role of osteoporosis on fracture fixation is still controversial, mainly due to the lack of strong clinical evidence correlating the osteoporotic condition with implant failure [3, 4]. Nevertheless, numerous biomechanical experiments [5-8] suggest that osteoporosis may jeopardizes the outcome of fracture fixation. In fact, a peri-implant bone bed of low quality presents less bone surface to anchor the implant as well as a weaker bone structure to receive the loads coming from the implant, therefore reducing the so-called initial (or primary) implant stability [9, 10]. A compromised initial stability may not allow a proper healing process around the implant, with a typical problem being the formation of weak layer of fibrous tissue between the implant and the anchoring bone [11].
A second key aspect is the effect of osteoporosis on the dynamical process of bone remodeling around the implant, which is ultimately responsible for the long-term stability of the bone-implant system. It is well accepted that osteoporosis impairs bone remodeling, especially in the peri-menopausal years [12]; briefly, both bone formation and resorption are increased with the latter being higher and therefore leading to bone loss. In general, trabecular bone, being exposed to more remodeling events due to its greater surface, suffers from a larger bone loss than cortical bone. Nevertheless, osteoporosis alters also cortical bone remodeling mainly by increasing bone resorption at the inner endocortical surface without a corresponding increase in bone formation at the outer periosteal location [13]. Such modifications are observed both in humans [14] as well as in animal models for osteoporotic bone loss [15]. Implant placement also modifies the remodeling process due to several reasons acting at different time scales including the presence of the implant itself which often trigger de novo bone formation on its surface [16], the unavoidable microdamage produced by inserting the implant which is belied to increase bone remodeling in the peri-implant bone [17] and the loading redistribution which may induces stress shielding and consequently bone resorption [18]. Whether and to which extent osteoporotic modifications in bone remodeling interfere with the normal regeneration mechanisms in the peri-implant bone is still not well elucidated. Obviously, such scenario cannot be assessed in a systematic way in human patients; thus, animal models are the preferred pre-clinical research approach. Implant osseointegration and anchorage in poor quality bone and in compromised metabolic conditions associated with osteoporosis have been investigated in ovariectomized (OVX) and orchiectomized (ORX) animals as those methods are widely used to simulate the high turnover scenario with negative bone balance occurring in osteoporotic patients. However, the results of such studies are quite controversial: for instance, some authors have found bone formation around implants to be significantly less in OVX animals compared to control groups [19, 20] while other have reported no significant differences [21-23] or differences occurring only in the early stages of osseointegration [24]. One common limitation of the abovementioned studies is the use of histology-based dynamic histomorphometric analysis to measure bone remodeling. This technique does not allow a proper characterization of bone resorption and provides information only at end time points.

Therefore, a better understanding of the bone remodeling process following
implantation, which ultimately affects the peri-implant bone regeneration and maintenance, is essential for improving our understanding of implant anchorage and stability in osteoporotic bone. In the present study, we investigated the changes in bone formation and resorption as well as the corresponding architectural modifications following implantation in a mouse model mimicking estrogen-deficient bone loss. For that purpose, we used a previously developed framework combining in vivo micro-CT imaging with novel metal-ceramics implant to overcome the metal artifacts induced by standard endosseous metal implants [25]. Specifically, this work aimed to investigate the influences of osteoporosis on the time evolution of bone remodeling and the consequential modifications in bone architecture occurring at three different locations within the same bone: the trabecular bone, the endocortical surface and the periosteal surface of cortical bone.

4.1.2 Materials and methods

Animal experiment

The animal experiment is based on 12-week old female C57BL/6J mice (JANVIER, Saint Berthevin Cedex, France). The mice were ovariectomized bilaterally (OVX group, n=9) to provoke bone loss and structural deterioration similar to the estrogen-deficient scenario in osteoporotic patients. A sham-ovariectomized group (SHM, n=8) underwent surgery but the ovaries were not removed. After a recovery period of 9 weeks which allowed estrogen-deficient bone loss to reach a plateau [15], special needle-shape implants (Composite Metal Technology Limited, Hampshire, United Kingdom) were inserted into the sixth caudal vertebra (CV6) of both OVX and SHM animals. Implants and implantation procedure were already described in details elsewhere [25]. In short, the implantation site was located with fluoroscopic imaging and the small metal-ceramic implants (diameter of 0.5 mm and length of 1.5 mm) with a sharp tip were inserted using a well-established pinning procedure [26]. Before implantation, the implants were coated with a titaniferous layer of about 30 nm (pfm medical titanium gmbh, Nuremberg, Germany) and sterilized. Implantation was performed under isoflurane anesthesia and the mice were sacrificed 6 weeks after implant insertion. All in vivo animal procedures were approved by the local authorities (Kantonales Veterinäramt Zürich, License No. 190/2010, Switzerland).
4.1 Effect of osteoporosis on bone response following implantation

**In vivo micro-CT imaging and image processing**

The entire CV6 was scanned nine consecutive times using *in vivo* micro-CT (vivaCT 40, Scanco Medical, Brüttisellen, Switzerland). The first two scans were performed before the OVX/SHM surgery and 9 weeks after surgery (which corresponded to one day before implantation). Following implantation, the CV6 containing the implant was scanned right after implant insertion (week 0), and weekly for the following 6 weeks (i.e., week 1 – week 6). The scanning procedure followed an optimized protocol previously developed [25]. Briefly, the images were acquired at a nominal isotropic resolution of 10.5 µm, with an integration time of 350 ms, 500 projections and no frame averaging. The peak voltage and the current of the micro-CT were set to 55 kVp and 145 µA, respectively. The total scanning time for imaging the entire vertebra with the implant was about 15 minutes. With the imaging parameters used here, the radiation dose estimated by the manufacturer corresponded to ca. 480 mGy per scanning session. Based on previous knowledge, the total dose delivered within the 16-week experiment was not expected to cause substantial modifications of bone architecture and remodeling [27-29].

Image processing involved several steps which have been developed and refined in earlier works on *in vivo* micro-CT imaging of mouse vertebrae [15, 30, 31]. The virtual reconstructions of the vertebra were first aligned along the cranio-caudal axis using rigid registration [32]. A Gaussian filter (support 1, sigma 1.2) was applied to the grey-scale images to reduce the noise. A globally fixed threshold, corresponding to 31.6% of the maximum grey value, was used to separate the bone-implant system from the background. Regions of bone formation and bone resorption were obtained by superimposing sequential scans of the same vertebra [30] within a two-week time interval (i.e., week 2 was overlaid with week 0, week 3 with week 1, week 4 with week 2, etc.). Although weekly information were available, a two-week time interval was preferred to increase the reproducibility of the measured bone formation and resorption regions [25]. Single formed and resorbed voxels were removed to decrease registration and partial volume errors [25, 33]. Implant was separated from the surrounding bone thanks to a semi-automatic approach which involved minimal user interaction [25]. Cortical, trabecular and full bone compartments were automatically detected with an algorithm described elsewhere [29]. Within the cortical compartments (without the growth plates), we distinguished between periosteal and endocortical surfaces, which were identified by extracting
the outer and inner surface voxels of the cortical shell, respectively (Figure 4.1a). The endocortical surfaces comprised voxels in contact with the marrow space as well as with intracortical pores. Being interested in the changes close to and far from the implant, the different bone compartments were further divided into a peri-implant (i.e., bone located up to 420 µm away from implant surface) and a distant (i.e., bone located more than 420 µm away from implant surface) region [25].

**Animal model of bone loss**

Bone architectural parameters measured right before OVX/SHM surgery and 9 weeks after surgery included: full tissue volume including the growth plates (TV), full length along the distal-proximal axis of the entire CV6, cortical thickness (Ct.Th), cortical area fraction (Ct.Ar/Tt.Ar), cortical porosity (Ct.Po), the area of periosteal and endocortical surfaces, trabecular bone volume fraction (BV/TV),

![Figure 4.1: Periosteal and endocortical surfaces (a) and trabecular bone (b) of mouse vertebra with an implant.](image)
4.1 Effect of osteoporosis on bone response following implantation

Figure 4.2: Three dimensional visualization of bone formation and bone resorption sites over time of representative ovariectomized (OVX) mouse.

Remodeling sites in the trabecular compartment

Remodeling sites at the periosteal and endocortical surfaces
Table 4.1: Bone architectural parameters before surgery and 9 weeks after surgery for ovariectomized (OVX) and sham-ovariectomized (SHM) mice. * denoted a significant difference (p < 0.05) before and after surgery within the same group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OVX Before surgery</th>
<th>OVX After surgery</th>
<th>Variation [%]</th>
<th>SHM Before surgery</th>
<th>SHM After surgery</th>
<th>Variation [%]</th>
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<tbody>
<tr>
<td><strong>Full bone</strong></td>
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<tr>
<td>TV [mm^3]</td>
<td>6.98 ± 0.19</td>
<td>7.63 ± 0.19</td>
<td>9.34 ± 0.66 *</td>
<td>7.19 ± 0.16</td>
<td>7.51 ± 0.17</td>
<td>4.49 ± 0.22 *</td>
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<tr>
<td>Length [mm]</td>
<td>3.87 ± 0.03</td>
<td>4.10 ± 0.03</td>
<td>5.91 ± 0.32 *</td>
<td>3.86 ± 0.04</td>
<td>3.92 ± 0.04</td>
<td>1.55 ± 0.18 *</td>
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<tr>
<td><strong>Cortical bone</strong></td>
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<tr>
<td>Ct.Th [mm]</td>
<td>0.16 ± 0.00</td>
<td>0.15 ± 0.00</td>
<td>-7.39 ± 1.13 *</td>
<td>0.16 ± 0.00</td>
<td>0.17 ± 0.00</td>
<td>7.47 ± 0.97 *</td>
</tr>
<tr>
<td>Ct.Ar/Tt.Ar [%]</td>
<td>43.77 ± 0.47</td>
<td>38.74 ± 0.64</td>
<td>-11.43 ± 1.55 *</td>
<td>44.69 ± 0.57</td>
<td>47.52 ± 0.74</td>
<td>6.37 ± 1.18 *</td>
</tr>
<tr>
<td>Ct.Po [%]</td>
<td>17.47 ± 0.25</td>
<td>19.95 ± 0.46</td>
<td>14.20 ± 2.20 *</td>
<td>16.93 ± 0.21</td>
<td>15.16 ± 0.26</td>
<td>-10.43 ± 1.05 *</td>
</tr>
<tr>
<td>Periosteal surface [mm^2]</td>
<td>16.82 ± 0.34</td>
<td>19.02 ± 0.47</td>
<td>13.01 ± 0.83 *</td>
<td>16.12 ± 0.35</td>
<td>16.18 ± 0.34</td>
<td>0.43 ± 0.34</td>
</tr>
<tr>
<td>Endocortical surface [mm^2]</td>
<td>12.76 ± 0.35</td>
<td>13.20 ± 0.39</td>
<td>3.45 ± 0.74</td>
<td>12.15 ± 0.25</td>
<td>11.25 ± 0.15</td>
<td>-7.32 ± 0.94 *</td>
</tr>
<tr>
<td><strong>Trabecular bone</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>BV/TV [%]</td>
<td>15.67 ± 0.61</td>
<td>10.37 ± 0.40</td>
<td>-33.35 ± 2.58 *</td>
<td>16.93 ± 0.63</td>
<td>21.90 ± 0.51</td>
<td>30.10 ± 3.51 *</td>
</tr>
<tr>
<td>Tb.Th [mm]</td>
<td>0.08 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>-11.02 ± 2.17 *</td>
<td>0.08 ± 0.00</td>
<td>0.09 ± 0.00</td>
<td>17.68 ± 1.95 *</td>
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<tr>
<td>Tb.N [1/mm]</td>
<td>2.43 ± 0.07</td>
<td>2.17 ± 0.04</td>
<td>-10.42 ± 1.62 *</td>
<td>2.51 ± 0.07</td>
<td>2.56 ± 0.07</td>
<td>2.02 ± 1.23</td>
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</tbody>
</table>
Effect of osteoporosis on bone response following implantation

trabecular thickness (Tb.Th) and trabecular number (Tb.N). All architectural parameters were calculated and reported according to standard guidelines [34]. Net bone change including formed and resorbed bone volume within the period of 9 weeks was measured at the periosteal surface, endocortical surfaces, and in the trabecular compartment.

**Bone architecture and bone remodeling following implantation**

Following implantation, bone architectural parameters including TV, full length, Ct.Th, Ct.Ar/Tt.Ar, Ct.Po, BV/TV, Tb.Th and Tb.N were measured at each time point during the *in vivo* monitoring period. Bone remodeling rates following implantation including bone formation rate (BFR) and bone resorption rate (BRR) were computed based on formed and resorbed voxels [13] within a two-week time interval (Figure 4.2), which allowed occurrence of sufficient changes for accurate detection with the current image resolution of the *in vivo* micro-CT. Additionally, mineral apposition rate (MAR), mineral resorption rate (MRR), mineralizing surface (MS), and eroded surface (ES) were computed. At the periosteal and endocortical surfaces, BFR and BRR were normalized to the bone surface area at each time point; while in the trabecular compartment they were normalized to the bone volume.

**Statistics**

Architectural and remodeling parameters were tested for significance between OVX and SHM using Mann-Whitney Rank Sum Test since the two groups in each timepoint did not always follow a normal distribution and nor did they always have equal variance. To test the significance between the first and last time point within the same group a paired t-test was used. P-values smaller than 0.05 were considered significant. All data are shown as mean ± standard error.

**4.1.3 Results**

**Structural modification after OVX surgery**

Ovaries removal deteriorates the structure of both cortical and trabecular bone in the mouse caudal vertebra CV6 (Table 4.1). Nine weeks after surgery, cortical thickness (Ct.Th) decreased significantly by -7.4% in OVX mice, which contrasted
with the 7.5% increase (p < 0.05) occurring in SHM. The cortical bone in OVX animals was not only thinner but also more porous (14.2% increase in Ct.Po, p < 0.05). Instead, Ct.Po in SHM mice diminished by -10.4% (p < 0.05). The periosteal surface expanded considerably in OVX (13.0%, p < 0.05) whereas stayed practically constant in SHM (0.43%, p > 0.05). The endocortical area showed only a minor enlargement in the OVX group (3.4%, p > 0.05) whereas it contracted by -7.3% (p < 0.05) in SHM animals. The loss of trabecular bone volume fraction (BV/TV) due to OVX amounted to -33.4% (p < 0.05) and it was reflected in the trabeculae getting thinner (-11.0% in Tb.Th, p < 0.05) and even disappearing from the trabecular network (-10.4% in Tb.N, p < 0.05). Conversely, SHM mice showed a pronounced increase in BV/TV (30.0%, p < 0.05) mainly due to thicker trabeculae (17.7% in Tb.Th, p < 0.05). Incidentally, OVX vertebrae were also significantly longer and larger than SHM bones.

**Modifications in bone architecture following implantation**

Table 4.2 shows the architectural parameters of full, cortical and trabecular bone for OVX and SHM following implantation at the first (week 0) and last (week 6) time point of the *in vivo* monitoring. Full TV and full bone length for both OVX and SHM increased significantly with time, indicating that implant placement and the monitoring process did not prevent bone growth. In the peri-implant region, the cortical shell was thickened in both groups with cortical thickness (Ct.Th) increasing significantly over time (5.0% for OVX and 14.4% for SHM). An increase was also observed in cortical area fraction (Ct.Ar/Tt.Ar) for both groups (2.9% for OVX and 7.3% for SHM, p > 0.05). Additionally, the peri-implant cortical bone became less porous over time as shown by the decreasing cortical porosity (Ct.Po) especially in SHM where a significant decrease of -13.7% was found, while in OVX Ct.Po decreased slightly for -1.4% (p > 0.05). Conversely, bone loss was observed in the trabecular compartment with a decreasing bone volume fraction (BV/TV) in both groups (-6.1% for OVX, p > 0.05; -11.0% for SHM, p < 0.05); although trabecular thickness (Tb.Th) showed an increase (11.7% for OVX and 17.4% for SHM, p < 0.05), trabecular number (Tb.N) dropped over time especially in SHM (-14.7%, p < 0.05). In the distant region, Ct.Th did not show significant change comparing week 0 and week 6, while Ct.Ar/Tt.Ar decreased over time in both groups (-3.8% for OVX and -5.8% for SHM, p < 0.05); bone loss in the trabecular compartment...
4.1 Effect of osteoporosis on bone response following implantation

Table 4.2: Bone architectural parameters at the first (week 0) and last (week 6) time point for ovariectomized (OVX) and sham-ovariectomized (SHM) mice. *p < 0.05 denoted a significant difference between week 0 and week 6 within the same group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OVX</th>
<th>SHM</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 6</td>
<td>Variation [%]</td>
<td>Week 0</td>
</tr>
<tr>
<td>Full bone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV [mm³]</td>
<td>7.48 ± 0.19</td>
<td>8.00 ± 0.16</td>
<td>7.10 ± 0.70 *</td>
<td>7.27 ± 0.16</td>
</tr>
<tr>
<td>Length [mm]</td>
<td>4.09 ± 0.03</td>
<td>4.16 ± 0.03</td>
<td>1.72 ± 0.31 *</td>
<td>3.91 ± 0.04</td>
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</tbody>
</table>

Peri-implant region

Cortical bone

<table>
<thead>
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<th>OVX</th>
<th>SHM</th>
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<tbody>
<tr>
<td>Ct.Th [mm]</td>
<td>0.17 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>4.98 ± 2.20 *</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Ct.Ar/Tt.Ar [%]</td>
<td>43.35 ± 1.43</td>
<td>44.42 ± 1.45</td>
<td>2.90 ± 3.09</td>
<td>50.64 ± 1.40</td>
</tr>
<tr>
<td>Ct.Po [%]</td>
<td>23.77 ± 0.81</td>
<td>23.38 ± 0.77</td>
<td>-1.37 ± 2.57</td>
<td>21.61 ± 0.67</td>
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Trabecular bone

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OVX</th>
<th>SHM</th>
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</tr>
</thead>
<tbody>
<tr>
<td>BV/TV [%]</td>
<td>7.55 ± 0.80</td>
<td>7.15 ± 0.83</td>
<td>-6.07 ± 4.08</td>
<td>14.33 ± 1.97</td>
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<tr>
<td>Tb.Th [mm]</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>11.72 ± 3.38 *</td>
<td>0.09 ± 0.01</td>
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<tr>
<td>Tb.N [1/mm]</td>
<td>2.22 ± 0.10</td>
<td>1.98 ± 0.15</td>
<td>-10.90 ± 4.65 *</td>
<td>2.57 ± 0.13</td>
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</table>

Distant region

Cortical bone

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OVX</th>
<th>SHM</th>
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</thead>
<tbody>
<tr>
<td>Ct.Th [mm]</td>
<td>0.13 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>2.83 ± 1.57</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Ct.Ar/Tt.Ar [%]</td>
<td>40.74 ± 1.64</td>
<td>39.14 ± 0.72</td>
<td>-3.83 ± 1.83 *</td>
<td>49.56 ± 1.06</td>
</tr>
<tr>
<td>Ct.Po [%]</td>
<td>22.96 ± 0.49</td>
<td>23.04 ± 0.50</td>
<td>0.58 ± 2.38</td>
<td>17.69 ± 0.40</td>
</tr>
</tbody>
</table>

Trabecular bone

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OVX</th>
<th>SHM</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>BV/TV [%]</td>
<td>12.73 ± 0.59</td>
<td>11.71 ± 0.64</td>
<td>-7.08 ± 5.12</td>
<td>25.64 ± 0.86</td>
</tr>
<tr>
<td>Tb.Th [mm]</td>
<td>0.06 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>9.41 ± 2.55 *</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Tb.N [1/mm]</td>
<td>2.51 ± 0.02</td>
<td>2.19 ± 0.04</td>
<td>-12.70 ± 1.62 *</td>
<td>2.76 ± 0.09</td>
</tr>
</tbody>
</table>
Figure 4.3: Cortical thickness (Ct.Th) in the peri-implant (a) and distant region (b), as well as trabecular bone volume fraction (BV/TV) in the peri-implant (c) and distant region (d) at each time point following implantation for ovariectomized (OVX) and sham-ovariectomized (SHM) mice. * p < 0.05 denoted a significant difference between groups, # p < 0.05 showed significant different comparing the first and the last time point.
was more pronounced compared to the peri-implant region in SHM due to the decreasing Tb.Th (-3.1%, p > 0.05) and Tb.N (-6.6%, p > 0.05), while in OVX Tb.Th increased for 9.4% (p < 0.05) and Tb.N dropped for -12.7% (p < 0.05) over the monitoring period.

Figure 4.3 shows the full time evolution of Ct.Th and BV/TV for OVX and SHM in the peri-implant and distant region following implant placement. In the peri-implant region, Ct.Th started to increase from week 2 for OVX and from week 3 for SHM, with the final thickening (i.e., week 0 – week 6) in SHM (14.4%) being significantly higher than in OVX (5.0%). The increase in cortical thickness was contrasted by a loss of trabecular bone, which was not greater than 10% over 6 weeks and with no significant differences between OVX and SHM. In the distant region, Ct.Th had also a slight increasing trend for both groups but the final thickening of the cortex was less pronounced (around 2% – 3%) than in the peri-implant bone and there were not significant differences between the two groups when comparing week 0 to week 6. However, at intermediate time points (i.e., from week 1 to week 3) OVX animals had higher percentage variations in Ct.Th than SHM. Interestingly, BV/TV in the distant region dropped by 29% in SHM (week 0 – week 6, p < 0.05) whereas it stayed practically constant in OVX.

Modifications in bone formation and bone resorption following implantation

Figure 4.4 shows the bone remodeling rates following implant placement for OVX and SHM in the peri-implant region for the three locations analyzed: periosteal surface, endocortical surface, and trabecular bone. Bone formation rate (BFR) was transiently elevated for both groups in the peri-implant region of periosteal surface, endocortical surface, and trabecular bone (Figure 4.4a, 4.4b and 4.4c). At the periosteal surface, BFR initially increased, reached a peak in week-interval 2-4 (0.046 µm³/µm²/d for OVX and 0.088 µm³/µm²/d for SHM) and then decreased over time to about 0.013 µm³/µm²/d in the last week-interval for both groups (Figure 4.4a). Endocortical BFR showed similar pattern to the periosteal surface for both groups, with the value being higher by roughly a factor of 2 for OVX and 3 for SHM, resulting at significantly higher BFR in SHM than in OVX (Figure 4.4b). In trabecular bone, the slightly elevated BFR peaked in week-interval 2-4 (2.0 %/d for OVX and 1.6 %/d for SHM, Figure 4.4c) and OVX animals showed slightly higher values than SHM.
**Figure 4.4:** Rates of bone formation, bone resorption and net remodeling for ovariectomized (OVX) and sham-ovariectomized (SHM) mice in the peri-implant region of the periosteal surface (a, d and g), endocortical surface (b, e and h), and trabecular bone (c, f and i). * p < 0.05 denoted a significant difference between groups, # p < 0.05 showed significant different comparing the first and the last time point.
4.1 Effect of osteoporosis on bone response following implantation

Bone resorption rate (BRR) was very high in SHM only at the endocortical surface and for the first two time intervals; over the following weeks similar values (around 0.080 µm³/µm²/d) were observed in BRR for both OVX and SHM (Figure 4.4c). Very low resorption was observed on the periosteal surface both in SHM and OVX (Figure 4.4d) whereas in the trabecular compartment OVX animals showed a higher BRR for all the time interval considered (Figure 4.4f). Mineral apposition rate (MAR), mineral resorption rate (MRR), mineralizing surface (MS), and eroded surface (ES) in the peri-implant region were reported in the supplementary material (Table S4.1).

The net effect of bone formation and bone resorption in the peri-implant region was presented as net remodeling rates, which were similar for both groups at the periosteal surface (0 – 0.06 µm³/µm²/d, Figure 4.4g) and in the trabecular compartment (-0.5% – 0.1%, Figure 4.4i); while at the endocortical surface net rate in SHM was initially lower (-0.011 µm³/µm²/d for OVX and -0.152 µm³/µm²/d for SHM in week-interval 0-2, p < 0.05) but then was elevated and became significantly higher than in OVX in week-interval 2-4 (0.061 µm³/µm²/d for OVX and 0.241 µm³/µm²/d for SHM) and 3-5 (-0.016 µm³/µm²/d for OVX and 0.141 µm³/µm²/d for SHM, Figure 4.4h).

Figure 4.5 shows the bone remodeling rates for OVX and SHM following implantation in the distant region, again for periosteal, endocortical and trabecular surfaces. A general observation is that the values of bone formation and resorption rates here were lower than in the peri-implant region except for trabecular BRR which had comparable magnitudes. At the periosteal surface, similar formation pattern was observed in OVX and SHM: BFR increased to reach a peak at about 0.005 µm³/µm²/d in week-interval 1-3 and 2-4, and then dropped to practically no formation in the last two week-intervals (Figure 4.5a). Endocortical BFR was initially higher than periosteal BFR and then decreased over time in both groups, with the value in OVX (decreasing from 0.019 µm³/µm²/d to 0.009 µm³/µm²/d from week-interval 0-2 to 4-6) being significantly lower than in SHM (decreasing from 0.030 µm³/µm²/d to 0.016 µm³/µm²/d from week-interval 0-2 to 4-6) except for the first week-interval (Figure 4.5b). Trabecular BFR for OVX was initially elevated (1.5 %/d in the first week-interval) and then decreased over time to about 0.7 %/d in the last two week-intervals. In SHM, BFR showed no significant change over time and varied between 0.4 – 0.7%/d (Figure 4.5c).
Figure 4.5: Rates of bone formation, bone resorption and net remodeling for ovariectomized (OVX) and sham-ovariectomized (SHM) mice in the distant region of the periosteal surface (a, d and g), endocortical surface (b, e and h), and trabecular bone (c, f and i). * p < 0.05 denoted a significant difference between groups, # p < 0.05 showed significant different comparing the first and the last time point.
Periosteal BRRs were very small both in OVX and SHM indicating practically no resorption on that surface away from the implant (Figure 4.5d). Conversely, endocortical BRR were up to a factor 6 higher than periosteal rates and, specifically in the first two week-intervals BRR in SHM was significantly higher than in OVX, but started from the third week-interval, it decreased and was not statistically different from OVX (Figure 4.5e). Trabecular BRR in the two groups was again very similar and fairly elevated at initial weeks of the monitoring period (up to 1.9 %/d for OVX and 2.0 %/d for SHM) and decreased linearly over time to about 1.0 %/d for both groups (Figure 4.5f). MAR, MRR, MS, and ES in the distant region were reported in the supplementary material (Table S4.1). Net remodeling rates were similar for OVX and SHM in the distant region of the periosteal (around 0 for both groups, Figure 4.5g) and endocortical surface (ranging from -0.012 – -0.036 µm³/µm²/d, Figure 4.5h), but not in the distant trabecular bone where net remodeling rate for SHM was significantly lower (up to -1.6 %/d for SHM in week-interval 1-3) than for OVX (remaining constant at around -0.5 %/d) in the first three time-intervals (Figure 4.5i).

4.1.4 Discussion

The aim of this study was to investigate the changes in bone architecture and bone remodeling (including formation and resorption) taking place following implant insertion in osteoporotic bone. Although it has been shown that low bone quality impaired implant anchorage [9, 10], the time evolution of bone formation and resorption following implantation, which directly affects the peri-implant bone quality, was not yet well elucidated, especially in osteoporotic bone where the remodeling process is unbalanced. Here, using a mouse caudal vertebra model mimicking estrogen-deficient bone loss, we investigated both bone formation and bone resorption with the presence of an implant in a noninvasive time-lapsed manner, at the periosteal, endocortical and in the trabecular bone. The sixth caudal vertebra (CV6) of ovariectomized mice was chosen as a model system to investigate whether osteoporotic bone has still the ability to adapt its remodeling process to allow peri-implant bone changes which facilitate implant integration and anchorage. Firstly, the cortical and trabecular bone loss in OVX confirmed that the CV6 of ovariectomized mice was an effective model to study osteoporotic bone loss [15]. The structural deterioration observed in OVX was a result of the remodeling
imbalance with accelerated resorption and impaired formation, a well-known symptom of estrogen deficiency [35].

Considering the structural changes in peri-implant bone, OVX animal showed a reduced ability to thicken their cortical bone in response to implant placement, which indicated a lower bone-implant contact and presumably reduced fixation strength [6]. The fact that trabecular BV/TV decreased substantially in the distant bone of SHM animals but not of OVX was, at first, quite unexpected. However, considering the absolute values of BV/TV (instead of its percentage variations) it was clear that OVX animals had an initial bone volume fraction which was almost a factor of two smaller than SHM. Therefore, it may be that the bone volume was so low that, under the same level of physiological mechanical stimulations, the strains were much higher in OVX than in SHM and therefore further significant bone loss was prevented.

Changes in bone architecture were ultimately due to the modifications in bone remodeling following implantation. Transient elevated bone remodeling rates for both OVX and SHM indicated that formation was accelerated following implantation especially in the peri-implant region, even in osteoporotic bone where formation was impaired before implantation. Most interestingly, the ability of accelerating formation in osteoporosis was lower than in healthy bone. In the peri-implant region, both periosteal and endocortical formation were transiently elevated for both OVX and SHM, which was in agreement with a previous study on healthy bone [36]. Higher BFR was observed in SHM compared to OVX, especially at the endocortical surface where significant difference was found in every week-interval. Resorption was also transiently increased only in SHM, compensating the high BFR until week-interval 2-4, leading to a higher Ct.Th in SHM compared to OVX only after week 4 following implantation. Close to the implant in the trabecular compartment, transient increase was observed for BFR and BRR in both groups, with BRR being higher than BFR except for week-interval 2-4, leading to a decrease in trabecular BV/TV. In the distant region, formation was also transiently increased at the periosteal and endocortical surface, with the value lower than in the peri-implant region by at least a factor of 5; resorption was only initially elevated in SHM and was lower here compared to the peri-implant bone for both groups. The limited increase of formation rates in the distant region led to a slight but not significant increase in the cortical thickness. In the distant region of trabecular bone the initially
Effect of osteoporosis on bone response following implantation

Elevated BFR in OVX contributed to the relatively stable BV/TV, while the constantly low BFR in SHM resulted in a decreasing BV/TV.

There were some limitations of the presented study that need to be mentioned. First of all, the mouse vertebral model used in the presented study does not represent human cortical bone: adult mice are still growing in length while human cortical bone stops growing in early adulthood. Moreover, although it was reported that most of the bone loss in osteoporotic cortical bone in human was caused by intracortical bone remodeling which increased the porosity, even if intracortical remodeling were present also in mice, it was not possible to monitor it in this study due to the smaller pore size in mouse cortical bone and the limited image resolution of the in vivo micro-CT. Secondly, bone remodeling was monitored only after implantation, while the remodeling behavior before implantation or without the implant was not characterized on the same animal. However, in a previous work we compared the remodeling behavior on genetically identical mice of the same age with and without the implant and we could demonstrate that in the distant region bone formation was not affected by the presence of the implant whereas, to some extent, bone resorption was [25]. Therefore we concluded that the chosen distant region could be considered as a control scenario at least for the bone formation parameters. Nevertheless, this lack of a suitable control for bone resorption did not affect the investigation of the difference between OVX and SHM in bone remodeling and bone architecture.

In conclusion, the presented work showed that cortical bone played an important role in bone-implant integration in both healthy and osteoporotic bone, with cortical thickness increasing significantly following implantation especially close to the implant. This increase was mainly contributed by an accelerated bone forming phase at the endocortical surface. This acceleration was limited in osteoporotic bone and thereby reduced the bone-implant contact. The obtained knowledge may provide useful guideline in bone remodeling regulation and mechanical control for developing advanced treatments for osteoporotic fracture fixation with improve implant anchorage and stability.

Acknowledgements

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advices on the image-based micro-FE analysis. The technical supports from Dr. Michael Schirmer (pfm medical titanium gmbh, Nuremberg, Germany) and Stephen Cooke (Composite Metal Technologies, Hampshire, United Kingdom) are gratefully acknowledged. ZL acknowledges support of the Chinese Scholarship Council and DR of the IOF-SERVIER Young Investigator Research Grant as well as of the ECTS Postdoctoral Fellowship.
### Table S4.1: Mineral apposition rate (MAR), Mineral resorption rate (MRR), Mineralizing surface (MS), and Eroded surface (ES) for OVX and SHM in peri-implant and distant region at the first (week 0) and last (week 6) time point.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OVX</th>
<th>SHM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 6</td>
</tr>
<tr>
<td>Peri-implant region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periosteal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAR [µm/d]</td>
<td>1.440 ± 0.164</td>
<td>1.954 ± 0.076</td>
</tr>
<tr>
<td>MRR [µm/d]</td>
<td>3.366 ± 0.442</td>
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</tr>
<tr>
<td>MS [%]</td>
<td>40.093 ± 2.407</td>
<td>47.000 ± 1.615</td>
</tr>
<tr>
<td>ES [%]</td>
<td>13.757 ± 1.741</td>
<td>8.326 ± 1.218</td>
</tr>
<tr>
<td>Endocortical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAR [µm/d]</td>
<td>1.604 ± 0.050</td>
<td>1.872 ± 0.092</td>
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<tr>
<td>MRR [µm/d]</td>
<td>2.830 ± 0.298</td>
<td>2.095 ± 0.033</td>
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<tr>
<td>MS [%]</td>
<td>45.972 ± 3.761</td>
<td>33.275 ± 2.071</td>
</tr>
<tr>
<td>Trabecular</td>
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<td></td>
</tr>
<tr>
<td>MAR [µm/d]</td>
<td>1.730 ± 0.059</td>
<td>1.791 ± 0.085</td>
</tr>
<tr>
<td>MRR [µm/d]</td>
<td>2.649 ± 0.140</td>
<td>2.069 ± 0.084</td>
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<tr>
<td>MS [%]</td>
<td>34.384 ± 1.874</td>
<td>26.968 ± 2.192</td>
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<tr>
<td>ES [%]</td>
<td>24.522 ± 1.216</td>
<td>28.702 ± 1.596</td>
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<tr>
<td>Distant region</td>
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<tr>
<td>Periosteal</td>
<td></td>
<td></td>
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<tr>
<td>MAR [µm/d]</td>
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<td>1.400 ± 0.026</td>
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<tr>
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<td>1.704 ± 0.032</td>
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<td>MS [%]</td>
<td>33.038 ± 2.810</td>
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<tr>
<td>ES [%]</td>
<td>8.436 ± 0.823</td>
<td>4.727 ± 0.518</td>
</tr>
<tr>
<td>Endocortical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAR [µm/d]</td>
<td>1.435 ± 0.015</td>
<td>1.344 ± 0.030</td>
</tr>
<tr>
<td>MRR [µm/d]</td>
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<tr>
<td>MS [%]</td>
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<td>23.432 ± 1.166</td>
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<tr>
<td>Trabecular</td>
<td></td>
<td></td>
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<tr>
<td>MAR [µm/d]</td>
<td>1.557 ± 0.012</td>
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</tr>
<tr>
<td>MRR [µm/d]</td>
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<tr>
<td>MS [%]</td>
<td>35.204 ± 2.921</td>
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<tr>
<td>ES [%]</td>
<td>25.739 ± 1.710</td>
<td>22.693 ± 0.996</td>
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</table>
Chapter 4 Bone response following implantation in disease and treatment

References


4.1 Effect of osteoporosis on bone response following implantation


Chapter 4 Bone response following implantation in disease and treatment


4.1 Effect of osteoporosis on bone response following implantation


4.2 Effect of loading on osteoporotic bone response following implantation

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in preparation
as “The effect of mechanical loading on peri-implant bone formation and resorption”

Abstract:
Controlled mechanical loading has been suggested as an anabolic treatment to improve bone quality and facilitate implant anchorage in fracture fixation, especially in osteoporotic bone where implant stability may be jeopardized by the poor bone quality and compromised systemic condition of the patient. However, development of proper loading protocols requires better understanding of the influencing mechanisms of mechanical loading on the response of bone around the implant. In this study, in vivo micro-computed tomography was used to assess the load-induced changes in bone remodeling and architecture following implantation in a mouse model mimicking estrogen-deficient bone loss. Image-based micro-finite element analysis was applied to investigate the time evolution of the strength of the whole implanted bone as well as to investigate and mechanical regulation of bone remodeling. Our results showed that in the implanted bone mechanical loading increased bone formation rate and decreased bone resorption rate by modulating the bone surface under formation (mineralizing surface) and resorption (eroded surface) in both trabecular and, to a less extent, in cortical bone. We also found that the average thickness of formed bone packets and the average resorption depth of resorbed bone remained unaltered under loading. The load-induced changes in bone remodeling led to an increase in bone mass represented by the elevated trabecular
bone volume fraction and cortical thickness, both structural changes presumably contributing to the enhanced strength of the whole bone under loading. One additional important result was that, in the peri-implant region the mechanoresponsiveness of the bone was reduced by the presence of the implant. The obtained knowledge may provide useful guideline for optimizing the loading protocols in treatment regimens to facilitate peri-implant bone regeneration and implant anchorage in osteoporotic fracture fixation.

Keywords:
Peri-implant bone remodeling, osteoporosis, mechanical loading, in vivo micro-computed tomography, micro-finite element analysis

4.2.1 Introduction

Osteoporosis is one of the most common musculoskeletal diseases among the elderly population. It is characterized by a structural and material deterioration due to bone loss and decreased bone quality, often resulting in an increased probability of fracture [1]. Such fractures usually require fixation with implants, and the long-term success of fracture fixation depends on a stable implant anchorage into the bone stock [2]. However, implant anchorage in osteoporotic bone may be jeopardized by the poor bone quality and compromised systemic condition of the patient [3], as suggested by a large number of animal studies [4-7]. Effective treatments for improving implant anchorage and bone-implant stability is nowadays considered an unmet medical need in osteoporotic fracture fixation.

Healthy bone is able to adapt its mass, structure and material properties to changes in the mechanical environment through the process of bone remodeling [8]. Such ability is generally maintained in aged [9, 10] and even in osteoporotic bone [11]. In fracture fixation, once the initial inflammatory reaction is over, the biomechanical integrity of the bone-implant construct is attained and maintained through bone remodeling. This process not only controls the formation of a mature bone tissue at the bone-implant interface that can transmit load effectively [12] but also allows peri-implant bone to partially reconfigure to house the implant. The application of external controlled mechanical loading has been shown to interact with several physiological mechanism within the bone cells [13] usually resulting in a net gain of
bone mass [9, 14] and stiffness [15]. Therefore, controlled mechanical loading is considered an effective anabolic treatment to improve peri-implant bone quality and to facilitate implant anchorage especially in osteoporotic bone.

External loading can be applied directly to the implant, a scenario which has been extensively investigated in dentistry in the context of early loading of orthodontic implant [16] with the final aim to increase the bonding strength at the bone-implant interface. In this situation, a judicious loading protocol has to be chosen as excessive loading can lead to micromotion and, consequently, to the formation of a weak fibrous tissue layer at the bone-implant interface [17, 18]. A second option is to administer mechanical loading not directly to the implant itself but to the implanted bone with the goal of increasing bone mass and fixation strength within the peri-implant bone bed. In this context, mechanical loading can be applied at the whole bone length scale or only selectively at the level of the implanted bone. The former is the strategy adopted in the so-called whole body vibration (WBV) methods: in studies using healthy as well as osteoporotic animal models, mechanical stimulation in the form of low (~ 1-10 Hz) or high (~ 10-100 Hz) frequency vibrations has been proven to increase peri-implant bone mass in cortical [19, 20] as well as in trabecular [21, 22] bone. Conversely, the possible benefits of local mechanical stimulation, i.e. loading applied exclusively at implanted bone, on the response of peri-implant bone has been less investigated [23]. In general, bones subjected to external mechanical loading respond by a combination of increased bone formation and decreased bone resorption [11, 24] resulting in a net augmentation of bone strength, mainly in the direction of the applied load [14]. The presence of the implant alters the remodeling process: both bone formation and bone resorption are substantially increased especially in the first weeks following implant insertion [25-27] and these changes are generally stronger close to the implant than in the peri-implant bone [25, 26]. In the complex context of peri-implant bone regeneration, it is not clear whether and to which extent the ability of bone to promptly respond to mechanical loading is maintained. For instance, Zhang and colleagues [23] observed a positive effect of local compressive vibrational loading only very close to the implant and particularly at the bone-implant interface, but neither peri-implant bone nor bone further away from the implant seemed to be significantly affected.

Therefore, the global aim of this study was to investigate the response of the implanted bone to the local application of external mechanical stimulation. For that
purpose, we used a combined experimental-computational approach. Specifically, in vivo micro-CT imaging was used to assess the load-induced changes in bone remodeling and bone architecture following implantation in a mouse model mimicking estrogen-deficient bone loss. After OVX-related bone loss, implants were inserted in the mouse caudal vertebra and a loading protocol was followed to induce an anabolic response on the implanted bone. Supportive image-based high resolution finite element analysis was performed to correlate the local mechanical environment with the measured bone formation and bone resorption events. The specific aims of this work were: i) to investigate the effect of mechanical loading on bone remodeling (including both bone formation and bone resorption) and the consequential modifications in bone architecture in the peri-implant bone (i.e., close to the implant) as well as in the whole implanted bone; ii) to check whether the global mechanoresponsiveness of the bone at the whole organ level is compromised by the presence of the implant and iii) to report possible impairments in mechanoresponsiveness within the peri-implant bone.

4.2.2 Materials and methods

Animal procedures and in vivo loading

To induce bone loss and structural deterioration, ovariectomy was performed bilaterally on twelve 12-week old female C57BL/6J mice (JANVIER, Saint Berthevin Cedex, France). After a recovery period of 9 weeks, which allowed bone loss to reach a plateau, cylindrical implants were inserted in the sixth caudal vertebrae (CV6, Figure 4.6A) of mice using a well-established pinning procedure [28]. The implants were made of an aluminum matrix composite material and had fairly small dimensions (i.e., diameter of 0.5 mm and length of 1.5 mm) (Composite Metal Technology Limited, Hampshire, United Kingdom). A titaniferous layer of about 30 nm was deposited on the surface of the implants using plasma enhanced chemical vapor deposition (pim medical titanium gmbh, Nuremberg, Germany) to enhanced biocompatibility. Two stainless steel pins (Fine Science Tools, Heidelberg, Germany) were inserted in the adjacent vertebrae of CV6 (i.e. CV5 and CV7, Figure 4.6A) for the application of mechanical loading. Implantation sites were located with fluoroscopic imaging and implantation was performed under isoflurane anesthesia.
Figure 4.6: (A) *In situ* mechanical loading applied to CV6 through the adjacent vertebrae CV5 and CV7. (B) Time plan of the loading and imaging protocol with the 7 scanning time points during the monitoring period.

After implantation, three weeks of healing period was allowed before the administration of external loading. For that purpose, the animals were randomly divided into two groups: a group subjected to external mechanical loading (CML, N=6), and a control group without external loading but only subjected to physiological loading (CTR, N=6). For the CML group, mechanical loading was applied to CV6 through the two stainless steel pins in CV5 and CV7 (Figure 4.6A), as previously described [14, 28]. Briefly, a sinusoidal force of 8N was applied at the frequency of 10 Hz for 3000 cycles (5 minutes), three times a week, for 4 weeks. During the loading treatment the mice were anesthetized with isoflurane. For the CTR group, the CV6 was fixed in the loading device without applying loading and the mice were under anesthesia for 5 minutes. All *in vivo* animal procedures were
approved by Kantonales Veterinäramt Zürich, Switzerland (License No. 190/2010) and were performed according to the animal welfare regulations and guidelines in Switzerland.

**Imaging and image processing**

Figure 4.6B shows the time plan for the *in vivo* monitoring using vivaCT 40 (Scanco Medical, Brüttisellen, Switzerland). The entire CV6 was scanned right after implantation (week 0) and at week 1.5 to assess bone changes induced by implant placement. One scan was then performed at the starting point of the loading regime at week 3 and, during loading, 4 consecutive scans were performed weekly for 4 weeks. A nominal isotropic resolution of 10.5 µm was used in the scans, with an integration time of 350 ms, 500 projections and no frame averaging. The total scanning time for imaging the entire vertebra with the implant was about 15 minutes.

The peak voltage and current of the micro-CT were set to 55 kVp and 145 µA, respectively. A beam hardening correction algorithm provided by the manufacturer [29]. The grey-scale images of the CV6 were aligned using rigid registration [30], Gaussian filtered to reduce noise (sigma 1.2, support 1), and segmented to separate the bone-implant system from the background using a global fixed threshold (31.6% of the maximum grey value), corresponding to 555 mg HA/cm³. By rigid registration of the scans obtained at two subsequent time points, regions of bone formation and bone resorption within the time interval considered were identified [31]. A filter for removing single formed and resorbed voxels was implemented to decrease registration and partial volume errors [15]. The implant was segmented from surrounding bone using a semi-automatic approach previously described [25]. It is worth noticing that the first two voxels of bone in contact with the implant were not considered because of the influence of beam hardening and partial volume effects. The trabecular and cortical bone were also selected in a fully automatic manner based on previously developed algorithms [14, 32]. In both trabecular and cortical compartments, a peri-implant bone region was defined as a cylindrical region around the implant starting right at the implant surface and extending for 420 µm away from that surface. A distant bone region was defined by subtracting the peri-implant region from the corresponding trabecular or cortical compartments [25].
Evaluation of bone architecture and bone remodeling

Bone architectural parameters were measured at each time point during the \textit{in vivo} monitoring. The parameters include: full tissue volume (TV), full length along the distal-proximal axis of the entire CV6, trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th) and trabecular number (Tb.N), cortical thickness (Ct.Th), cortical bone area (Ct.Ar), cortical area fraction (Ct.Ar/Tt.Ar) and cortical porosity (Ct.Po). All architectural parameters were calculated and reported according to standard guidelines [33].

Bone remodeling rates following implantation were computed based on formed and resorbed voxels by registration of the scans obtained at two time points [31]. Specifically, during the three-week recovery period, the scans obtained at week 1.5 were registered on scans at week 0, and scans at week 3 on scans at week 1.5. In the loading regime, the scans were registered considering a two-week time interval as it yielded more reproducible measurements, especially for values within the peri-implant bone region [25]. The calculated remodeling parameters were: bone formation rate (BFR), bone resorption rate (BRR), mineralizing surface (MS), eroded surface (ES), mineral apposition rate (MAR), and mineral resorption rate (MRR).

Finite element analysis

Three-dimensional (3D) micro-FE models were generated by converting the \textit{in vivo} micro-CT images into finite element meshes of identical cubic elements using Parosol [34]. Virtual intervertebral disks were added to the proximal and distal ends of the segmented mouse vertebra to facilitate load application and to avoid unrealistically high strain regions on the bone surface [11, 28]. A Young’s modulus of 14.8 GPa was assigned to the voxels representing bone and disks [14], and 130 GPa was assigned to the voxels representing the implant. A Poisson’s ratio of 0.3 was assigned to both bone and implant. Axial load was applied through the distal intervertebral disk while the proximal disk was fixed (Figure 4.12A): a physiological load assumed to be 4N [35] was applied to the CTR group at all the time points and also to the CML group at week 0 and at week 1.5; an external load of 8N was applied to the CML group starting from week 3. The simulated strain energy density (SED), defined as the elastic energy associated with the tissue deformation per unit volume was used in this study to characterize the local mechanical environment.
Chapter 4 Bone response following implantation in disease and treatment

The whole bone strength of the implanted bones was calculated, as previously described by Pistoia and colleagues [36], based on the amount of voxels strained above a given critical strain.

**Mechanical regulation of bone remodeling**

The mechanical regulation of the load-induced bone remodeling process was investigated by linking the occurrence of surface remodeling events to the local mechanical environment according to a previously established methodology [11]. In brief, sites of bone formation and bone resorption identified by image registration of week 5 over week 3 were projected onto the surface of the scan at week 3. Mean SED values were calculated at sites of formed, resorbed, and quiescent bone within the trabecular and cortical compartments. Furthermore, SED frequency distributions were computed by “binning” the SED values in intervals having the size of 1% of the maximal SED on the surface; within each SED interval, the relative percentage of surface voxels being formed, quiescent and resorbed was derived. In this procedure, formation, resorption and quiescent sites were “rescaled” to have the same amount of voxels in order to eliminate the influence of differences in the total volume of formed, resorbed and quiescent voxels [11]. Therefore the results can be interpreted as the conditional probability of a remodeling event to take place within a given time interval and for a given level of mechanical strain. The remodeling probabilities were fit by exponential functions using non-linear regression analysis.

**Statistics**

For all the reported parameters, a paired t-test was used to assess significant differences between the first and the last time point within the same group. Mann-Whitney Rank Sum Test was used to test the significance between groups since not all the data were normally distributed nor did they always have equal variance. Repeated measurements ANOVA were used to analyze significant difference between groups for the absolute change between the first and the last time interval. P-values smaller than 0.05 were considered significant. All data are shown as mean ± standard error.
4.2 Effect of loading on osteoporotic bone response following implantation

4.2.3 Results

Effect of mechanical loading on bone architecture

Figure 4.7 shows the time evolution of selected parameters representing trabecular and cortical bone structure. During the recovery period following implantation (i.e., week 0 to week 3), similar changes in bone architecture were observed for both loaded and unloaded animals as expected since mechanical loading started only after week 3. During the loading phase (denoted by the grey area), substantial increases in both trabecular and cortical bone volume were observed for the CML group. Specifically, trabecular bone volume fraction (BV/TV) for the CML group increase linearly with a slope of around 5% per week whereas in the CTR group BV/TV showed no significant changes over time (Figure 4.7A). The observed behavior of BV/TV was due to a considerable increase in trabecular thickness (Tb.Th) rather than in trabecular number (Tb.N): in fact Tb.Th increased over time for both groups (45% for CML and 27% for the CTR, week 0 – week 7, p < 0.05, Figure 4.7B) whereas Tb.N decreased by roughly 15% for both groups (week 0 – week 7, p < 0.05, Figure 4.7C). In cortical bone, both cortical thickness (Ct.Th) and cortical area fraction (Ct.Ar/Tt.Ar) increased linearly in CML group but at different speed (Figure 4.7D and 4.7E) yielding final percent difference: 20% and 12% for Ct.Th and Ct.Ar/Tt.Ar, respectively (week 0 – week 7, p < 0.05). In the CTR group Ct.Th also showed an increase with time (8%, week 0 – week 7, p < 0.05) whereas Ct.Ar/Tt.Ar stayed virtually constant. Interestingly, mechanical loading also decreased cortical porosity (Figure 4.7F) as indicated by the 26% decrease (week 0 – week 7, p < 0.05) observed in the CML group which contrasted with the absence of statistically significant variations in the CTR group. Reported in Table 4.3 are the absolute values of architectural parameters relative to whole bone, trabecular and cortical bone at the first (week 0) and the last (week 7) time point. The absolute differences between week 0 and week 7 were higher for the CML group than for the CTR group for the following parameters: trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), cortical thickness (Ct.Th), cortical bone area (Ct.Ar), cortical area fraction (Ct.Ar/Tt.Ar), and cortical porosity (Ct.Po). No significant difference was found between groups in the absolute changes over time for full tissue volume (TV), full length, and trabecular number (Tb.N).
Figure 4.7: Time evolution of bone architectural parameters for the loading (CML) and the control (CTR) group. (A) Trabecular bone volume fraction (BV/TV), (B) Trabecular thickness (Tb.Th), (C) Trabecular number (Tb.N), (D) Cortical thickness (Ct.Th), (E) Cortical area fraction (Ct.Ar/Tt.Ar), (F) Cortical porosity (Ct.Po). * p < 0.05 denoted a significant difference between groups, # p < 0.05 showed significant different comparing the first and the last week. The grey area denotes the loading period.
Effect of loading on osteoporotic bone response following implantation

Table 4.3: Bone architectural parameters at the first (week 0) and the last (week 7) time point for the loading (CML) and the control (CTR) group. * p < 0.05 denoted a significant difference between groups for the absolute difference between week 0 and week 7.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTR</th>
<th></th>
<th>CML</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 7</td>
<td>Week 0</td>
<td>Week 7</td>
</tr>
<tr>
<td><strong>Full bone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV [mm³]</td>
<td>7.340 ± 0.139</td>
<td>8.029 ± 0.145</td>
<td>7.497 ± 0.170</td>
<td>8.179 ± 0.200</td>
</tr>
<tr>
<td>Length [mm]</td>
<td>4.173 ± 0.031</td>
<td>4.268 ± 0.028</td>
<td>4.219 ± 0.032</td>
<td>4.313 ± 0.026</td>
</tr>
<tr>
<td><strong>Trabecular bone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV/TV [%]</td>
<td>10.249 ± 0.515</td>
<td>10.790 ± 0.649</td>
<td>10.256 ± 0.360</td>
<td>13.695 ± 0.499 *</td>
</tr>
<tr>
<td>Tb.Th [mm]</td>
<td>0.063 ± 0.002</td>
<td>0.081 ± 0.004</td>
<td>0.065 ± 0.005</td>
<td>0.093 ± 0.003 *</td>
</tr>
<tr>
<td>Tb.N [1/mm]</td>
<td>2.435 ± 0.056</td>
<td>2.085 ± 0.049</td>
<td>2.327 ± 0.048</td>
<td>1.994 ± 0.031</td>
</tr>
<tr>
<td><strong>Cortical bone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ct.Th [mm]</td>
<td>0.136 ± 0.002</td>
<td>0.148 ± 0.006</td>
<td>0.138 ± 0.001</td>
<td>0.166 ± 0.002 *</td>
</tr>
<tr>
<td>Ct.Ar/Tt.Ar [%]</td>
<td>37.825 ± 0.903</td>
<td>38.612 ± 1.497</td>
<td>38.610 ± 0.406</td>
<td>43.913 ± 0.411 *</td>
</tr>
<tr>
<td>Ct.Po [%]</td>
<td>21.670 ± 0.474</td>
<td>20.892 ± 0.970</td>
<td>21.532 ± 0.223</td>
<td>18.189 ± 0.253 *</td>
</tr>
</tbody>
</table>
Effect of mechanical loading on bone formation and resorption

Trabecular bone

Following implantation, in trabecular bone higher bone formation and lower bone resorption were observed during the loading regime in the CML group compared to the CTR group (Figure 4.8). Firstly, during the recovery period, both groups had very similar bone formation rate (BFR) and bone resorption rate (BRR). The initially high BFR decreased by roughly -32% when going from week 1.5 to week 3 (BFR was 1.85 %/d at week 1.5 and 1.25 %/d at week 3) for both groups. In the subsequent loading stage, BFR in the CML group stayed at around 1.25 %/d during the first three weeks and decreased to 0.95 %/d at the last week. On the contrary, BFR in the CTR group showed an almost linear decrease to a final value of 0.57 %/d (Figure 4.8A). BFR in the CML group was significantly higher (p<0.05) than in the CTR group during the entire loading phase. The higher BFR in the CML group was essentially due to a larger MS as evident when comparing the time evolution of MS (Figure 4.8B) and of MAR (Figure 4.8C). In the CTR group, MS decreased significantly over time from 31% to 22% (Figure 4.8B). Mechanical loading did not affect MAR which decreased over time (from 2.1 μm/d to 1.3 μm/d) for both groups (Figure 4.8C) in a significant manner.

After implant placement and before the beginning of the loading protocol, CTR and CML had also very similar bone resorption rate (BRR, Figure 4.8D). In fact, BRR was initially elevated and reached a peak at week 3 (1.76 %/d for CML and 1.70 %/d for CTR). Subsequently, BRR decreased and, eventually, was 26% lower in the loading group than in the control group. Like mineral apposition rate, also mineral resorption rate (MRR) was not affected by mechanical loading, and it decreased significantly over time from 2.8 μm/d to 1.7 μm/d for both groups (Figure 4.8F). Conversely, eroded surface (ES) showed a loading-dependent behavior with mechanical stimulation being able to reduce it by approximately 18% with respect to the CTR group (Figure 4.8E). Taken together, these results suggested that the load-induced variations in BRR are essentially due to the modifications in ES.

Cortical bone

In cortical bone, higher bone formation and lower bone resorption in the loading stage were also observed in the CML group when compared to the CTR group, but
Figure 4.8: Time evolution of trabecular bone remodeling parameters for the loading (CML) and the control (CTR) group. 

(A) Bone formation rate (BFR), (B) Mineralizing surface (MS), (C) Mineral apposition rate (MAR), (D) Bone resorption rate (BRR), (E) Eroded surface (ES), (F) Mineral resorption rate (MRR). * p < 0.05 denoted a significant difference between groups, # p < 0.05 showed significant difference comparing the first and the last week. The grey area denotes the loading period.
Figure 4.9: Time evolution of cortical bone remodeling parameters for the loading (CML) and the control (CTR) group. (A) Bone formation rate (BFR), (B) Mineralizing surface (MS), (C) Mineral apposition rate (MAR), (D) Bone resorption rate (BRR), (E) Eroded surface (ES), (F) Mineral resorption rate (MRR). * p < 0.05 denoted a significant difference between groups, # p < 0.05 showed significant different comparing the first and the last week. The grey area denotes the loading period.
the differences between the two groups were less pronounced than for trabecular bone (Figure 4.9). Following implantation, BFR was transiently elevated and peaked at week 3 (again with very similar values between the two groups: 0.45 %/d for CML and 0.47 %/d for CTR). After the peak, BFR decreased for both groups, with the value of BFR being significantly higher for the CML group (0.28 %/d) than for the CTR group (0.20 %/d) only at the last week (Figure 4.9A). Similar to trabecular bone, the behavior of BFR in cortical bone was mainly due to time changes in MS but only at later stages, i.e. starting from week 6 (Figure 4.9B and 4.9C).

A transient peak in BRR at week 3 was also present in cortical bone (Figure 4.9D) and again both groups showed, in the following weeks, a decreasing behavior with loading inducing BRR to decrease faster. In analogy to trabecular bone, ES in the cortical compartment was also responsive to mechanical loading (Figure 4.9E) whereas MRR (Figure 4.9F) was not.

**Effects of mechanical loading on bone remodeling and architecture in the peri-implant bone**

After investigating the global mechanoresponsiveness of the implanted bone, we evaluated whether bone was also able to respond to the external mechanical loading in the peri-implant region. Figure 4.10 summarized the bone remodeling behavior and the consequential architectural changes in the peri-implant region of trabecular and cortical bone. Peak shaped curves characterized by initially high remodeling values, distinct peaks always occurring at week 3 (except for BRR in the CTR group which peaked at week 5) and subsequent decreasing trends were observed for both BFR (Figure 4.10A and 4.10D) and BRR (Figure 4.10B and 4.10E) within trabecular as well as cortical bone. Most noticeably, the behavior of the two groups was fairly similar also after the administration of mechanical loading with only minor differences (even tough never statistically significant) especially in BRR within trabecular bone (at week 7 slightly smaller in CML, Figure 4.10B) and BFR in cortical bone (at week 7 slightly higher in CML, Figure 4.10D). These results indicate a reduced ability of the peri-implant bone to respond to external mechanical loading. However, the interplay of BFR and BRR in the CML group was still more favorable than in the CTR group, allowing a larger increase in cortical thickness (Figure 4.10F) and, interestingly, even small differences in bone formation and bone resorption rates could be reflected into larger variations in bone structure. This is
Figure 4.10: Bone remodeling and the consequential architectural changes in the peri-implant region for the loading (CML) and the control (CTR) group. (A) Trabecular BFR, (B) Trabecular BRR, (C) BV/TV, (D) Cortical BFR, (E) Cortical BRR, (F) Ct.Th. * p < 0.05 denoted a significant difference between groups, # p < 0.05 showed significant different comparing the first and the last week. The grey area denotes the loading period.
4.2 Effect of loading on osteoporotic bone response following implantation

Figure 4.11: Mechanical regulation in the loading group (CML): percentage difference of mean SED at formation and resorption sites compared to the mean quiescent SED in (A) trabecular and (B) cortical bone, Conditional probability of formation and resorption in (C) trabecular and (D) cortical bone.

most obvious when looking at the significant increase in cortical thickness (Figure 4.10F) and at the “prevention” of loss in trabecular bone volume fraction (Figure 4.10C).

**Mechanical regulation of bone remodeling**

To further investigate the mechanical control of bone remodeling in the implanted bone, we correlated the local surface remodeling events with the mechanical environment in the whole trabecular bone as well as cortical bone. In loaded mice,
Table 4.4: Coefficients of the conditional probability curves for formation and resorption in Figure 4.11.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Trabecular</th>
<th>Cortical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation: ( F = y_0 + a \cdot (1 - \exp(-b \cdot \text{SED}/\text{SEDMAX})) )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( y_0 )</td>
<td>27.710</td>
<td>23.158</td>
</tr>
<tr>
<td>( a )</td>
<td>32.166</td>
<td>23.169</td>
</tr>
<tr>
<td>( b )</td>
<td>0.049</td>
<td>0.122</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.948</td>
<td>0.955</td>
</tr>
</tbody>
</table>

| Resorption: \( R = y_0 + a \cdot \exp(-b \cdot \text{SED}/\text{SEDMAX}) \) |            |          |
| \( y_0 \)   | 19.419     | 28.194   |
| \( a \)     | 45.628     | 31.733   |
| \( b \)     | 0.448      | 0.672    |
| \( R^2 \)   | 0.907      | 0.816    |

Mechanical regulation of the bone remodeling process within the trabecular compartment was evident (Figure 4.11A): mean SED at formation sites was significantly higher than mean SED at quiescent sites and, at the same time, mean SED at resorption sites was significantly lower than mean SED at quiescent sites. The corresponding conditional probabilities of remodeling events within the trabecular compartment (Figure 4.11C) allowed to characterized the details of the mechanical regulation which can be summarized in the following two essential features: bone resorption was more strictly controlled than bone formation as the variation of the resorption probability was steeper with SED (indicating by the higher coefficient \( b \) inside the exponent for resorption probability, Table 4.4), and the presence of some remodeling events not directly mechanically regulated (19% of the total resorption events and 30% of the total formation events, indicating by the coefficient \( y_0 \), Table 4.4). Mechanical regulation within the cortical compartment was less evident, in line with the smaller effect on the remodeling behavior. There, in the CML group, mean SED at formation sites was significantly higher than mean SED at quiescent sites; however, no significant differences were found between mean SED at quiescent and resorption sites (Figure 4.11B). The corresponding remodeling probabilities within the cortical compartment (Figure 4.11D) indicated an increased amount of non-targeted bone resorption (28% of the total resorption events, Table 4.4) and a slight decrease in non-targeted bone formation (23% of the total formation events, Table 4.4) compared to the trabecular bone.
4.2 Effect of loading on osteoporotic bone response following implantation

**Whole bone strength**

Figure 4.12 shows the image-based micro-FE simulations used to compute the time behavior of whole bone strength. Following implantation, the whole bone strength showed a slight increase at week 1.5 and then decreased at week 3 ($p > 0.05$) for both groups (Figure 4.12B). In the loading regime, the whole bone strength increased 20% over time for the CML group and was significantly higher in the entire loading regime than for the CTR group, where the whole bone strength did not show significant change over time (Figure 4.12B). Correlations were found for the CML group between changes in the whole bone strength and changes in BV/TV (Figure 4.12C, $R = 0.91$, $P < 0.01$), as well as between changes in the whole bone strength and changes in Ct.Th (Figure 4.12D, $R = 0.85$, $P < 0.05$).

### 4.2.4 Discussion

The presented study evaluated the mechanoresponsiveness of bone in the presence of an implant and demonstrated a locally reduced mechanoresponsiveness within the peri-implant bone which, however, did not compromise the global mechanoresponsiveness of the whole implanted bone. In fact, although mechanical stimuli are known to be anabolic and have therefore been used as physical therapy to promote bone strength [13], the reaction of damaged or implanted bone to loading may be different than intact bone [37], moreover the effects of loading depend on the type of bone and location of the damage [38]. Therefore, optimization of the loading protocols to facilitate fracture as well as peri-implant healing requires better understanding on the influence of mechanical loading not only on the whole bone but also on the peri-implant region.

Mechanical loading increased bone formation rate and decreased bone resorption rate in both trabecular and cortical compartment by modulating the bone surface under formation or resorption, while the average thickness of the formed bone packets and the average resorption depth were not altered. These findings are in agreement with previous studies, where trabecular bone in intact caudal vertebrae [14] and cortical bone in intact tibiae [10] were investigated using the same mouse strain. Comparing to intact bone, bone remodeling following implantation was characterized by transiently elevated remodeling rates which showed a peak three weeks after implant insertion and then decreased. Mechanical loading slowed the
Chapter 4 Bone response following implantation in disease and treatment

Figure 4.12: Micro-FE analysis of whole bone strength for the loading (CML) and the control (CTR) group. (A) Illustration of axial loading applied on the caudal vertebra through virtual intervertebral disks, and simulated SED distribution on the cross-section of the vertebra. (B) Whole bone strength over time. (C) Correlation between changes in whole bone strength and changes in trabecular bone volume fraction ($BV/TV$) for the CML group. (D) Correlation between changes in whole bone strength and changes in cortical thickness ($Ct.Th$) for the CML group. * $p < 0.05$ denoted a significant difference between groups; # $p < 0.05$ showed significant different comparing the first and the last week; the grey area denotes the loading stage.
4.2 Effect of loading on osteoporotic bone response following implantation

decreasing trend of BFR and accelerated the decrease of BRR, resulting in higher BFR and lower BRR for the loading group compared to the control group. By relating MS with recruitment of new osteoblasts and MAR with osteoblastic activity, the load-induced bone formation seems to be initiated by the enhanced osteoblastic differentiation. Similarly, by relating ES with differentiation of osteoclasts and MRR with osteoclastic activity, the decreased bone resorption under loading can be attributed to an inhibition of osteoclastic differentiation. These results are in agreement with in vitro studies investigating the cellular mechanism of load-induced bone cell activities: mechanical stimuli directly up-regulated osteoblastic differentiation while decreasing osteoclastogenesis-associated key soluble factors [22, 39] and increasing secretion of osteoprotegerin, an osteoclastogenesis inhibitor [40], thereby leading to a net down-regulation of osteoclastic differentiation.

In agreement with the increased BFR and decreased BRR caused by mechanical loading, more bone was formed and less bone was resorbed in both trabecular and cortical area as shown qualitatively in the three dimensional registered images (Figure 4.13); moreover, quantitative results showed net bone gain in both trabecular and cortical bone subjected to load. Trabecular bone volume fraction (BV/TV) for the CML group showed a linear increase after loading started, while for the CTR group BV/TV did not change over time. In the peri-implant bone, mechanical loading triggered an increased in cortical thickness and prevented a loss of trabecular bone volume. These two structural changes would be beneficial for the mechanical stability of the bone-implant system [41, 42].

Intact bone has been shown to adapt to loading: load-induced increase in bone volume fraction gradually reduced the strain signal in bone (subjected to the same load), and thereby reduced the effect of loading on remodeling rates [15]. In this study, along with the changes in bone architecture, adaptation to load was also observed: the load-induced higher BFR dropped by the end of the loading stage; the decreasing trend of BRR caused by loading slowed down after two weeks of loading. Nevertheless, by the end of the four-week loading phase, BFR was still higher and BRR was still lower for the CML group than for the CTR group (except for BRR in cortical bone), indicating that more time was needed to accomplish the load adaptation process.

The mechanical regulation of bone remodeling around the implant was shown by linking the occurrence of the remodeling events to local mechanical stimuli
Figure 4.13: Three dimensional visualization of bone formation and bone resorption sites over time of representative mouse for the control (CTR) and the loading (CML) group.
4.2 Effect of loading on osteoporotic bone response following implantation

computed in the loading mice. The main finding of this analysis was that bone formation and bone resorption were mechanically regulated in the trabecular compartment whereas bone resorption showed less regulation in the cortical compartment. This could be explained by the possible higher amount of microdamage induced by implant placement in cortical bone which needs to be removed before the physiological mechanical control could take over.

Image-based micro-FE analysis showed that whole bone strength decreased slightly (p > 0.05) 3 weeks after implantation for both groups, which could be linked to the small drop in BV/TV plausibly attributed by remodeling (resorption) of the fast formed bone in the healing site [43]. In the loading phase, whole bone strength for the CML group was increased by 20%. The increase in whole bone strength for the CML group was due to both an increasing BV/TV (R = 0.91, p < 0.01) as well as Ct.Th (R = 0.85, P < 0.05). In a previous study using intact caudal vertebrae of the same mouse strain, however, correlation was only found for changes in BV/TV but not for changes in Ct.Th [14]. This suggested the importance of cortical bone in implant anchorage: cortical bone allows higher contact surface compared to the implant than trabecular bone [12, 44], and plays a major role in the mechanical stability of the bone-implant system [42, 45]. This is especially true in the peri-implant region, where bone and implant have direct contact. In this study peri-implant Ct.Th was significantly elevated while peri-implant BV/TV was not changed under mechanical loading, indicating that the loading regime was able to efficiently improve the bone-implant stability by modulating the most critical component, cortical bone.

There were some limitations in this study which should be considered. First of all, the three weeks of recovery period before administration of loading was selected based on previous studies on similar mice [14, 28, 43]. Nevertheless, optimization of the loading protocol requires the choice of a suitable starting point: if loading starts before bone-implant integration micromotion may be induced at the bone-implant interface triggering bone resorption [46, 47] as well as the formation of a weak fibrous tissue layer [17, 18], whereas the therapeutic effect of loading may be compromised if loading is inadequately delayed [48]. Therefore, the starting point of the loading protocol used in this study may be further optimized to achieve premium outcome. Secondly, in the first three weeks the remodeling parameters were calculated in a time interval of 1.5 weeks, while in the loading phase a time
interval of two weeks was used. As the calculated remodeling rates are dependent on the chosen time interval, this could lead to an underestimation of the rates: if at a site bone was resorbed and then formed (or was formed and then resorbed) within the given time interval, no change would be detected and thereby this remodeling behavior could not be recorded. Nevertheless, comparisons between groups were done within the same time intervals and therefore the influences of the chosen time interval were reduced to the least extent. One additional limitation is that we did not directly compute the strength of the bone-implant system. This could be done either with biomechanical experimental testing at the last time point or by micro-FE simulations [49]. However a preliminary validation study should be performed before using the latter strategy. In conclusion, we gained insights into the load-induce bone changes around an implant and its mechanical regulation on the tissue level. The knowledge obtained in this study help in optimizing the loading protocols in treatment regimens to facilitate implant anchorage in osteoporotic fracture fixation.

Acknowledgements

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References

4.2 Effect of loading on osteoporotic bone response following implantation


Chapter 4 Bone response following implantation in disease and treatment


[34] C. Flaig, P. Arbenz. A scalable memory efficient multigrid solver for micro-


4.2 Effect of loading on osteoporotic bone response following implantation


Chapter 5

Synthesis
Background

Osteoporosis is prevalent among the elderly population and often causes devastating bone fractures, which requires long-term fixation with implants in order to restore the function of the fractured site [1]. The long-term success of fracture fixation greatly depends on the early bone response following implantation, which regenerates the bone tissue at the implantation site to ultimately attain implant integration into the host bone. However, the poor bone quality and the compromised systemic conditions caused by osteoporosis may impair the early implant integration and jeopardize the long-term stability of the bone-implant system [2].

The bone remodeling process is a critical player in the complex biological response of bone following implant placement. During bone remodeling, in the peri-implant bone, damaged bone tissue is resorbed by osteoclasts and new bone is locally laid down by osteoblasts, leading to an adaptation in mass and structure of the peri-implant bone which is eventually responsible for the implant integration [3]. Despite its importance, the process of bone remodeling following implantation and the consequential modifications in bone architecture remain poorly understood. Moreover, bone remodeling is mechanically controlled with the occurrence of bone formation being higher at sites with high strains and bone resorption being dominant at sites less strained [4]. Therefore, controlled external mechanical loading has been proposed as a therapy to tune the process of bone remodeling in order to favor bone formation with the ultimate goal of enhancing implant integration [5, 6]. However, in order to develop effective loading protocols for osteoporotic fracture fixation, a more fundamental understanding of the interplay between mechanical loading and bone formation/resorption at the local tissue level in the peri-implant bone is mandatory.

The introduction of in vivo micro-computed tomography (micro-CT) and the latest development on image registration techniques provided access not only to the time evolution of changes in bone architecture, but also to the local identification and quantification of bone remodeling over time, including bone formation and bone resorption [7-9]. However, the application of these techniques for the investigation of bone response around metallic implants used in clinics is limited by the marked image artifacts caused by the implant [10].
Chapter 5 Synthesis

In this context, the main aim of this thesis was to develop a method allowing *in vivo* monitoring of the spatio-temporal changes in living bone following implantation, including bone remodeling and bone architecture. The method was then employed to characterize the response of osteoporotic bone and to answer the clinically relevant question whether mechanical loading could be used to improve peri-implant bone regeneration in osteoporotic bone.

**Novel method for understanding bone response following implantation**

The main achievement of Chapter 3 was the establishment of a novel *in vivo* monitoring technique to investigate the bone response following implantation over time using *in vivo* micro-CT imaging combined with a mouse model. Although *in vivo* micro-CT has been used to measure the architecture of bone around metal implants [10, 11], the image quality was greatly reduced by the image artifacts caused by the metallic implant material [10]. Those artifacts can even be amplified by several factors of the *in vivo* scan: the radiation dose has to be limited; implant orientation relative to the X-ray beam is difficult to optimize; animal movement might occur during the scan. To cope with those artifacts a fairly large region around the implant is excluded from the quantitative analysis [12]; however, the bone response in this region is crucial for understanding implant integration and consequently, mechanical stability [2]. In the present thesis, the use of the low-density high stiffness composite material implants effectively eliminates the image artifacts; moreover, thanks to the high stiffness of the composite implant, the implantation procedure was adapted from a well-developed pinning protocol at the Institute for Biomechanics, which was specifically designed for inserting small implants into mouse vertebrae [13]; additionally, the titanium coating on the surface of the implant enhanced biocompatibility. A detailed analysis of bone architecture and bone remodeling was conducted in both cortical and trabecular bone, which were further divided into a cylindrical peri-implant region close to the implant and a distant region far from the implant. An *ex vivo* reproducibility study showed that both bone architecture and bone remodeling assessed by the proposed technique were highly reproducible in both peri-implant and distant regions, given that the image registration was done for *in vivo* scans within two-week time intervals. By using this technique, it was possible to assess the interplay between bone resorption and bone formation following implantation in both cortical and trabecular bone, and further associate the remodeling behavior with the spatio-temporal changes of bone architecture in each
animal, which was not possible using conventional histomorphometric analysis [14]. After implant placement, a quick and substantial thickening of the cortical shell was observed in the peri-implant region, which was a result of the transiently high bone formation rate in this region; in trabecular bone, however, a persistent loss was found especially close to the implant due to the continuous elevated bone resorption rate. Additionally, by comparing bone remodeling following implantation with the remodeling behavior measured in previous studies on age-matched mice [7, 15], we found that the effect of the implant on bone formation was mainly localized within the peri-implant bone whereas on bone resorption it could extend over the entire vertebra.

**New insight into bone response following implantation in osteoporosis**

The major finding of *Chapter 4.1* was the quantitative description of the spatio-temporal changes in bone remodeling and bone architecture following implantation in an osteoporotic scenario. Although it has been shown that implant integration is impaired by osteoporotic conditions including reduced bone mass [16, 17] and low bone mineral density [18], more fundamental understanding on the influence of osteoporosis on the bone regeneration process following implantation should lead to safe and efficient treatments for osteoporotic fracture fixation. With this in mind, we investigated the bone response following implantation in a mouse model mimicking the estrogen depleted bone deterioration in osteoporosis [19] by using the *in vivo* monitoring technique developed in *Chapter 3*. The investigation was performed at three different locations within the same bone: the trabecular surface, the endocortical surface and the periosteal surface of cortical bone. Following implantation, an accelerating bone forming phase was observed mainly in the peri-implant endocortical bone, leading to an increase in cortical thickness close to the implant in both estrogen depleted and healthy control mice. Contrarily, trabecular bone experienced a decrease in the entire area with the reduction in healthy control mice being higher than in estrogen depleted mice. These results indicated that the accelerating bone forming phase and the consequential increase in the thickness of peri-implant cortical bone were responsible for the implant integration and the stability of the bone-implant system. This was in agreement with a previous study which elucidated the critical role of cortical bone in stabilizing the bone-implant construct using pull-out simulations [20]. Moreover, we observed an impaired integration process in osteoporotic bone with both the bone formation and the
increase in cortical thickness being limited, leading to a reduced bone-implant contact compared to healthy bone, whereas it was previously reported that the remodeling process following implantation was similar in healthy and osteoporotic rats by using dynamic histomorphometry [14]. Our study showed advantages by acquiring time-lapsed measurements on the same animal using the in vivo monitoring technique, which greatly reduced the influence due to biological variation between individual animals and therefore were more sensitive in detecting changes over time.

Understanding load-induced changes in bone response following implantation

The major finding of Chapter 4.2 was the combined anabolic (increased bone formation) and antiresorptive (decreased bone resorption) effect of controlled external mechanical loading on osteoporotic bone in the presence of an implant, in both trabecular and cortical bone. Moreover, these load-induced changes in bone remodeling around an implant were found to be associated with the bone surface under formation or resorption, but not with the thickness of the formed bone packets or the resorption depth. As a result of the changes in bone remodeling, both trabecular bone volume fraction and cortical thickness were significantly increased under loading, leading to the enhanced strength of the whole bone-implant construct. Furthermore, we observed a locally reduced mechanoresponsiveness in the peri-implant bone as no statistically significant difference in the remodeling rates was detected between loaded and control animals. Nevertheless, the global mechanoresponsiveness of the whole implanted bone was not compromised and the load-induced changes in bone remodeling, bone architecture and whole bone strength suggested corresponding enhancements in the integration and stability of the implant. To our knowledge, this is the first time the detailed process of load-induced bone remodeling was characterized around a metallic implant, both in the peri-implant bone and at the whole bone level. Indeed it has been shown that intact bone responded to external mechanical loading by a combination of increased bone formation and decreased bone resorption, resulting in a net increase of bone mass [4, 7]. However, in the complex context of peri-implant bone regeneration [21], only one previous study reported a net increase of bone at the bone-implant interface when compressive vibrational loading was applied to the implanted bone [22]. The obtained knowledge in load-induced bone response following implantation in our study may guide the development of novel loading treatments for enhancing implant integration in osteoporotic fracture fixation.
**Limitations and future research**

Firstly, we used special implants which are different from standard endosseous implants used in clinics. Therefore, it is not clear whether they induce the same peri-implant bone response as steel or titanium implants widely used in the clinical setting. Nevertheless, the elastic modulus of the implant material was similar to that of titanium and the implant surface was coated with a biocompatible titanium-based layer. In order to better simulate the clinical reality, screw implants may be used in future studies, as it has been shown that optimized thread design can enhance bone-to-implant contact as required for the low bone quality conditions [23]. However, the implantation procedure and the segmentation method to separate bone and implant in image processing would need to be adjusted and verified due to the changes in the implant shape.

Considering the monitoring process, one limitation is the dependency of the remodeling parameters on the chosen time interval for measuring the remodeling process. On one hand, the chosen time interval should be sufficiently large for producing reproducible outcomes due to the limited resolution of the *in vivo* micro-CT; on the other hand, when the remodeling rates are calculated in a large time interval, more intermediate remodeling events cannot be captured, leading to an underestimation of the rates. In our studies, reproducible outcomes were achieved by using a two-week time interval [21]; nevertheless, *in vivo* scans were taken weekly to detect the changes in bone architecture which may be quite fast especially close to the implant.

For the chosen osteoporotic animal model, the estrogen depleted mouse caudal vertebra may not represent the osteoporotic conditions in human bone. Firstly, the caudal vertebrae still show growth in adult mice as the epiphysis do not fully close, while human bones stop growing in early adulthood. Secondly, mice lack a well-developed Haversian remodeling system in the skeleton, which is the main cause of the increased cortical porosity in osteoporotic patients [24]. Nevertheless, mouse model shows advantages here because of the inbred strain which limits biological variation and thereby reduces the required number of animals in the experiments. Moreover, the choice of mouse caudal vertebrae as implantation site enabled us to image the full bone including both cortical and trabecular compartments within a reasonable scanning time.

Finally, the main limitation of the loading protocol used in *Chapter 4.2* was the lack
of optimization in the starting point of the loading regime. Here, the chosen three weeks of recovery period before the loading regime were based on previous experiences in our group on intact caudal vertebrae (i.e., without the implant) of the same mouse strain [7]. However, it has been shown that fractured bone and intact bone responded to load differently [25]. In the presence of an implant, loading starting before implant integration may induce micromotion at the bone-implant interface which initiates bone resorption [26, 27], whereas the therapeutic effect of loading may be compromised if loading is inadequately delayed [28]. Therefore, although the current loading protocol clearly showed both anabolic and antiresorptive effects, the starting point of the loading regime can be further optimized in order to achieve favorable outcome.

Conclusions

In conclusion, the present thesis has led to a deeper understanding of the precise role of bone remodeling and mechanical loading in the bone regeneration process following implant placement, which is essential for the development of effective treatments for osteoporotic fracture fixation. The thesis comprises three important achievements. Firstly, a novel in vivo approach for monitoring and quantifying the time evolution of bone response following implantation, including the detailed process of bone remodeling and the consequential changes in bone architecture. Secondly, the novel approach was employed to assess the spatio-temporal changes in bone response following implant placement in an osteoporotic scenario, within three different locations of the same bone including trabecular bone, endocortical and periosteal surface of cortical bone. Thirdly, controlled external mechanical loading was demonstrated to be beneficial for the bone response following implantation in an osteoporotic scenario by increasing bone formation and decreasing bone resorption; additionally, the detailed characterization of the local mechanical environment gave new insights in the relationship between bone remodeling and mechanical strains in the presence of the implant.

References


