In vivo and ex vivo µCT evaluations of biodegradable poly(3-hydroxybutyrate) implants in a rat model

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Graz, April 2014 *Gustav Schmöller*

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Abstract

Introduction: Biodegradable materials have first been introduced to medicine in the form of resorbable sutures and small fixation devices. By now, a widespread range of materials enables even complex and heavy load applications. The advantages of biodegradable materials are obvious: reduction of secondary surgeries, avoidance of common complications known from conventional metallic devices and potential for optimization for special applications, i.e. pediatric fracture fixation.

At present, biodegradable materials can be divided into polymers, metals and ceramics, of which polymers are the most versatile group due to the great variety of materials and diverse mechanical and chemical properties. Polyhydroxyalcanoates and poly(3-hydroxybutyrate) (PHB) in special belong with the most promising polymeric materials available for biodegradable applications in medicine today.

Methods, materials and animals: Three blends of PHB, featuring varying amounts of the heavy metal zirkonium and the resorbable bone substitute Herafill® were established and shaped as cylindric pins (1,6mm diameter, 8mm length). These pins were transcortically implanted in 5 week old Sprague-Dawley rats´ femora and their surface and volume monitored in vivo over a 9 month period using micro computed tomography (μCT). Several explanted pins were also scanned using μCT in an ex vivo setting for validation reasons.

Results: Due to 3D-processing issues of in vivo μCT scans, only ex vivo scans produced validly quantifiable data. Though, in vivo scans showed no obvious signs of degradation even after 9 months of dwelling time, which was confirmed by ex vivo evaluations. Actually, both volume and surface of the pins had even increased in relation to calculated preimplantational values and degradational behavior was thus denied.

Discussion: PHB already possesses good properties for pediatric osteosynthesis that can even be enhanced by techniques such as blending. Still, important factors must be taken into account, i.e. slow degradation, sterilization issues, general biocompatibility and fibrous encapsulation of implants. For in vivo evaluations of degradable implants, μCT has proven to deliver adequate results, though the used composites presented density properties too similar to those of bone to be certainly differentiated from surrounding tissue in vivo. Hence, only ex vivo scans could be regarded as valid data. Also, some pins appeared to be inhomogeneously blended, yet this had no effect on degradation properties.

Conclusion: The exceptionally slow degradation of all three blends of PHB cannot meet the requirements modern osteosynthesis puts on a biodegradable implant in a growing skeleton. Hence, without major progress in material sciences and enhancement of degradation properties, PHB cannot be recommended for the use in biodegradable pediatric fracture fixation devices

Zusammenfassung

Einleitung: Biodegradierbare Materialien wurden in der Medizin erstmals in Form resorbierbaren Nahtmaterials und kleiner Fixierungsutensilien eingesetzt. Derzeit ermöglicht eine große Auswahl an verschiedenen Materialien selbst komplexe Anwendungen unter großer Belastung. Die Vorzüge biodegradierbarer Materialien sind offensichtlich: eine Reduzierung von Sekundäreingriffen, die Vermeidung von Komplikationen konventioneller Implantate aus Metall und das Potential zur optimierten Anpassung an besondere Anwendungen wie etwa die operative Frakturversorgung in der Pädiatrie.

Zum jetzigen Zeitpunkt lassen sich biodegradierbare Materialien in Polymere, keramische Werkstoffe und Metalle unterteilen, wobei Polymere aufgrund ihrer großen Vielfalt und dementsprechend unterschiedlichster chemischer und mechanischer Eigenschaften die vielseitigste Gruppe darstellen. Polyhydroxyalkanoate und Poly(3-Hydroxybutyrat) im Speziellen gehören dabei zu den vielversprechendsten polymerischen Materialien die heutzutage für medizinische Anwendungen verfügbar sind.

Methoden, Materialien und Versuchstiere: Es wurden drei Verbundstoffe auf der Basis von PHB, mit dem Schwermetall Zirkonium und dem abbaubaren Knochenersatzstoff Herafill® in unterschiedlichen Gewichtsanteilen, hergestellt und als zylindrische Pins mit einem Durchmesser von 1,6 mm und einer Länge von 8mm geformt. Diese Pins wurden 5 Wochen alten Sprague-Dawley Ratten transkortikal in beide Femora implantiert und ihre Oberfläche und ihr Volumen in vivo über einen Zeitraum von 9 Monaten mit einem Mikro Computertomographen (µCT) kontrolliert. Einige explantierte Pins wurden zu Validierungszwecken auch in einer ex vivo Konfiguration gescannt.

Resultate: Aufgrund von Problemen bei der 3D-Aufbereitung der in vivo µCT Scans konnten nur aus den ex vivo Scans valide messbare Daten gewonnen werden. Dennoch zeigten schon die in vivo Scans selbst nach 9 Monaten Verweildauer keine offensichtlichen Degradationsmerkmale, was durch die ex vivo Auswertungen nur bestätigt wurde. Tatsächliche waren sowohl Volumen als auch Oberfläche der Pins in Relation zu den prae implantationem errechneten Werten angestiegen und ein Abbauverhalten damit ausgeschlossen.

Diskussion: PHB selbst besitzt schon gute Eigenschaften für die Osteosynthese bei Kindern, diese können durch Techniken wie das Blenden mit anderen Materialien sogar

noch verbessert werden. Jedoch müssen auch wichtige Faktoren berücksichtigt werden, so etwa die langsame Degradation, Sterilisierungsprobleme, die generelle Biokompatibilität und fibröse Enkapsulation der Implantate. Für die in vivo Bewertung von degradierbaren Implantaten hat sich das µCT als geeignet erwiesen, die verwendeten Verbundstoffe wiesen jedoch Dichteeigenschaften auf, die so eng an denen von Knochen liegen, dass eine sichere Unterscheidung zu umgebendem Gewebe in nicht gewährleistet werden konnte. Deswegen konnten nur Daten aus ex vivo Untersuchungen als valide Ergebnisse betrachtet werden. Darüber hinaus erschienen einige Implantate inhomogen im Sinne der Materialvermischung, was jedoch keinen Einfluss auf die Degradationseigenschaften hatte.
Conclusio: Die außergewöhnlich langsame Degradation aller drei Verbundstoffe kann den hohen Anforderungen der modern Osteosynthese an biodegradierbare Implantate im wachsenden Skelett nicht gerecht werden. Ohne eindeutigen Fortschritt in der Materialforschung und ohne Verbesserung des Degradationsverhaltens kann PHB also nicht für die Verwendung in der pädiatrischen Frakturversorgung empfohlen werden.

Inhaltsverzeichnis

1 Introduction

Implants of all types – ranging from minimally invasive pins over versatile screws and plates to large intramedullary rods – belong to the most important medical devices in modern orthopedic surgery. Due to their superior mechanical properties and a lack of utilizable alternatives, up-to-date implants are most commonly made from metallic alloys containing stainless steel or titanium as their main component.

On the one hand, stainless steel is distinguished by its low price, availability, fabrication properties, biocompatibility and strength. Titanium on the other hand features an even higher strength per density, corrosion resistance, inertness to biologic environment, enhanced biocompatibility, a more moderate elastic modulus and a high rate of integrity with the bone, though its costs exceed those of steel by far (1). Other than most materials, titanium does not contain calcium phosphate and has been shown to calcify in vitro, its osteoinductive properties being described to cause difficulties in implant removal. Both steel and titanium are known to release substances considered as toxic or at least questionably tolerable to the human body, i.e. nickel, aluminum and vanadium (1). Furthermore, steel shows only limited resistance to microbial attack and titanium features lower frictional strength often causing micro abrasion, so both materials may lead to host inflammatory reactions at times. For this reason, surface modifications as coating or topographic alteration are estimated to further ameliorate biocompatibility issues in both materials (1).

As general disadvantages, metallic devices interfere with radiologic imaging techniques and offer potential for migration, breakage, implant loosening, long term physiological reaction or growth restriction. Most importantly, metallic implants usually have a higher elastic modulus, the *Young´s modulus*, and therefore are stiffer than bone. Unfortunately, this characteristic feature may cause "stress shielding" – the implant takes all load off the bone and decreases the stimulus needed for accurate healing to a minimum – and result in weakening or resorption of the bone, implant loosening and even refracture after implant removal. One possible way to avoid stress shielding is lowering the Young´s modulus of conventional materials by alloying (1-4).

Material	Young’s modulus (GPa)	Fracture toughness (MPa)
human cortical bone	3-20	3-6
stainless steel	190	50-200
titanium and Ti alloys	110-116	55-115

Table 1: mechanical properties of conventional materials for implants (1)

At present, adapted metallic implants are in use for osteosynthesis in pediatrics, though children must not be understood as “small adults”. The ongoing growth of bone describes the main issue in pediatric fracture fixation, for which reason pediatric implants and the whole therapeutic setting have to meet requirements that can be neglected in adults. For one part, the healing process happens faster in a growing skeleton, so implants require constant monitoring and sooner explantation than in adults. For another part, ossification of bones takes place during childhood and mechanical properties of the bone strongly depend on the biological age of the child. Finally, as long as epiphyseal closure has not yet taken place, fractures and other irritations – i.e. surgery and improperly used implants – may irreversibly damage growing bone and result in growth irregularities. (5, 6)

Fracture healing in growing bone is understood to happen in three phases: (i) the inflammatory phase takes place as a reaction to fracture associated haematoma; from a fibrinous basis a collageneous net builds a basic structure and essential proteins for the formation of new bone are produced; (ii) during the reparative phase that lasts several weeks, mechanically inferior callous forms from both enchondral and intramembraneous ossification and bridges the gap in the fractured bone; (iii) finally, the remodeling phase can last months or even years, during which callous is replaced by fully adequate bone. The final result of healing and remodeling of the bone mainly depends on location of the fracture, proximity to the joint, three-dimensional orientation of the fragments and skeletal age (7).

All of these reasons, besides the parameters shown in [figure 1], must be taken into account to prevent complications when pediatric fractures necessarily indicate surgical treatment.

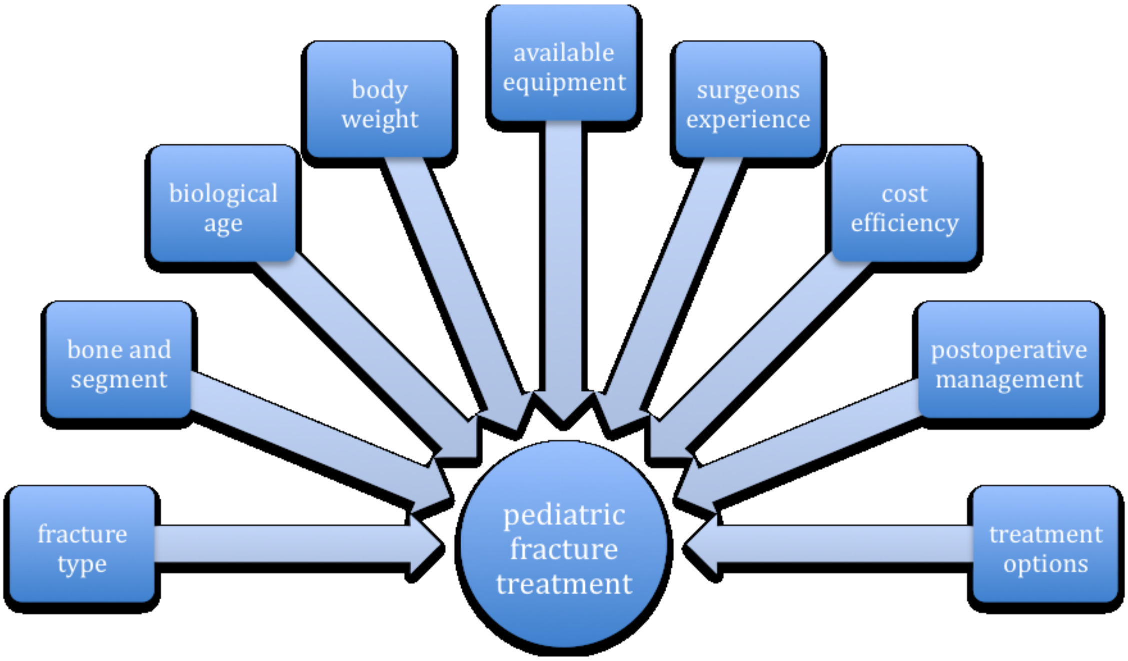

Figure 1: Important parameters for treatment of fractures during childhood (8)

Common implants usually have to be explanted after fulfilling their medical use and therefore cause further physical and mental stress especially in young patients through anesthesia, invasive procedures, immobilization and hospitalization. As every surgery involves high costs, reducing the number of surgeries also represents an important health care issue. With the rise of the chemical industry during early twentieth century, biodegradable materials emerged and first made their way into surgery in the form of resorbable sutures. Soon the idea emerged to replace metallic devices with biodegradable devices that could meet the exact needs and hence reduce common complications and secondary surgeries for implant removal (9).

To successfully use biodegradable materials for fracture fixation in children, specific demands must be met. Firstly, a replaced fracture essentially requires sufficient stability over the whole period of fracture healing to guarantee optimal fixation of all fragments. Secondly, the material must by all means offer optimal biocompatibility features. And thirdly, the device has to allow for gradual load transfer and regeneration of the bone while granting full degradation over a period of 12 to 15 months (10).

Various biodegradable materials with metallic, ceramic or polymeric character offer numerous new possibilities and display a great variety of chemical and physical properties. This leads to the assumption, that each single orthopedic requirement could be fulfilled by

an optimized degradable material while providing complete resorption and consequently minimizing need for secondary surgical intervention. Hence, the thought is obvious, that the specific requirements of pediatric implants – such as grade of stiffness, gradual load transfer and biocompatibility – can be met most precise by biodegradable materials.
While in the wide group of polymeric materials polyglycolic acid (PGA), polylactic acid (PLA) and their subgroups and co-polymers have initially attracted the biggest interest, over the last decades the focus has shifted towards more versatile chemical families like the polyhydroxyalkanoates (PHA) (11).

As the best characterized representative of PHA, poly(3-hydroxybutyrate) (PHB) shows promising potential for the use as biodegradable material in pediatric orthopedics. Not only does PHB show convincing thermoplastic and mechanical properties similar to conventional plastics and elastomers, but it also undergoes full degradation without leaving toxic or harmful remnants. The natural occurrence of 3-hydroxybutyrate and its oligo- and polymers in human blood and tissues only augments the high biocompatibility of PHB. A common technique for further optimization of chemical and mechanical properties is blending PHB with various biodegradable materials like other polymers (i.e. 3-hydroxyvalerate), bioceramics (i.e. calciumsulphate [$CaSO_4$]) or even metals (i.e. magnesium). PHB may also be produced from renewable resources without being dependent from fossil feedstock, though the high production cost outlines the biggest drawback, making PHB far more expensive than conventional plastics at the present time. Nevertheless, recent research results suggest a major decrease in production costs for the near future due to new production techniques and cheaper basic materials (11-13).

For all the above reasons, PHB proves to be a promising material in the development of fully biodegradable devices for osteosynthesis in children and therefore is the material of interest in the present study.

1.1 Biodegradable polymeric implants

The idea of self-resorbing devices for internal fixation in medicine first came up in the 1960ies, when Kulkarni et al described polylactic acid and its properties, stating that it is

„…*a highly interesting product as a synthetic surgical repair material…*" (9). Over the following decade, basic research for application in humans took place and an important group around Scandinavian researchers Rokkanen and Böstman was formed. They were the first to experimentally fix ankle fractures using polylactide-glycolide copolymer implants and released first results in 1985 (14). For almost 30 years now, this group has continued research on the topic of biodegradable materials for medical applications and has published important studies almost annually.

During the late 80ies and early 90ies, Hope et al performed clinical trials on the treatment of displaced elbow fractures of either the lateral condyle, the medial epicondyle or the olecranon in children. They used bioresorbable pins of polyglycolic acid and compared the results to those achieved by using conventional metallic Kirschner wires, showing that PGA (Polyglycolic acid) pins did not lead to complications like infection, soft-tissue-ossification and the need for removal, but therefore were followed in one of 13 cases by avascular necrosis and premature fusion of the epicondyle (15).

During the 1990ies, the use of and research on biodegradable polymers globally intensified, preparing the way for new applications of resorbable implants and devices like pins, screws and cords of biodegradable materials found their way into orthopaedics. On the one hand, Hoffmann reported of indications like disintegration of the acromioclavicular joint, ligamentous lesions of cruciated ligaments in the knee, diverse fractures located close to or in big joints and arthroscopic fixation of osteochondral flakes (16). On the other hand, Pihjalamäki et al published their results of treating small-fragment fractures and osteotomies in 27 adults with self reinforced PLA (polylactic acid) pins (17). Böstman´s study on the economic factors of avoiding implant removal by using absorbable fixation devices concluded that only a slight economic benefit could be achieved at that time, as the removal of metallic implants was and still is not necessary in every case, whereas biodegradable implants usually cause high costs in production (18).

By 2000, Rokkanen et al could look back on a total of 3200 orthopedic patients treated with resorbable devices such as cylindrical rods, screws, tacks, plugs, arrows, and wires. As the most common indication they mentioned the displaced malleolar fracture in adults, while in children the transphyseal fixation with small-diameter polyglycolide pins was performed most commonly (19). In the same year, the Dutch surgeons van der Elst and

Patka described the state of the art in biodegradable fracture fixation, stating that – due to chemical and mechanical limitations – PGA and PLA outlined the most promising materials for internal fixation devices in medicine (20).

Around the turn of the century it was a well known fact, that biodegradable implants may lead to local foreign-body reactions, when Böstman and Pihjalamäki reviewed pre-clinical and in vitro studies as well as their own clinical observations. While in previous studies the incidence of adverse tissue responses to implants made of PGA varied considerably, their own observations showed only rare foreign body reaction in both PGA (5,3%) and PLA (0,2%), depending strongly on the location of injury and implant. (21,22). Also in 2000, the Swiss researcher Gogolewski outlined the meaning of biodegradable materials in trauma and bone surgery, highlighting the need for modification of existing materials concerning production, molecular characteristics and degradation time (23). Middleton and Tipton went one step further and verbalized their demands on the ideal biodegradable material: few to none toxic or inflammatory reaction in the body, complete and traceless metabolisation, acceptable shelf life, easy procession and sterilization. All this should happen in the context of matching mechanical properties and degradation time of the material with the needs of the medical application (24).

By 2001, the range of clinical applications for biodegradable implants had extended, being used most commonly for the fixation of soft tissue in sports medicine, for example arthroscopic fixation of rotator cuff lesions and meniscal repair. Ciccone et al also mentioned acceptable results even for fracture fixation, but stated, that widespread acceptance of biodegradable devices was still limited by concerns about their initial fixation strength (25). Following Gogolewskis criteria for resorbable implants, Slongo saw promising potential for the use in paediatric traumatology due to short healing times, less demand for long-term stability, easy adjuvant immobilization through plaster and reduction of pain and costs (3).

By analyzing the patients' perception of 100 adults treated with resorbable fracture fixation devices, Mittal et al proved, that a demand for resorbable implants existed not only from the professional, but also from the patients´ side of view (26).

Due to recurring reports of side effects and inflammation associated with biodegradable implants, the issue of biocompatibility was highlighted in a review study performed by Nuss and von Rechenberg. Throughout literature they had found the appearing of foreign body giant cells discussed most controversially as an indicator of inflammation and biocompatibility in the implantation site, but they came to the conclusion that "*…every unnatural implant induces a natural foreign body response, thus, it is not the response by itself but its extent and intensity on which the biocompatibility of a medical device is determined. Therefore, any definition of biocompatibility related to a certain material should be based on quantitative evaluation of the cell population surrounding the implant, not just on the types of cells that occur.*". As an ultimate measure of biocompatibility, they suggested the quantification of granuloma and fibrous tissue in the implantation site (27).
In 2011 Amini et al. published collected information on short-term and long-term effects of biodegradable implants used in orthopedics, giving an overview on foreign body reaction (FBR), that – regardless of inertness or non-toxicity – usually is associated with implantation of any biomaterial. As for long-term complications of chronic FBR, they mentioned osteolysis, fibrotic encapsulation and even sarcoma. Although tolerable levels of inflammation are vital to the healing process, chronic inflammatory processes will lead to malfunction of an implant, so they saw the main challenge for biodegradable devices in moderating the long-term FBR. Besides degradation properties of the material, physical properties like wettability and geometry of the implant seem to play a main role in FBR and therefore in the general success of the implant (28).

Many companies are currently involved in the biodegradable material industry and a variety of devices and implants constructed from different materials are commercially available. In orthopedics, biodegradable implants are being used in the form of pins, conventional screws and interference screws, cords, anchors, thermoplastic foils, meniscal reconstruction devices, plates, intramedullary fixation devices and even vascular scaffolds. Their composition and the mode of reinforcement vary according to the application they are intended for. Usually, biodegradable polymers are very rigid when processed in their pure form, so self-reinforcement – one device is composed of multiple, differently shaped and processed parts of the same material – and reinforcement with other materials such as glass, carbon fibre or even other biodegradable materials became a popular way of strengthening biodegradable implants and optimizing them for their intended application (29).

Nowadays, biodegradable devices are most commonly used as low load bearing implants in dental, maxillofacial and orthopedic surgery, for example as pins for fixation of small-fragment-fractures in hand and ankle. Even pediatric neurosurgeons and interventional cardiologists have found advantages in using biodegradable plates and completely degradable, transient vascular scaffolds (30-32).

In their 2008 tutorial for degradable polymers used for osteosynthesis, Swiss researchers Eglin and Alini gave an up-to-date overview of materials in use, their degradation properties, new trends in biodegradable device technologies – polymer coating, bioactive and composite devices – and important issues such as stability and infection. In their eyes, the main reason for preoccupation towards the use of biodegradable devices in daily orthopedic routine is a lack of significant prospective trials documenting their efficacy. Also, they expect that next generation biodegradable materials may rather be bioactive than passive, show better results in vivo and offer potential for new therapies (4).

Currently, the latest version of the *"Standard Guide for Assessment of Absorbable Polymeric Implants"*, composed as standard F2909-12 by the American Society for Testing and Materials (ASTM), is available internationally since 2012. On 13 pages it includes descriptions of possible applications, information on processing and analyses of raw materials and even finished products (33).

Because of the limited range of clinically approved applications and materials and a distinctive skepticism in clinical routine, biodegradable devices cannot meet all current needs and expectations yet. However, hopes may be entertained that intense research can lead to the development of optimized biodegradable, eventually even bioactive devices for almost any kind of medical application.

1.2 Biodegradable materials

Materials intended for internal use in the human body while causing only minimal adverse reactions are called biomaterials. First generation biomaterials, which first emerged during the early 1980ies, were meant to match specific requirements and purposes in living tissue – usually replacement or reinforcement – with their most common feature being inertness. Based on minimal reactivity with their biological surroundings, this means they have to be neither toxic, nor pathogenic in vivo and need to induce only minimal inflammatory response (34). Second generation biomaterials are defined as being either biodegradable or bioactive, which describes their ability to interact with a biological environment in terms of biological response and tissue/surface bonding. Finally, third generation biomaterials are designed to combine both of these characteristics, so that bioactive materials are being made degradable and degradable materials are being made bioactive by means of stimulating specific cellular responses. In all biomaterials, biocompatibility is a main feature for success (34-37). Generally, biomaterials can be subdivided into three main groups: ceramics, metals and polymers (37).

1.2.1 Biodegradable ceramics

Throughout literature, calcium orthophosphates (CaP) represent the most common resorbable ceramic biomaterial, being mainly used as bone substitutes in the form of cements and concretes or as coatings on medical devices for internal application (38-40). As their solubility in water is one of their main characteristics, different kinds of CaP have unique behavior in vivo, but their biodegradability may be very precisely predicted. In general, the solubility of the material compared to the solubility of the bone's mineral part defines its degradation capacities (38).

While low-temperature CaP, gained from aqueous solutions, are mostly used as starting material for high-temperature processing, high-temperature CaP like Hydroxyapatite (HA), β-tricalcium phosphate (TCP) and their composites have been perfectly integrated into clinical routine, mainly in the form of calcium phosphate cements (CPC). For applications requiring faster degradation, α-TCP seems to be better suited. Though, usually not degradation properties, but bioactive qualities – i.e. gradual release of incorporated drugs – determine the use of bioceramics in bone surgery, on this account CPC devices may be found in the implantation site even years after initial surgery. Also, bioceramics as well as conventional ceramics, are known to have poor mechanical properties and therefore seem to be suited as material for load bearing devices only with reservations (38-40).

1.2.2 Biodegradable metals

Metallic alloys have been used as internal fixation devices for many decades. Even though eventual disintegration of implants could be witnessed as undesirable side effect, only recently research on their biodegradable properties has attracted increasing attention. The most promising metallic materials for biodegradable implants are iron (Fe) and magnesium (Mg). As both of them physiologically occur in the human body, their metabolism is well investigated and their biodegradable properties are being examined at the present time (41-46).

While pure iron was found to degrade too slow and its mechanical properties appear unsuitable for internal fixation devices, new design strategies tend to combine Fe with other metals, non-metals and rare earths such as manganese (Mn), carbon (C) and palladium (Pd) (41). Also, alloying with iron's own degradation product iron(III)oxide (Fe_2O_3) seems to have beneficial effects on strength and degradation rate of Fe-based implants (42).

Magnesium, on the other hand, has already been subject to multiple studies on biodegradable implants, the first clinical experiments giving account of its biodegradable properties were already conducted during early 20th century (43). Some of its advantages are its relatively low weight, its high mechanical strength and fracture toughness, an elastic modulus and compressive yield strength similar to that of natural bone, the fast rate of degradation and its osteoconductive properties. Though its pure form is known to degrade too fast for load bearing implants, alloys with manganese (Mn), zinc (Zn), calcium (Ca) and yttrium (Y) have shown better in-vitro and in-vivo results (44, 10). One important issue for the use of magnesium in implants is the changing amount of impurity elements in industrially refined magnesium. Natural composition of extracted magnesium and diverse procedures of casting and refining are the most common reasons for unintended impurities and contamination (45). Another objectionable factor is located in the degradation processes of magnesium: in biologic environments, its corrosion is usually accompanied by extensive production of soluble hydrogen gas, which on the one hand has no deteriorating effects in terms of toxicity or carcinogenesis, but on the other hand may negatively affect structural and mechanical properties of the surrounding tissue (43-49).

More recent studies suggest, that alloys combining both iron and magnesium may lead to the desired properties in mechanical strength and degradation, and even latest technologies, i.e. inkjet 3-D printing, show promising results in fabrication of metallic biodegradable implants (41, 49).

1.2.3 Biodegradable polymers

Since the first serious thoughts about bioresorbable polymeric implants emerged during the 1960ies, when Kulkarni et al. suggested to use polylactic acid (PLA) in surgical implants, a great variety of biodegradable polymeric materials has been developed and tested (2).

In terms of application, degradable polymers can be classified into three groups: (i) polymers for medical use, (ii) polymers for ecological use and (iii) polymers for dual use. In terms of origin, natural polymers can generally be distinguished from synthetic polymers (50). Flieger et al. went so far as to differentiate between synthetically obtained polymers, polymers produced through fermentation by microorganisms and polymers from chemically modified natural products (51). Their classification is presented in [figure 2]. Though, not all biodegradable polymers can be utilized as biomaterials for internal use in living tissue.

chemical synthesis

- polyglycolic acid
- polylactic acid
- polycaprolactone
- polyvinyl alcohol

fermentation by microorgansisms

- polyesters
- neutral polysaccharides
  - gellan gum
  - pullulan
  - laminarin and curdlan

chemical modification of natural products

- starch
- cellulose
- chitin and chitosan
- soy-based plastics

Figure 2: Biodegradable polymers sorted by production path (51)

According to Ulery et al, biodegradable polymers suitable for biomedical applications can be classified either as hydrolytically or encymatically degradable. Several degradable polymers – i.e. esters, anhydrides, acetals, carbonates, amides, urethanes and phosphates – are characterized by hydrolytically labile chemical bonds that allow them to be broken down without further influence, while others have bonds, that actually possess hydrolytic sensitive properties, but in fact require catalysis to show significant degradation under physiological conditions (11). For a detailed list of biodegradable polymers for medical use, see [figure 3].

hydrolytic degradation

- poly(α-esters)
  - polyglycolide
  - polylactide
  - poly(latide-co-glycolide)
  - polyhydroxyalcanoates
  - polycaprolactone
  - poly(propylene fumarate)
- polyanhydrides
- polyactetals
- poly(ortho esters)
- polycarbonates
- polyurethanes
- polyphosphazenes
- polyphosphoesters
- combinated polymers

encymatic degradation

- synthetic polyethers
- proteins and poly(amino acids)
  - collagen
  - elastin & elsatin-like polypeptides
  - albumin
  - fibrin
  - natural poly(amino acids)
  - synthetic poly(amino acids)
- polysaccharides
  - human origin
  - non-human origin

Figure 3: Biomedical polymers sorted by degradation (11)

Throughout literature of the past 30 years, the most important, most frequently described and most commonly used materials for bioresorbable internal fixation devices in medical applications were polyglycolic acid (PGA), polylactic acid (PLA), their copolymer poly(lactic-co-glycolic acid) (PLGA) and polydioxanone (PDS) (4, 11, 24-25, 37, 50-51).

1.2.3.1 Polyhydroxyalcanoates – PHA

Polyhydroxyalcanoates (PHA) are polyesters, that are produced from a wide range of different basic materials such as fossil resources (mineral oil, methane, hard coal etc.), renewable resources (starch, cellulose, sucrose etc.) and chemicals (proprionic acid, 4-hydroxy-butyric acid), from several byproducts (molasses, whey, glycerol) and even from carbon dioxide. As degradable biomaterials, they have mainly been used as an alternative to non-degradable plastics in products of everyday life, i.e. packaging films and disposable items, but also as basic raw material for optically active compounds. They are synthesized by a great variety of gram-positive as well as gram-negative bacteria and usually serve as an intracellular energy and carbon reserve. PHA represent an extensive group of completely biodegradable polymers, consisting of over 100 monomers and therefore being extremely versatile in terms of molecular density and chemical properties. Their key features are a high degree of polymerization and cristallinity, their insolubility in water and a piezoelectric function (53, 54).

Poly(3-hydroxybutyrate) (PHB) is a low molecular density, non-storage PHA found in the cytoplasm and cytoplasmatic membrane of many common bacteria such as *Escherichia coli*. It is also part of the cytoplasmatic membrane in several yeasts, plants and animals. Due to its common appearance, relatively simple production path and reproducibility, it has been very well characterized as a representative for PHA. Belonging with a sub-class of PHA called short-side-chain PHA (ssc-PHA), PHB and its copolymers have a highly crystalline, more brittle and less elastic appearance than so called medium-side-chain PHA (msc-PHA). For this reason, PHB in its pure form is only suitable for a limited range of applications (53, 54). Furthermore, it has a relatively high melting point of approximately 170°C and a glass transition temperature ranging between 0°C and 5°C. This means, that under physiologic circumstances, PHB is not completely solid, but in a solid-like, highly viscous state (54).

Recent studies have discussed further PHA and co-polymers, i.e. poly(4-hydroxybutyrate) (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) and poly(3-hydroxyoctanoate) (PHO). Several copolymers and composites show improved properties compared to PHB.

Primarily a higher mechanical stability and faster in-vitro degradation rates propose a superior suitability for the use in medical devices for internal application (55, 56).

Finally, PHA possess the biodegradability, biocompatibility and thermoprocessibility needed for medical implant applications and can be blended, surface modified or composited with other PHA, polymers, enzymes or inorganic materials for further adjustment of their specific properties (55).

1.3 Degradation properties

For ceramic materials, their solubility under physiologic conditions determines their degradation capacities (38). The crystallographic structure plays another important role in degradation, for which reason the chemically identical α-tricalcium phosphate (TCP) and β-TCP will degrade at completely different rates, only due to their microscopic structure. Furtheron, blending with different ions is known to either increase (i.e. CO_3^{2-}, Mg^{2+} or Sr^{2+}) or decrease (i.e. F^-) the solubility and biodegradability. Also, processing can have major influence on degradation, as low density and high porosity will accelerate absorption (38, 39).

In iron-based alloys, the anodic partial reaction ($Fe \rightarrow Fe^{2+} + 2e^-$) of Fe determines the degradation of the device. Iron then reacts with hydroxyl-ions formed through oxygen reduction ($H_2O + ½O2 + 2e^- \rightarrow 2OH^-$) and forms hydrous ferrous oxide ($FeO \cdot nH_2O$) or ferrous hydroxide ($Fe(OH)2$) as a visible corrosion product on the surface of the device. Degradation products usually have a layered structure, consisting of diverse compounds of iron, oxygen and hydrogen; this demonstrates the important role of oxygen in the degradation process of iron. Though, the layered structure builds a coating that prevents the body of the device from further fast degradation and the degradation rate of common iron devices therefore is to slow for clinical applications as fracture fixation (41).

The degradation of Magnesium is usually accompanied by formation of OH^- ions and gaseous hydrogen ($Mg + 2H_2O \rightarrow Mg^{2+} + 2OH^- + H2$). Thus, considerable gas accumulations in the surrounding tissue rule out the use in internal fixation devices (10, 57).

Investigations in several polymeric families showed remarkable differences in degradation rate and erosion mechanism. For this reason, their hydrolytic stability may vary twelve-fold as the comparison of very unstable polyphosphazenes and extremely stable polyamides shows. Polymer chemistry has significant impact on degradation rates, but moisture, temperature, pH and microbial activity of the environment also determine erosion properties of polymeric materials. Further important factors are water diffusion and monomer solubility or even size, surface and design of the final device (11, 53).

Additionally, the differentiation between surface and bulk erosion plays an important role in determining optimal materials for complex applications. Surface erosion is characterized by a high rate of polymer degradation and mass relief at the water-device interface and comparably low water diffusion into the body of the device, so that degradation happens almost exclusively at the surface. Bulk erosion on the other hand, shows the exact opposite reaction, so that water diffuses faster and mass loss takes place throughout the bulk of the device (11). Whether a device undergoes surface erosion, bulk erosion or a combination of both, is determined by its exact degradation behavior and the ratio of degradation time and stability. In hydrolytically degradable polymers, the labile bonds result in one product gaining a hydrogen atom and another gaining a hydroxyl group when broken up. Encymatically degradable polymers on the other hand, are theoretically sensitive to hydrolysis, while under physiological conditions only catalysis will implement their degradation. Ether or amide bonds can be seen as origin for the low degradation rates (11).

1.3.1 Specific degradation properties of PHB

Belonging with the hydrolytically degradable group of PHA [see table 3], PHB can undergo surface erosion because of its crystallinity and the hydrophobic properties of its backbone (11). As in all PHA [see chapter 1.2.3], the degradation of PHB depends on multiple environmental, chemical and structural factors and will happen upon exposure to living tissue, compost, soil or marine sediment. During degradation of PHB, carbon dioxide forms along with water in aerobic environment, in anaerobic environment methane can be found as a side product instead (53).

To be useable as a source for carbon, PHB and other PHA polymers may be metabolized and broken down to hydroxyacids by several microorganisms and a few bacterial serine hydrolases serving as PHA depolymerases – i.e. *Alcaligenes faecalis, Rhodospirillum rubrum, B. megaterium, A. beijerinckii, Pseudomonas lemoignei*. Their protein sequences contain a (i) signal sequence, (ii) catalytic domain with lipase box, (iii) linking domain and (iv) substrate-binding domain, acting as an adsorption site for polymer substrates. While PHA depolymerases usually have one substrate-binding domain, only recently a PHB depolymerase with two substrate-binding domains has been reported. This might result in enlargement of substrate specificity or enhancement of enzyme adsorption (53, 58).

PHB can also be subject to thermal degradation: while it shows no significant weight loss at the melting point of approximately 180°C, it will form volatile products when temperatures exceed 2000°C. During heating, the average molecular weight reportedly increases in the beginning due to condensation reactions, later on, chain scissions cause a major decrease in molecular weight (53).

Today, the in vivo degradation of PHB is understood to happen mainly hydrolytically [see chapter 1.2.3] and only subsequently for a small part under enzymatic influence (37).

1.4 Aim of the study

The present study aims at clarifying the usability of biodegradable PHB implants as intramedullary fracture fixation devices in pediatric orthopedics. For this cause, the in vivo degradation performance of pins made from three different blends of PHB was investigated in a growing rat model using micro computed tomography (μCT). The main focus was set on specific pediatric requirements, primarily directing at (i) a reasonable degradation velocity to ensure gradually decreasing load bearing of implants and (ii) an adequate duration for complete resorption of implants in living tissue to grant minimal influence on bone growth.

2 Materials, animals and methods

2.1 Materials and animals

The following chapter gives account of all materials and devices used throughout the whole study and provides exact description of experimental animals.

2.1.1 Implants

All of the implants – also related to as “pins” – had a crystalline structure with smooth, non-polished surface without coating and a cylindric shape with an estimated diameter of approximately 1,6mm and a length of 8mm. In accordance with these parameters, an initial volume of approximately 16,09mm^3 and a surface of approximately 44,23mm^2 were to be assumed.

Image 1: PHB pin prae implantationem

For our study, we used three different kinds of degradable implants, each of them based on PHB.

As in advance pins made of 100% PHB had proven not to be differentiable from the surrounding bone structure in μCT scans, three new PHB-based materials were synthesized at the Technical University of Graz and different substances for better visualization during μCT imaging and enhanced degradation properties were added:

Pins for group I were composed of PHB and 3 percent per weight (%w) zirkoniumdioxide (ZrO_2).
Zirkonium is a non-toxic heavy metal that physiologically occurs in the human body in low doses and usually is highly resistant to corrosion (59). In the present study its intended use was to enhance radiologic imaging properties of PHB and thus enable 3D evaluation of the implants.

Pins for group II were composed of PHB, 3%w zirkoniumdioxide and 10%w Herafill®, pins for group III were composed of PHB, 3%w zirkoniumdioxide and 30%w Herafill®.
Herafill® is a trademarked bone graft substitute produced and distributed by Heraeus® Medical GmbH Germany. It consists of $CaSO_4$ (calcium sulphate) and $CaCO_3$ (calcium carbonate) for the biggest part and is commonly used as a completely biodegradable substitute in bone defects that prevents ingrowth of soft tissue and provides an osteoconductive matrix for the formation of new bone at the same time with its own gradual resorption. Newer devices even use Herafill® as a biodegradable carrier for local application of antibiotics (60). Its intended use was to degrade faster than PHB, enlarge the surface of the implants and hence to accelerate degradation.

2.1.2 Surgery

The drills used during implantation were medical drills (Synthes®, Paoli, PA, USA), their diameter being slightly smaller than the pins' with 1,5mm.
For surgery we used standard surgical instruments: scalpels, tweezers and needle holders, for wound closure single-button-sutures of resorbable Vicryl® rapide 5-0 (Johnson&Johnson Medical GmbH, Norderstedt, Germany).

2.1.3 Experimental animals

All animal experiments were conducted under animal ethical respect and were authorized by the Austrian Ministry of Science and Research (accreditation number BMWF-66.010/0113-II/10b/2009). 134 male Sprague–Dawley rats with a body weight at a mean of 162,3g (at a range from 117g to 233g) and five weeks of age were used in this study. Rats were breeded at the Institute for Laboratory Animallore and Genetics at Himberg, Austria (Core Unit for biomedical research, Medical University of Vienna). For acclimatisation purposes, they were brought to the Hahnhof Institute for biomedical research at the Medical University of Graz, 7 to 14 days prior to the scheduled surgery date.
During the study, animals were housed in groups of four in clear plastic cages on standard bedding at 12 hour light/12 hour dark cycles with a supply of special rodent food („*Sniff*®”) and water ad libitum.

2.1.4 Pharmaceutics

In the tables below all pharmaceutics used during the study are listed.

Name	Active substance	Dose	Manufacturer
Forane®	Isoflurane	3,0 l/min	Abbot Laboratories Ltd, Kent, GB

Table 2: Inhalation narcosis

Name	Active substance	Dose	Manufacturer
Fentanyl®	Fentanyl	50µg/ml	Janssen-Cilag GmbH, Neuss, GER
Midazolam Delta®	Midazolam	1mg/ml	Deltaselect GmbH, Dreieich, GER
Domitor®	Medetomedin	1mg/ml	Pfizer Corp. Austria GmbH, Vienna, AT
Auqatears®	Carbomer	974P	Ciba Vision, Vienna, AT

Table 3:Anesthesia

Name	Active substance	Dose	Manufacturer
Narcanti®	Naloxon	400µg/ml	Torrex Chiesi Pharma GmbH, Vienna, AT
Anexate®	Flumazenil	100µg /ml	Roche Austria GmbH, Vienna, AT
Antisedan®	Atipamazol	5mg/ml	Pfizer Corp. Austria GmbH, Vienna, AT

Table 4: Antagonisation of anesthesia

Name	Active substance	Dose	Manufacturer
Dipidolor®	Piritramid	(4:50 glucose5%) :150 NaCl	Janssen-Cilag GmbH, Neuss, GER
Rimadyl®	Carprofen	0,5ml; 1:50 Nacl	Pfizer Corp. Austria GmbH, Vienna, AT

Table 5: Postoperative pain management

Name	Active substance	Dose	Manufacturer
Thiopental Sandoz®	Thiopental	2ml; 1:20 NaCl	Sandoz GmbH, Kundl, AT

Table 6: Euthanasia

2.1.5 Microcomputed tomography - µCT

As a possibility for non-invasive imaging and three-dimensional quantification of internal structures and implants, microcomputed tomography (µCT) was used for scanning pins in vivo as well as ex vivo. Scans were performed using an INVEON Siemens® µCT device and Siemens INVEON Acquisition Workplace® 1.2.2.2 (Siemens Medical Solutions, USA) at 70kV voltage, 500 µA current, and 1000 ms exposure time.

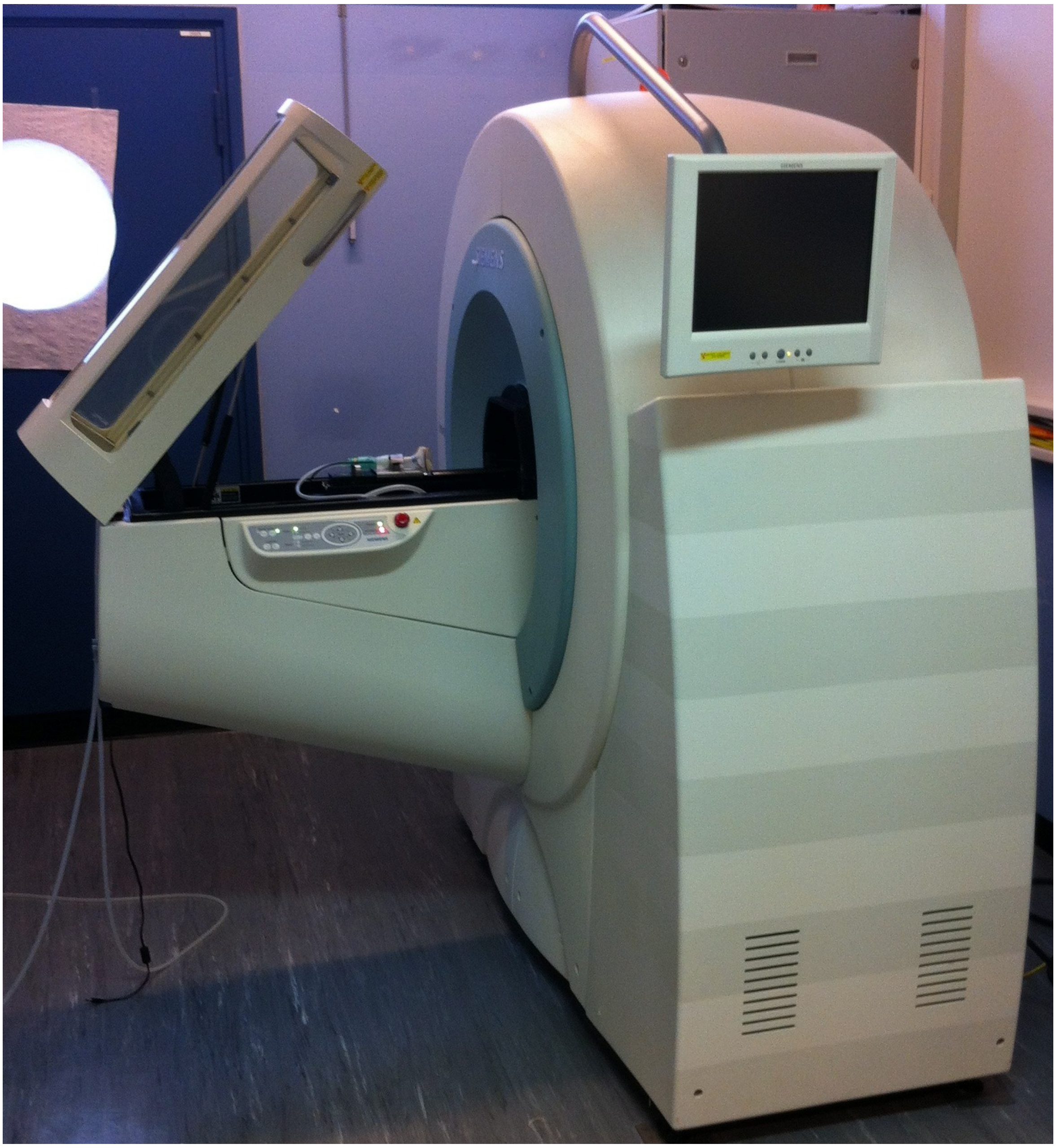

Image 2: μCT device

The acquired in vivo images were transferred to the medical image processing software MIMICS® 14.0 (Materialise NV, Leuven, Belgium) for 3D reconstruction and evaluation of pin degradation and condition of pin, bone and soft tissue.
Visualization and assessment of ex vivo data were executed in Siemens INVEON Research Workplace® 3.0 (Siemens Medical Solutions, USA).

2.2 Methods and study design

As chemically pure PHA had in advance proven not to be differentiable from bone tissue in small imaging µCT, three new composites based on PHB were established and used in three groups for comparison. The exact composition of these blends and the properties of the additives can be found in chapter [2.1.1 implants and surgery].

Each group consisted of 30 male Sprague-Dawley rats and 60 cylindrical pins. All rats were implanted two pins of the same material, one in the left and one in the right femur.

For every single pin in vivo µCT-scans were scheduled at 4 points during the study: (i) 1-7 days post operationem (PO), (ii) 3 months PO, (iii) 6 months PO and (iv) 9 months PO. At these scheduled times, several animals of each group were selected and euthanized by random and the implanted pins harvested (also referred to as “explantations”).

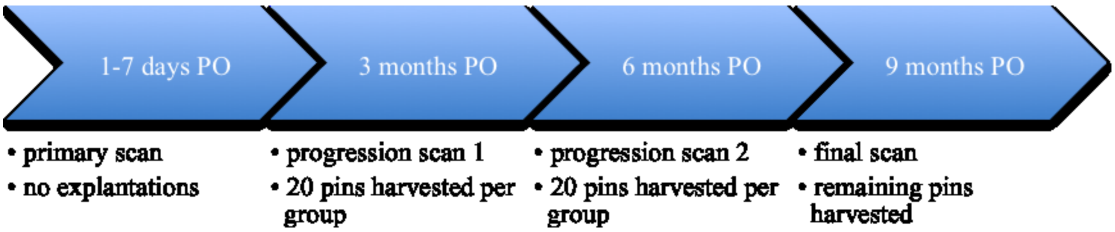

Figure 4: Time schedule of in vivo µCT scans and pin explantations

The harvested pins were externally examined for separate studies and then returned for validation µCT-scans in an ex vivo setting.

2.2.1 Surgical procedure

This chapter gives an overview on anesthesia, preoperative measures, the surgical process of pin implantation, postoperative management and euthanasia of experimental animals.

2.2.1.1 Anesthesia

We performed implantations at the Hahnhof Institute for biomedical research in a small animal operating room. After inhalation narcosis using Forane® (3.0 L/min; Abbot Laboratories Ltd, Kent, England), animals were weighed and subsequently underwent surgery. For continuous anesthesia and analgesia a subcutaneous injection of Fentanyl® (20µg/kg; Janssen-Cilag GmbH, Neuss, Germany), Midazolam Delta® (400µg/kg; DeltaSelect GmbH, Dreieich, Germany) and Domitor® (200µg/kg; Pfizer Corporation Austria GmbH, Vienna, Austria) was applied.

2.2.1.2 Immediate preoperative management

The operation field was cleared of all hair with a common shaver and the rats´ eyes moistened with AquaTears® (Novartis Pharma GmbH, Vienna, Austria) to prevent drying up during anesthesia.

2.2.1.3 Surgical procedure

First, the femur was approached through a later incision. After cut-down to the mid-diaphyseal region, the implantation bed was prepared using a medical drill (Synthes®, Paoli, PA, USA), its diameter of 1,5mm being slightly smaller than the implants'. The hole was positioned transcortically in a perpendicular angle to the longitudinal axis of the femur.

To achieve only minimal thermal irritation of the surrounding tissue, a low rotational speed of 200 rpm was selected and physiological saline irrigation continually applied to minimize frictional heat. To grant a uniform press fit, implants were inserted by gentle tapping until a transcortical placement could be ensured. Thereafter, the entire operation field was irrigated with physiological saline irrigation and the wound was closed in layers using single button stitches. Subsequently, the contra lateral femur underwent the exact same procedure.

Following the implantation, the animal was placed on a warming bed and the general anesthesia was antagonized by an intraperitoneal injection of a combination of Naloxone (120 µg/kg; Narcanti®, Torrex Chiesi Pharma GmbH, Vienna, Austria), Flumazenil (50 µg/kg; Anexate®, Roche Austria GmbH, Vienna, Austria) and Atipamezole (250 µg/kg; Antisedan®, Pfizer Corporation, Vienna, Austria) (10).

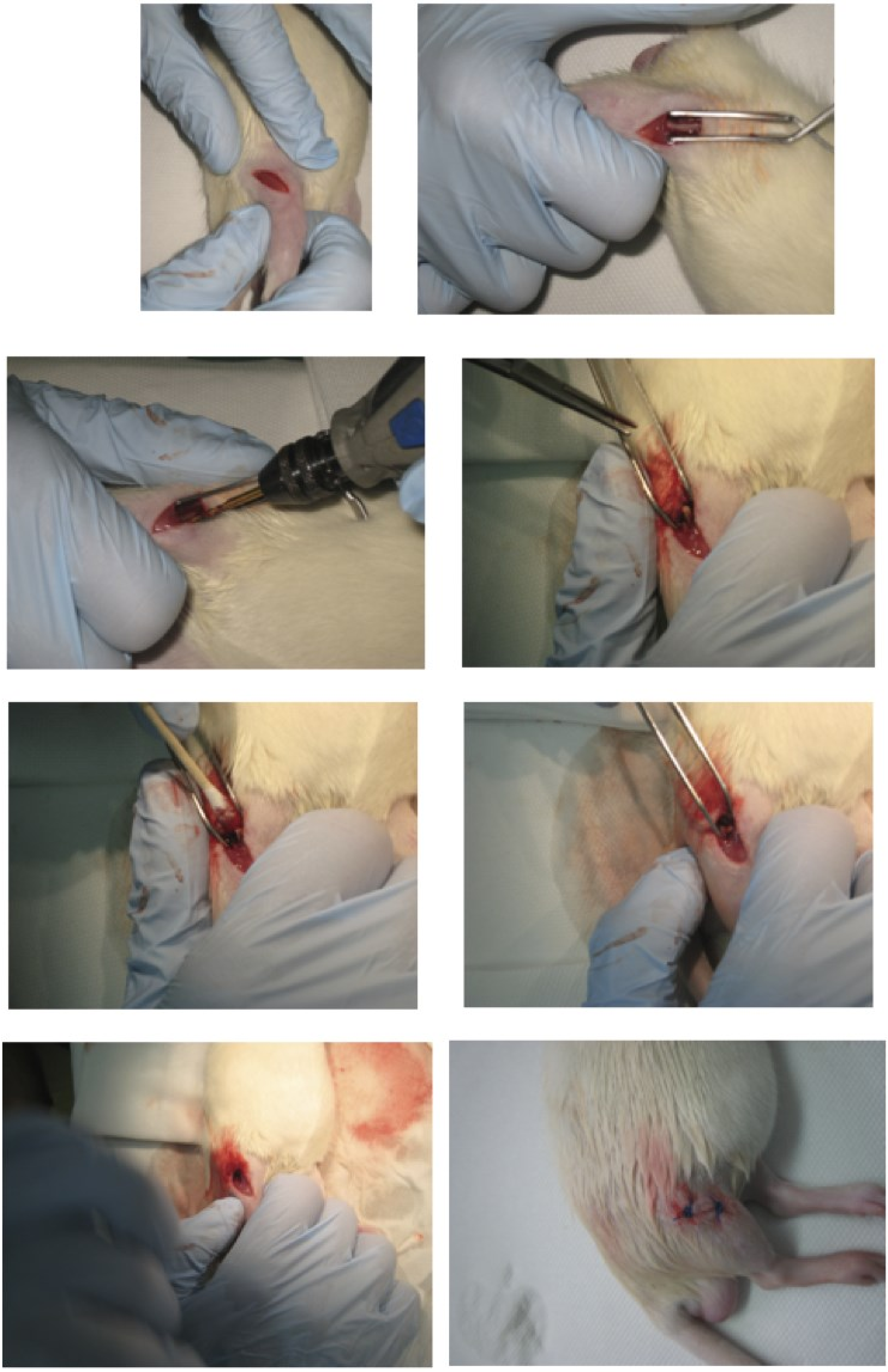

Image 3: Surgical procedure (friendly courtesy of Dr.in med. univ. K. Angerpointner)

2.2.1.4 Postoperative management

For immediate postoperative analgesia all animals received a single shot of Rimadyl® (200mg/kg; Pfizer Corporation GmbH, Vienna, Austria) and were then allowed free movement. To ensure further analgesia, Dipidolor® (Janssen-Cilag GmbH, Neuss, Germany) was added to the drinking water for 7 days postoperatively.

2.2.1.5 Euthanasia

After 3, 6, and 9 months, randomly selected animals of each group were sacrificed and the pins explanted for ex vivo scans. For this purpose, the rats were anesthetized with Forane®. When general anesthesia was reached, 2ml of a 1:20 solution of thiopental and saline solution were applied intracardially.

2.2.2 Microcomputed tomography - µCT

An INVEON Siemens® Preclinical microCT (µCT) (Siemens Medical Solutions, USA) was used for small animal imaging; all scanning was done at an energy of 70kV, a current of 500uA and 1000ms exposure time.

During in vivo scans, rats were anaesthetized (Isoflurane® inhalation narcosis) and strapped to the table to prevent movement artifacts. Obtained data sets were then named after the experimental animals, the side of implantation and the date of the scan.

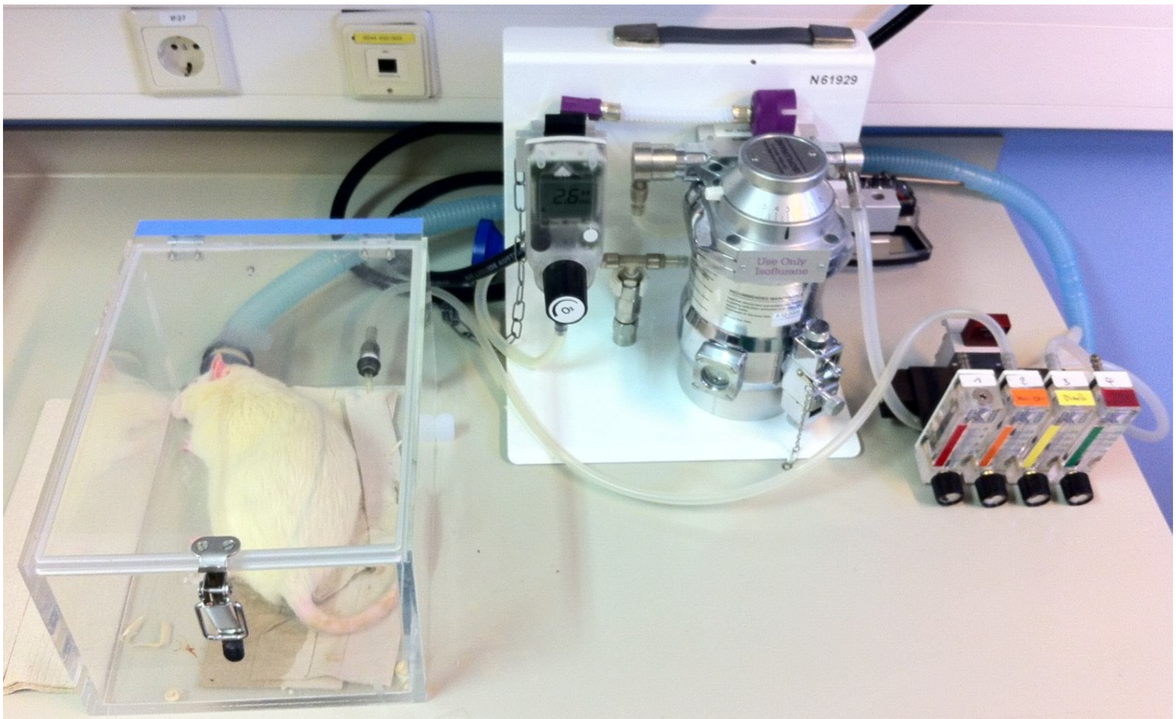

Image 4: Inhalation narcosis

For ex-vivo evaluation, several rats of each group were euthanized and the pins harvested at different times and then mechanically and histologically evaluated in a separated study. The harvested and cleaned pins were associated with the experimental animal and the corresponding implantation period. To grant recognizability during and after scans, they were fixed to the table with common adhesive tape in a predefined, L-shape pattern of four to five pins per scan. The naming of obtained data was made in accordance with the experimental animal, the side of implantation and the implantation period. As several pins were broken, they were excluded from scanning.

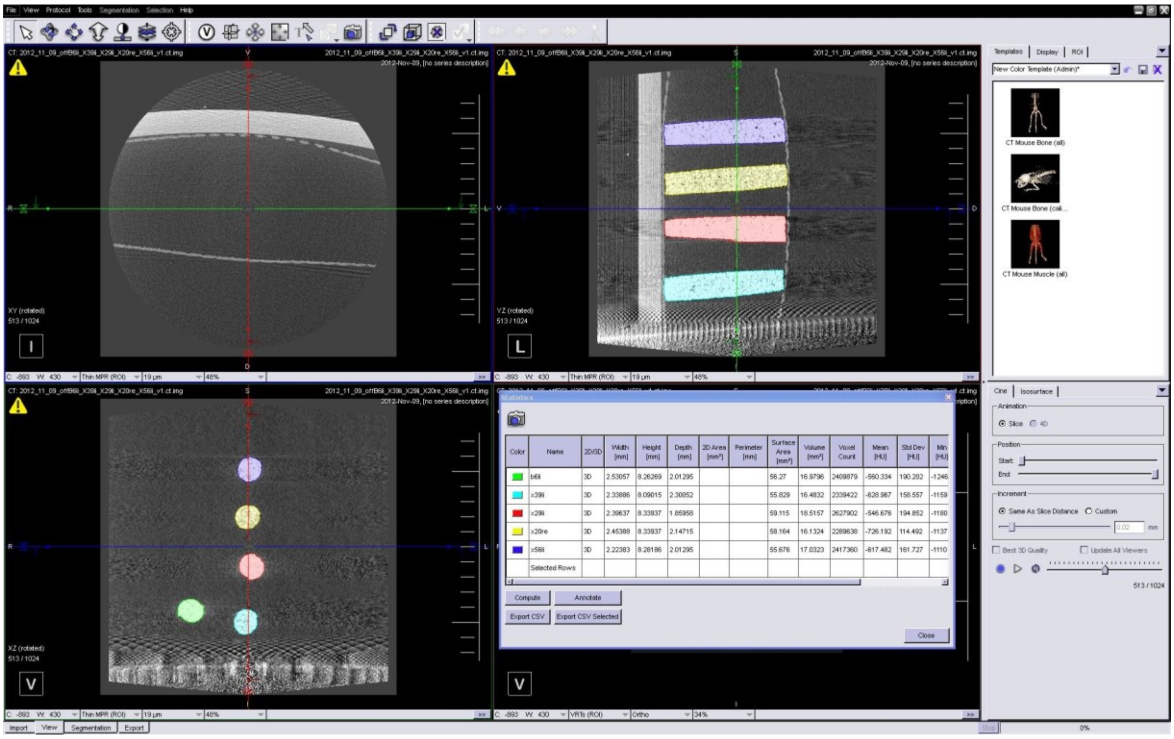

Image 5: L-shape scan pattern

Due to the differing animal size, the number of slices varied from scan to scan, between 90 and 120 slices were used for in vivo evaluation. Data were subsequently converted into DICOM format and as such imported into MIMICS® 14.0 (Materialise NV, Leuven, Belgium). Through image processing and 3D reconstruction tools, 3 dimensional models of the pins were constructed to enable automatic quantification of volume and surface of the pins.

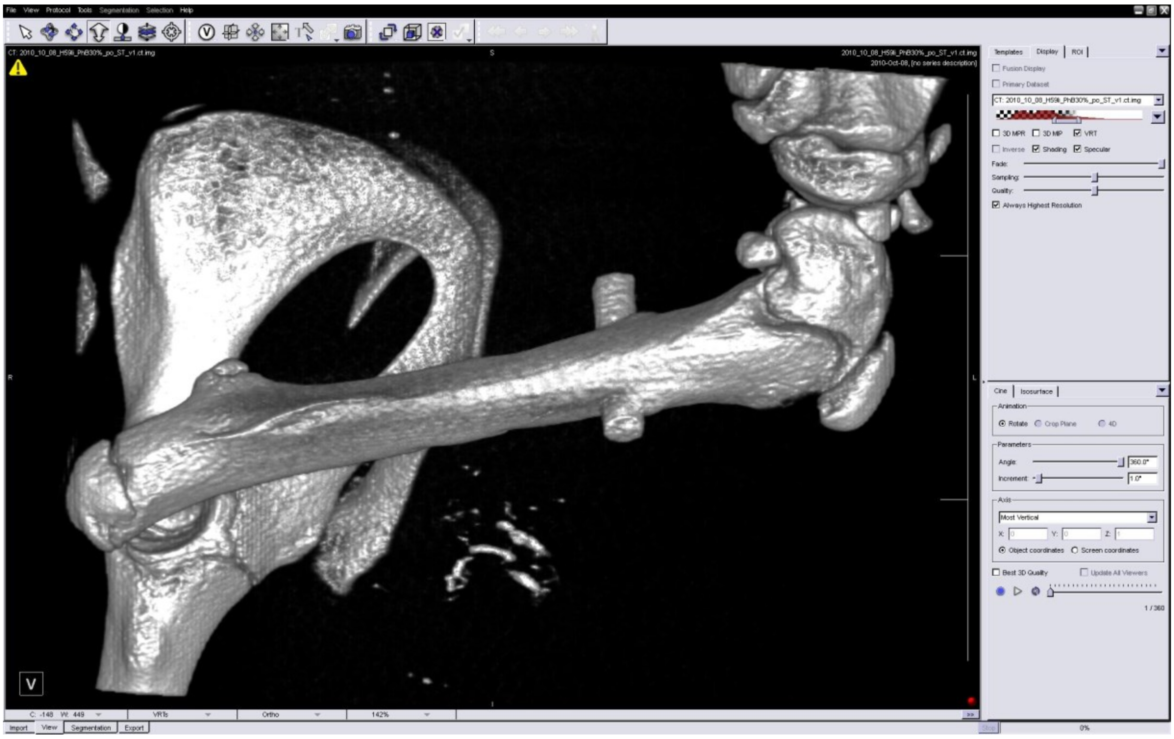

Image 6: raw 3D model of pin and bone in vivo

For ex vivo interpretation, scan data underwent evaluation in Siemens INVEON Research Workplace® 3.0 (Siemens Medical Solutions, USA), again image processing and reconstruction tools allowed to construct 3D models of pins and quantify their volume and surface.

2.2.2.1 Primary evaluation

Seven days post operationem, primary in vivo µCT scans were scheduled to evaluate pins concerning positioning and bone condition. To produce valid and comparable data, pins needed to grant the intended press fit and show bicortical placement as well as fully intact surrounding bone tissue. Also, a primary three-dimensional evaluation was intended to deliver reference data for the following scans.

2.2.2.2 Degradation

For grading the progress of implant degradation, the following terminology was established:

Inconspicuous: the implant did not show any change of shape or surface.
Surface degradation: superficial degradation and irregularities could be found.
Deep degradation: deep incisions and irregularities reaching to the middle of the pin and further could be found.
Degradation products: only scattered solid fragments, that would ensure no mechanic stability of the implant, could be found or no solid fragments at all.
Decomposed: no degradation products could be detected, the pin was completely dissolved and resorbed.

This terminology allows for a simple classification of degradation. In combination with the dwelling time of the implant in the biologic surrounding, a primary interpretation of degradation velocity can be made separate from three-dimensional evaluations of surface and volume.

2.2.2.3 Validation of measured pin properties

To validate the measuring of implant volume and surface obtained from in-vivo scans on the one hand, because of the difficult differentiation to bone in vivo on the other hand, harvested pins were scanned again after cleansing and close examination. The intention was to set both in and ex vivo scans in relation.

2.2.3 Statistics

The data acquired from μCT scans and 3d-reconstruction was processed through Microsoft Excel® and evaluated for the statistical analysis, which was performed using IBM® SPSS® Statistics 20.0.0 (IBM Corporation, Armonk, NY, USA). Pin volume (V, mm^3) and pin surface (S, mm^2) were evaluated for each sample.

For comparison of 3 different time points the Kruskal-Wallis test was applied as data did not show normal distribution according to the Kolmogorov-Smirnov test. Values were expressed as means with standard deviations (SD) and ranges. A p-value <0.05 was considered to be statistically significant.

3 Results

After reconstruction and migration of in vivo scan data to MIMICS® 14.0, differentiation between pins and surrounding bone tissue proved to be technically inaccurate in scans of all three groups. Despite of the blending with zirkoniumdioxide and Herafill® exceedingly similar density properties prohibited automatized distinction between implants and living tissue.

Though three-dimensional models looked promising when implants and bone were treated as a union, the 3D tools available could not deliver results appropriate for further interpretation of automatically separated implants. For this reason, manual line drawing between bone and implant in every single slice outlined the only possibility to gain any data at all from in vivo scans. Nevertheless, due to subjective interpretation during manual processing of single slices the surface of pins in three-dimensional reconstructions showed artificial irregularities not compatible with macroscopic and microscopic examination of harvested pins.

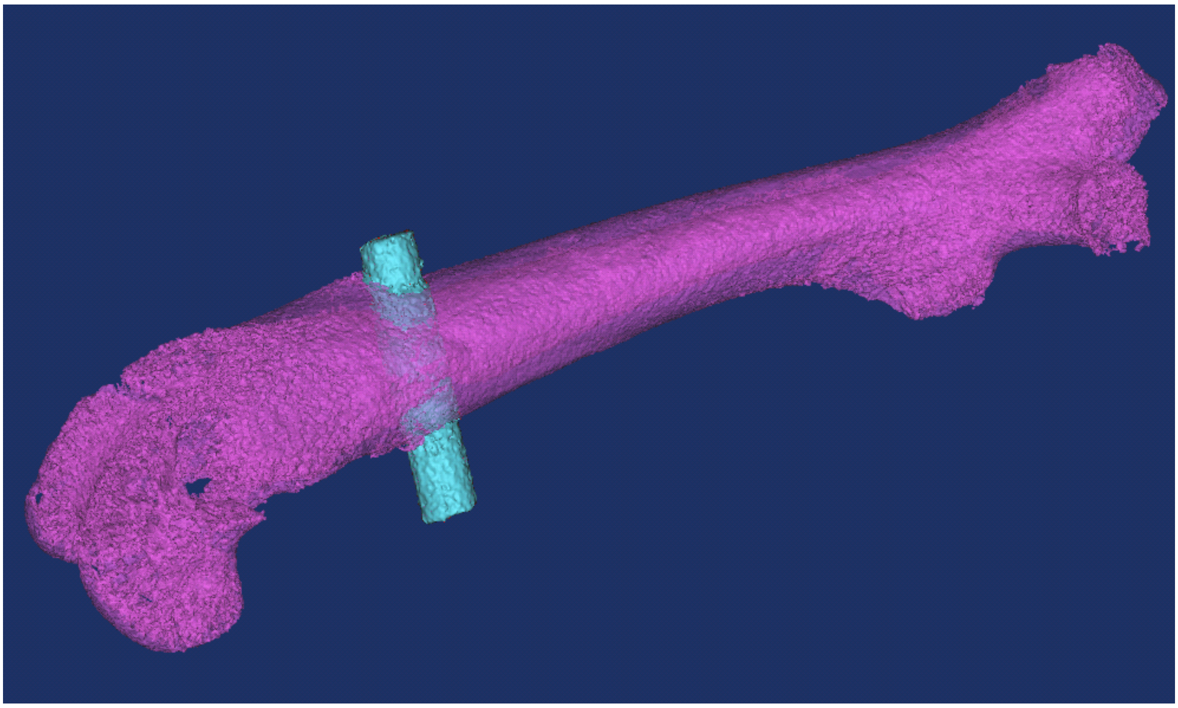

Image 7: in vivo 3D model of pin and bone after 3 months

For these reasons, in vivo scans and evaluations could not be used as a source for valid results. Though, a trend towards only minimal degradation could be detected in subjective examination of µCT data.

Ex vivo scans on the other hand – due to the absence of surrounding bone tissue – allowed more exact three dimensional quantification of pins' volumes and surfaces in INVEON Siemens Research Workplace® 3.0. For valid evaluations, only ex vivo scans were taken into account, as they created more accurate and reproducible results.

As primary in vivo scans after 1-7 days produced no valid basic results, initial dimensions of 1,6mm in diameter and 8mm in length for a cylindrical implant were used to calculate basic values for both surface and volume of the pins. Based on these calculations, a volume of approximately 16,09mm^3 and a surface of approximately 44,23mm^2 were set in relation to data gained from ex vivo scans and three-dimensional interpretations.

3.1 Pin degradation

As only a limited amount of pins could be returned from external examination and hence used for ex vivo evaluations, the exact numbers of pins scanned in an ex vivo setting are indicated in [table 7]. Of all pins returned for ex vivo scanning, 1 pin of group I (2,2%), 1 pin of group II (4,5%) and 3 pins of group III (13,6%) were found to be broken and consecutively excluded from further interpretation. Breakage could not be traced back to degradation processes in any of the broken pins though.

Group	Material (percent by weight)	3m	6m	9m
I	PHB + 3%w ZrO_2	10	8	9
II	PHB + 3%w ZrO_2 + 10%w Herafill®	8	6	8
III	PHB + 3%w ZrO_2 + 30%w Herafill®	6	8	8

Table 7: Number of intact pins per group and dwelling time available for ex vivo scanning

In a first macroscopic examination, the pins did not show obvious evidence of degradation. After 3D-processing in INVEON Siemens Research Workplace® 3.0, none of the pins used for ex vivo scanning displayed superficial irregularities, deep incisions or even solid fragments caused by degradation at all. As no changes of shape or surface could be detected in any scan or 3D model, all pins were classified as *inconspicuous* based on the predefined classification illustrated in chapter [2.2.3.2 Degradation].

Image 10: macroscopic view on pin post explantationem after 6 months in vivo

Results of three-dimensionally processed ex vivo scans were not specially evaluated from a statistic point of view, as degradation processes were obviously not present. Collected data was only presented in means (M) with ranges (R) and standard deviations (SD).

Mean values of the first ex vivo scans after three months of implantation differed from calculated basic values. Contrary to presumptions about the degradation of implants in vivo, implants had increased in both volume and surface.

3m	**Volume**			**Surface**		
	M	R	SD	M	R	SD
Calculated	16,09mm^3	-	-	44,23mm^2	-	-
Group I	16,89mm^3	15,69 – 17,79mm^3	0,877	59,91mm^2	53,04 – 81,51mm^2	8,668
Group II	17,67mm^3	16,78 – 19,02mm^3	0,775	57,63mm^2	54,34 – 60,31mm^2	2,204
Group III	18,16mm^3	17,39 – 18,83mm^3	0,524	57,89mm^2	54,67 – 65,83mm^2	4,586

Table 8: Calculated values and Mean/Range/Standard deviation after 3 months in vivo

After 3 months in vivo, group I had gained 4,97% in volume and 35,45% in Surface.
Group II had gained 9,82% in volume and 30,30% in Surface.
Group II had gained 12.87% in volume and 30,88% in Surface.

6m	Volume			Surface		
	M	R	SD	M	R	SD
Calculated	16,09mm^3	-	-	44,23mm^2	-	-
Group I	16,26mm^3	14,72 – 17,2mm^3	0,914	60,76mm^2	55,83 – 63,46mm^2	4,689
Group II	17,24mm^3	16,58 – 17,82mm^3	0,517	56,17mm^2	53,31 – 57,84mm^2	1,755
Group III	17,98mm^3	17,13 – 18,59mm^3	0,49	56,62mm^2	54,26 – 60,84mm^2	2,134

Table 9: Calculated values and Mean/Range/Standard deviation after 6 months in vivo

9m	Volume			Surface		
	M	R	SD	M	R	SD
Calculated	16,09mm^3	-	-	44,23mm^2	-	-
Group I	17,46mm^3	15,13 – 18,72mm^3	1,073	60,03mm^2	55,67 – 65,5mm^2	2,86
Group II	17,58mm^3	16,12 – 18,93mm^3	0,7	57,09mm^2	54,46 – 60,12mm^2	2,29
Group III	18,28mm^3	17,55 – 19,39mm^3	0,681	57,49mm^2	54,23 – 62,09mm^2	2,927

Table 10: Calculated values and Mean/Range/Standard deviation after 9 months in vivo

When comparing results obtained from groups with longer dwelling times in vivo with those of the first ex vivo scans after three months, neither continuous changes in surface or volume, nor relevant degradation could be monitored.

Furthermore, a direct comparative look at progression charts from group I (PHB + 3%w ZrO_2), group II (PHB + 3%w ZrO_2 + 10%w Herafill®) and group III (PHB + 3%w ZrO_2 + 30%w Herafill®) shows no relevant difference in degradation behaviour between blends of PHB, Zirkoniumdioxide and Herafill® in varying proportions.

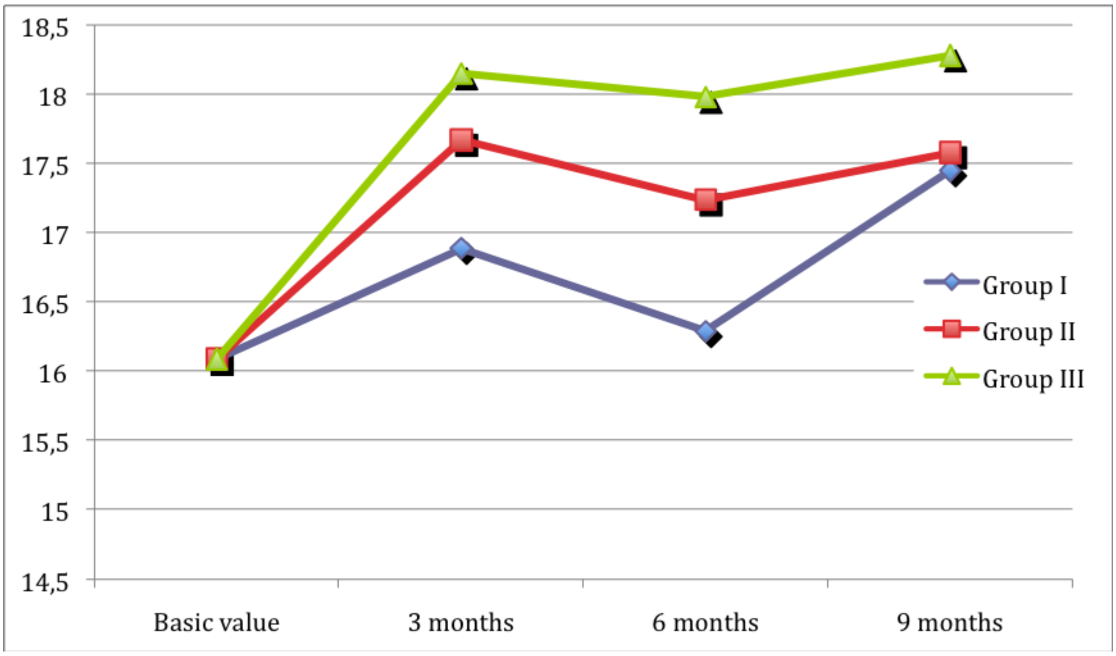

Figure 5: Progression chart of volume in mm³

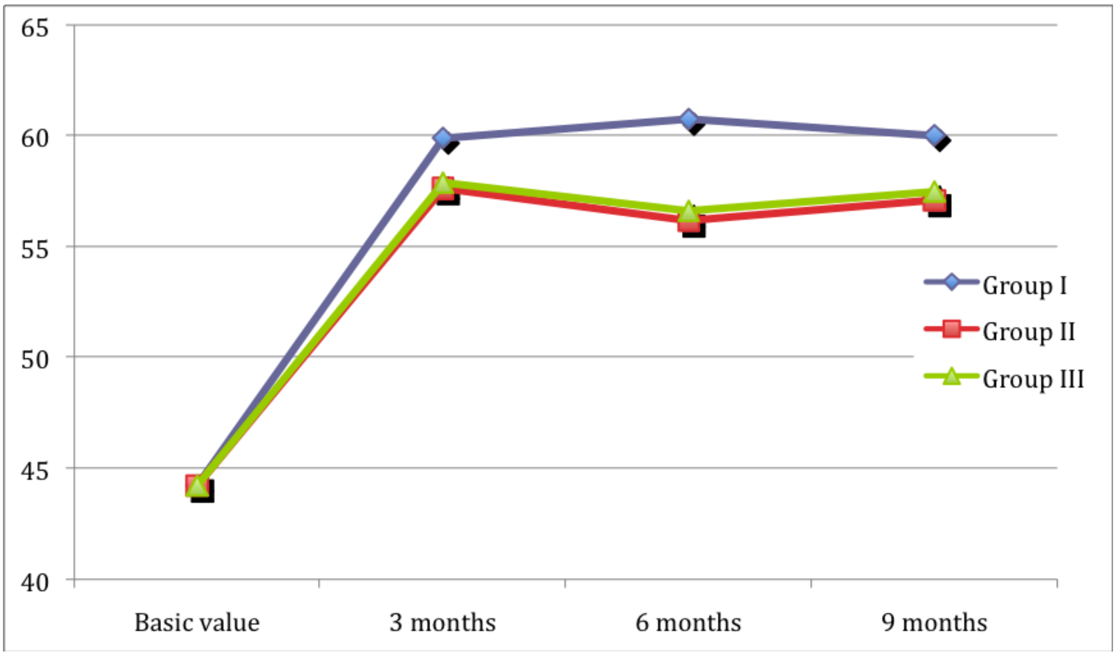

Figure 6: Progression chart of surface in mm²

Objective interpretation of all data gained from ex vivo scanning – as well as subjective interpretation of data gained from in vivo scans – could not verify significant degradation of implants over a dwelling period of 9 months in all three groups.

3.2 Problems of corrosion

PHB degrades relatively slow in vivo (56). In vivo scans that were performed during this study have not produced technically quantifiable results. Though, in several 3D models, in which pins have not been separated from bone tissue, the surfaces of pins always looked smooth to the naked eye and had no signs of corrosion such as incisions, hairline fractures or irregularities in shape or surface [see image 7].

In accordance with this observation, ex vivo results have shown no major sign of degradation or significantly quantifiable changes of volume or surface in any of the experimental groups of this study. Without a certain amount of degradation, usually distorting factors like breakage during implantation, improper fit in the bone or local inflammatory reactions have not had influence on the results.

In several μCT scans irregularities of density could be detected in the bulk of pins, which leads to the assumption that problems in mixing of composites may have occurred during manufacturing. The addressed pins did not produce results significantly deviating from those obtained from homogeneously blended implants though and were therefore included in the present study.

4 Discussion

The present study was performed with the intention of showing the in vivo degradation behavior of three similar PHB based blends using micro computed tomography (μCT) for follow-up in growing rats. These new blends, containing PHB as main material, zirconiumdioxide and Herafill® in variable proportions, entertained hopes of enhanced degradation properties and better suitability for the intended use in pediatric fracture fixation.

Not only for pediatric applications, where the number of surgical interventions is tried to be kept as low as possible, biodegradable implants outline an attractive alternative to conventional, non-degradable implants.

Though different concepts include a widespread variety of materials from a ceramic, metallic or polymeric background [see chapter 1.3], optimum properties of a biodegradable material for osteosynthesis in a growing skeleton have not yet been achieved. Due to its excellent biocompatibility, easy availability and a complete degradation in vivo, PHB presented a possible option for future biodegradable materials in medical use.

In a similar study that addressed metallic biodegradable implants based on magnesium, the μCT device proved to be excellently suitable for long term monitoring and three-dimensional evaluations of degradation properties of transcortically placed pins in growing rats. It enabled researchers to gain supplementary data without requiring extra animals (10). Several other studies have confirmed the usefulness and precision of μCT imaging over the last years, though three dimensional quantification of small volumes and surfaces is a relatively new aspect in radiologic examinations and therefore often seen with distinctive skepticism towards its reliability and validity (61-63).

For purposes of visualization in μCT imaging, zirkoniumdioxide was added to the implants used in our studies. At the same time, the fully degradable ceramic bone substitute Herafill® was added in two of three experimental groups in varying weight proportions to show its influences on the degradation properties of PHB in vivo. The assumption in adding a bone substitute was to increase the degradation rate of pure PHB by reducing the total content in the implant and hence increasing the surface of the remaining PHB (64). Also, when Wang et al. had performed studies on the in vitro degradation performance of hydroxyapatite reinforced PHB composites, their results featured higher mechanical stiffness and more suitable degradation properties obtained by blending with biodegradable ceramics (65).

Still, sterilization presents an outstandingly important issue for medical usability of degradable materials, as conventional methods used for traditional metallic devices have major impact on the mechanical properties of degradable devices (24, 66). The most noticeable differences are located in the physical and chemical properties of degradable polymers: even small amounts of moisture may lead to degradation, high temperatures on the other hand may lead to deformation. Moreover, in persistant implants surface sterilization usually is sufficient, while biodegradable implants need to be sterilized in their entirety because of the gradual resorption in the body. Also, sterilization remnants like gas can have major impact on the physical properties of polymers – especially in big surfaces. For the above reasons, sterilization methods as moist or dry heat, ethylene oxide, plasma and conventional radiation sterilization need to be avoided. As state-of-the-art with polymeric devices, gamma radiation can be used for sterilization without remnants, as long as temperatures are cooled down below 10° C (24, 66).

Surgery was well tolerated by the experimental animals, their full range of motion was given at any time – even immediately post operationem due to analgesia – and their daily behavior was not altered. Only common minimal signs of inflammatory reaction such as transient reddening of the skin and temporary swelling of the wound could be noticed as a result of surgery.
As PHB fully degrades to CO2 and H2O in vivo and 3-hydroxybutyrate and its polymers naturally occur in human blood and tissues, remnants of degradation were not found to cause further irritation of hard or soft tissue (12).

In vivo μCT scanning produced proper three dimensional models of implanted pins after 3, 6 and 9 months in vivo, pins of all groups did not show manifest signs of degradation. However, a quantification of surface and volume of implants was not possible in an objective and reproducible way because of exceedingly similar density properties of pins and surrounding bone, hence tools available in the used software could not achieve a clear differentiation between implant and tissue. Manual line drawing in single slices was possible, but results were obviously altered by subjective interpretation, which had major influence on automatically calculated values for surface and volume.

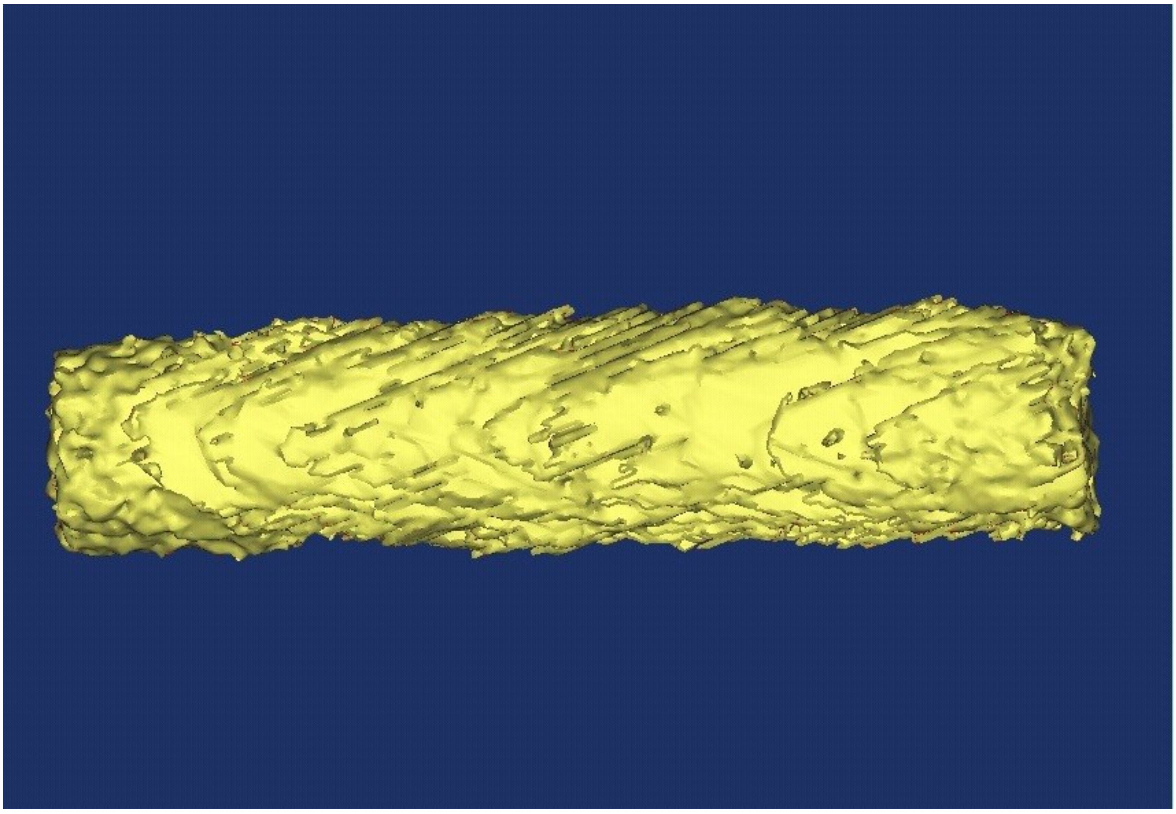

Image XX: irregular in vivo 3D model of pin without bone after 3 months

Ex vivo scanning on the other hand produced valid results. In none of the three blends degradation could be confirmed over the whole observation period. Contrarily, surface and volume of the pins were relatively constant in all scans, though the estimated values of approximately 16,09mm^3 in volume and approximately 44,23mm^2 in surface – outgoing from a cylindrical shape with 8mm in length and 1,6mm in diameter – were exceeded in all groups at all times.

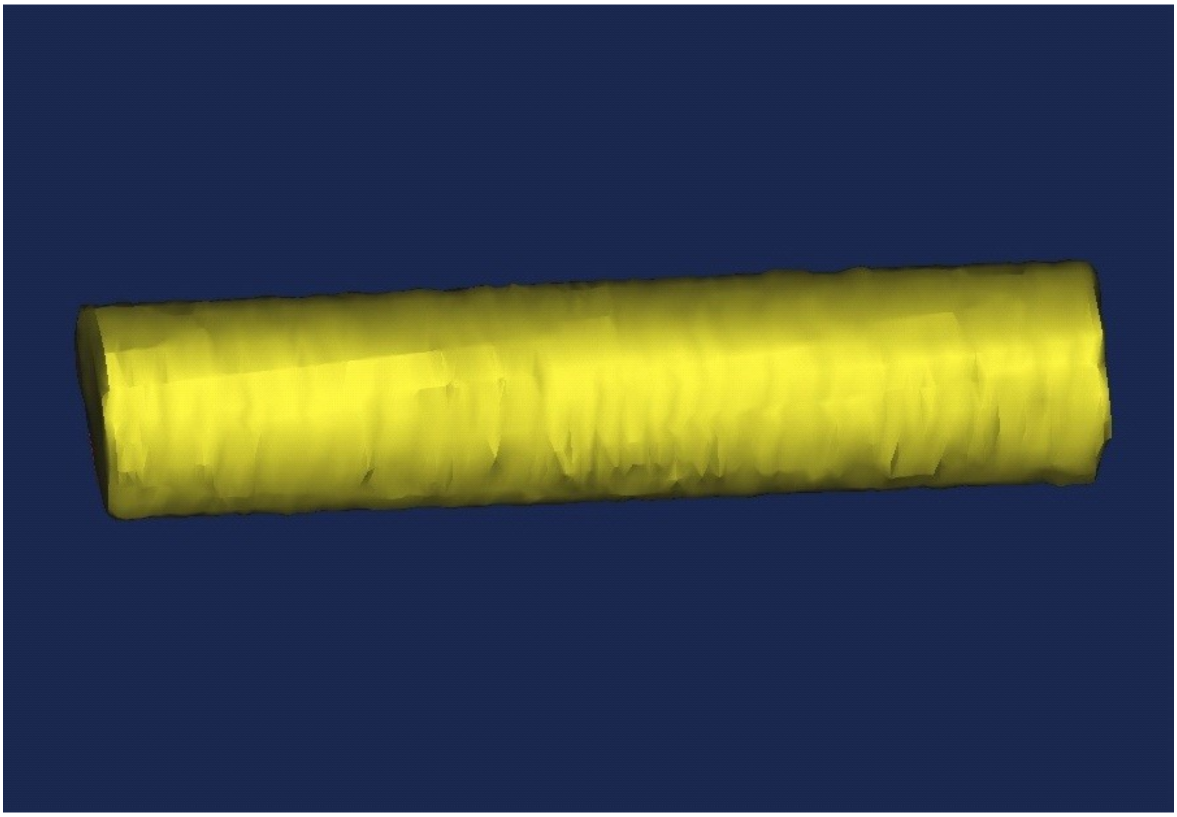

Image 9: regular ex vivo 3D model of pin without bone after 3 months

Initially, in vivo scans immediately post operationem were planned to deliver primary data for both surface and volume of every single pin, that could be set in relation to later scans. Without valid base data, this deviation from intended values cannot certainly be assigned with either pre implantational (i.e. manufacturing irregularities) or post implantational (i.e. dwelling of implants in vivo) factors. The slight variations that were detected between different pins of the same blend can be reduced to varying maceration processes in vivo and manufacturing irregularities [see chapter 3.2]. Also, intraobserver reliability depicts an issue that must not be completely neglected, as small reliability may be accountable for slight variations in final data.

In several scans, pins presented inhomogeneous areas with varying density. This leads to the thought, that problems of mixing PHB, zirconiumdioxide and Herafill® may have occurred during manufacturing and processing of the implants. However, the pins affected did not differ from other pins in terms of degradation, biocompatibility or mechanical properties.

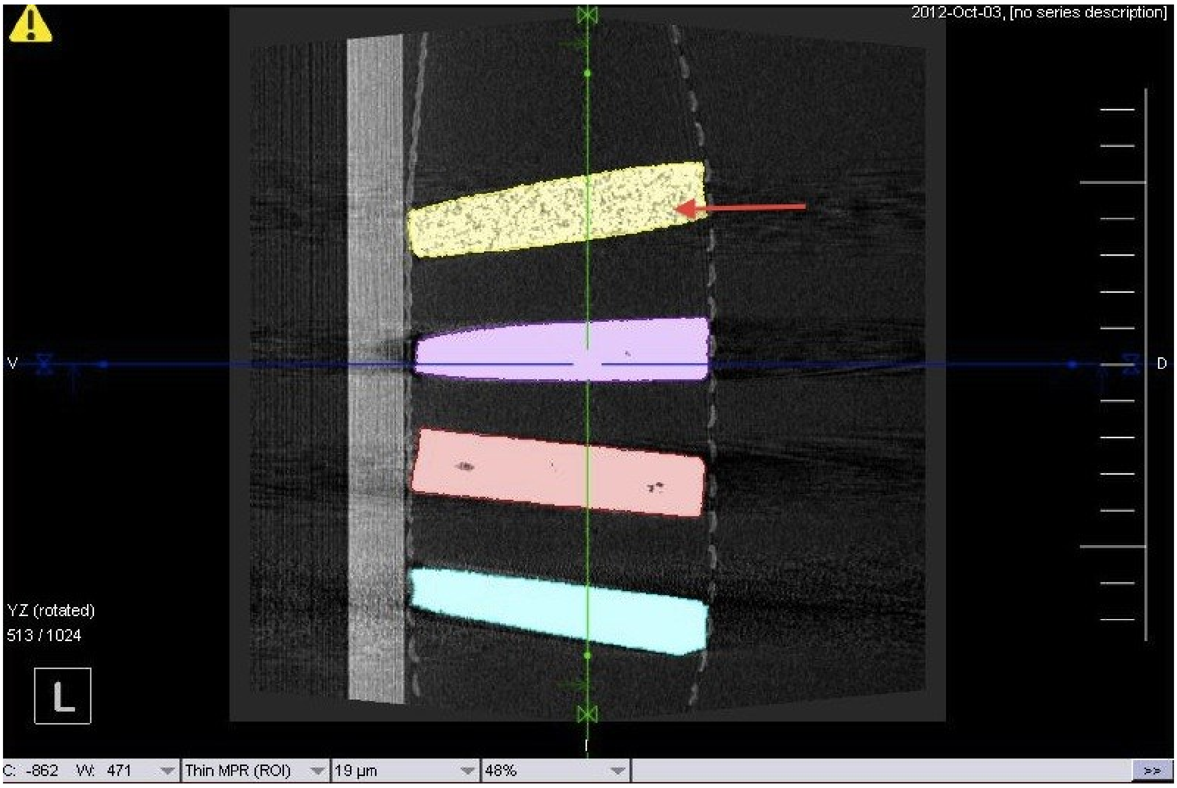

Image 11: inhomogeneous pin (yellow, indicated)

In vivo degradation properties of PHB are described throughout literature as relatively slow (55-56, 67-68). As shown in precedent studies, the degradation of PHB has proven to happen slower in vivo than that of PHB copolymer poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) and PLA degrading faster than both of them in terms of volume and molecular weight/density (67). Also, PHB leads to mild inflammatory reactions in vivo, causing fibrous encapsulation and hence further interfering with degradation when implanted extraosseously (56, 68).

In a study featuring subcutaneously implanted PHB, polylactide (PLA) and poly(3hydroxybutyrate-co-3hydroxyvalerate) copolymers (PHB-VA), PHB presented the least degradation, maintaining its initial appearance and stability over a 6 month period in vivo. Also, an extremely thin, vascularized fibrous capsule caused by mild inflammatory could be detected (67).

The described encapsulation as a sign of mild foreign body reaction (21, 27) of PHB implants and the resulting dissociation from the initially surrounding tissue may very likely be responsible for a decrease in degradation velocity, though the focus was not set on encapsulation in the present study and the very fine fibrous structures were hence not sought for. Also, further factors can affect biodegradability of PHB and hence call for closer examination. *Surface conditions* (i.e. surface area and hydrophilic properties), so called *first order structures* (i.e. chemical structure and molecular weight) and *high order structures* (i.e. glass transition temperature, melting temperature, elastic modulus and cristallinity) are among the most important influences. From this perspective, a relatively high melting temperature of approximately 170°C may be decisive for the slow degradation of PHB along with its highly crystalline morphology. As molecules in crystalline regions are more densely packed, they are less vulnerable to encymatic degradation than molecules in amorphous regions (69).

5 Conclusion

The intention of the study was to clarify the applicability of PHB as a material for self resorbing intramedullary fracture fixation in children, focusing on the quick degradation required to avoid severe complications in a growing skeleton. The present study has demonstrated the in vivo degradation properties of three different polymer based blends in a rat model. By using a µCT device, the degradation of transcortically placed implants was to be monitored in vivo in a non invasive manner. Besides the priorly known imperfect mechanical properties of PHB, the degradation rate of the three blends investigated proved to be definitely too slow for the desired application.

Over a 9 month period the first blend, containing PHB and zirkoniumdioxide, showed no remarkable signs of degradation.
The second and third blend additionally contained different amounts of the biodegradable bone substitute Herafill®. Over the same period, these blends did not exhibit any degradation either. The in vivo µCT results were confirmed by ex vivo high resolution µCT examinations.

Only significant advancement of PHB based materials could provide the properties required in pediatrics, if PHB is applicable as a biodegradable material for osteosynthesis devices at all.

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