OULU 2007
D 945

ACTA UNIVERSITATIS OULUENSIS

Johanna Tiainen

BIORESORBABLE PLAIN AND CIPROFLOXACIN-RELEASING SELF-REINFORCED PLGA 80/20 IMPLANTS' SUITABILITY FOR CRANIOFACIAL SURGERY

HISTOLOGICAL AND MECHANICAL ASSESSMENT

FACULTY OF MEDICINE,
DEPARTMENT OF SURGERY;
OULU UNIVERSITY HOSPITAL

ACTA UNIVERSITATIS OULUENSIS
D Medica 945

JOHANNA TIAINEN

BIORESORBABLE PLAIN AND CIPROFLOXACIN-RELEASING SELF-REINFORCED PLGA 80/20 IMPLANTS' SUITABILITY FOR CRANIOFACIAL SURGERY

Histological and mechanical assessment

Academic Dissertation to be presented, with the assent of the Faculty of Medicine of the University of Oulu, for public defence in Auditorium 1 of Oulu University Hospital, on November 16th, 2007, at 12 noon

OULUN YLIOPISTO, OULU 2007

Acta Univ. Oul. D 945, 2007

Supervised by
Professor Nureddin Ashammakhi
Docent Timo Waris

Reviewed by
Professor Alicia El Haj
Professor Yrjö T. Konttinen

ISBN 978-951-42-8575-2 (Paperback)
ISBN 978-951-42-8576-9 (PDF)
http://herkules.oulu.fi/isbn9789514285769/
ISSN 0355-3221 (Printed)
ISSN 1796-2234 (Online)
http://herkules.oulu.fi/issn03553221/

Cover design
Raimo Ahonen

OULU UNIVERSITY PRESS
OULU 2007

Tiainen, Johanna, Bioresorbable plain and ciprofloxacin-releasing self-reinforced PLGA 80/20 implants' suitability for craniofacial surgery. Histological and mechanical assessment

Faculty of Medicine, Department of Surgery, University of Oulu, P.O.Box 5000, FI-90014 University of Oulu, Finland, Oulu University Hospital, P.O. Box 10, FI-90029 OYS, Finland

Acta Univ. Oul. D 945, 2007
Oulu, Finland

Abstract

Ciprofloxacin was incorporated to plain bioresorbable self-reinforced polylactide/glycolyde 80/20 screws and tacks (ciprofloxacin releasing SR-PLGA). These implants were compared to otherwise similar conventional fixation devices. The effect of the ciprofloxacin addition on the pull-out force of screws and tacks was evaluated in human cadaver cranial bones. SR-PLGA tacks applied to cranial bone with a special applicator gun had a similar holding power as screws. Addition of the antibiotic compromised the strength of the screws so that ciprofloxacin-containing PLGA screws had lower pull-out strength than corresponding plain PLGA screws. Scanning electron microscopy showed that the fibrillar strip-like microstructure of plain SR-PLGA screws turned into a coarse uni-axial platelet-like pattern as a result of ciprofloxacin addition. It is concluded that this type of 4 mm long and 1.5 mm diameter ciprofloxacin-containing screws can only be used in non-load-bearing or slightly load-bearing applications. Tissue reactions elicited by plain bioresorbable self-reinforced polylactide/ glycolide (SR-PLGA) 80/20 screws were compared to similar but ciprofloxacin-releasing SR-PLGA fixation devices in rabbit cranial bone. Plain and ciprofloxacin-PLGA 80/20 screws elicited only mild inflammatory reactions upon implantation in rabbit cranial bone, but they did not interfere with osteoblast activity in up to 72 week long follow-up. Release of the antibiotic from ciprofloxacin-PLGA screws was gradual and the drug concentration in bone tissues was still higher at 8 weeks than the minimal inhibitory concentration (MIC) of ciprofloxacin for S. aureus (0.1–1.0 μg/g). Ciprofloxacin-releasing SR-PLGA screws can find clinical usage in the prevention of implant-related infections in osteofixation in craniomaxillofacial bones in non-load-bearing or slightly load-bearing applications. Larger 6 mm long and 2 mm diameter ciprofloxacin-releasing tacks had a similar holding power to cranial bone as conventional tacks. Tacks can be recommended for clinical use as the application procedure saves time and costs.

Keywords: pull-out strength, SR-PLGA, tissue reaction

To my family

Acknowledgements

This work was carried out at the Department of Surgery, Oulu University Hospital, in co-operation with the Department of Pathology, University of Oulu, Research Center and Institute of Biomaterials in Tampere University of Technology, from 2000 to 2006.

I am deeply grateful to my supervisor, Professor Nureddin Ashammakhi, M.D, Ph.D, FRCSEd, who supported and encouraged me during this work.

I owe my warmest thanks to my supervisor, Docent Timo Waris, M.D., Ph.D., for providing excellent conditions to do this work and for introducing me to scientific research.

My warmest thanks are also due to my co-authors: Docent Ylermi Soini, M.D., Ph.D., for giving invaluable expert assistance in the histological examinations: Minna Veiranto M.Sc., Esa Suokas Ph.D. (Tech.), Jouko Ilomäki M.Sc., Sanna Leinonen M.D., for helping with mechanical tests: Pertti Törmälä, MDhc, Ph.D. and Satu Länsman M.D., for giving critical comments for the articles.

I wish to thank Professor Jorma Hirvonen and Mr Jorma Kokkonen for technical help in collecting bone samples, Jyri Koort, M.D., PhD for helping with concentration measurements, Ms Seija Seljanperä and Mr Veikko Lähteenmäki for advice with animal experiments, Mrs Tanja Kuusisto for help with the histological specimens, Mrs Liisa Kärki and Mrs Seija Leskelä for technical assistance in preparing the figures and Mr Pasi Ohtonen for consultation on statistics.

During these years, the support of my family has been extremely valuable. I wish to thank my parents and my sisters for their support and encouragement throughout my life.

Finally I wish to express my loving thanks to my fiancé Markki Palve.

This research was financially supported by the Technology Development Center in Finland, the European Commission, the Academy of Finland, and the Ministry of Education (Graduate School of Biomaterials and Tissue Engineering).

Oulu, May 2007 Johanna Tiainen

Abbreviations

BG	bioactive glass
CO_2	carbondioxide
CT	computerized tomography
D	dekstro (optical isomer)
im	intramuscular
iv	intravenous
L	levo (optical isomer)
µg	microgram (10^{-6}g)
MPa	mega (10^6) Pascal (N/m^2)
MRI	magnetic resonance imaging
MRSA	methicillin resistant Staphylococcus aureus
N	newton (kgm/s^2)
Pa	Pascal (N/m^2)
PGA	polyglycolic acid or polyglycolide
PLA	polylactic acid or polylactide
PLLA	poly-L-lactic acid
PDLLA	poly-D/L-lactic acid
PLGA	polylactic/glycolic acid, copolymer of polylactide and polyglycolide
PMMA	polymethylmethacrylate
SEM	scanning electron microscopy
SR	self-reinforced
T_g	glass transition temperature (^oC), temperature at which changes of material phases between the crystalline (glassy) and liquid phase occur
T_m	melting point

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I Tiainen J, Leinonen S, Ilomäki J, Suokas E, Törmälä P, Waris T & Ashammakhi N (2002) Comparison of the Pull-out Forces of Bioabsorbable Polylactide/glycolide Screws (BioSorb and LactoSorb) and Tacks: a Study on the Stability of Fixation in Human Cadaver Parietal Skull Bones. J Craniofac Surg 13(4): 1-6.

II Tiainen J, Veiranto M, Suokas E, Törmälä P, Waris T, Ninkovic M & Ashammakhi N (2002) Bioabsorbable ciprofloxacin-containing and plain self-reinforced polylactide-polyglycolide 80/20 screws: pull-out strength properties in human cadaver parietal bones. J Craniofac Surg 13(3): 427-433.

III Tiainen J, Soini Y, Törmälä P, Waris T & Ashammakhi N (2004) Self-reinforced polylactide-polyglycolide 80/20 screws take more than 1½ years to resorb in rabbit cranial bone. J Biomed Mater Res [Appl Biomat] 70B(1): 49-55.

IV Tiainen J, Soini Y, Suokas E, Veiranto M, Törmälä P, Waris T & Ashammakhi N (2006) Tissue Reactions to Multifunctional Bioabsorbable Ciprofloxacin-Releasing Polylactide-Polyglycolide 80/20 Screws in Rabbits' Cranial Bone. J Mater Sci Mater Med 17(12): 1315-1322.

V Tiainen J, Veiranto M, Koort J, Suokas E, Kaarela O, Törmälä P, Waris T & Ashammakhi N (2006) Bone Tissue Concentrations of Ciprofloxacin Released from Biodegradable Screws Implanted in Rabbits Skull. Eur J Plast Surg (Accepted).

VI Tiainen J, Knuutila K, Veiranto M, Suokas E, Törmälä P, Kaarela O, Waris T & Ashammakhi N (2006) Multifunctional Bioabsorbable Ciprofloxacin-Releasing Polylactide-Polyglycolide 80/20 Tacks: Assessment of Pullout Strength in Human Cadaver Cranial Bone. (Manuscript).

Contents

1 Introduction

Bioresorbable polymers have been in clinical use as sutures for the last three decades. Since the 1960s continuous research and development in the field of biomaterials has led to the introduction of reliable bioresorbable internal fixation devices (Eppley and Sadove 1995b, Habal *et al.* 1999, Eppley *et al.* 1997a). At the advancing front of successful implants are the new self-reinforced (SR) devices, which have been produced and used in orthopedic, trauma (Rokkanen 1998, Törmälä *et al.* 1998), hand (Waris *et al.* 2004), thoracic (Länsman *et al.* 2002) and craniomaxillofacial (Ashammakhi *et al.* 2001) surgery. The biocompatibility of polyglycolide (PGA) and polylactide (PLA) and their copolymer (PLGA) has been demonstrated in several studies (Ashammakhi *et al.* 2001, Nordström *et al.* 1998, Jain 2000). The copolymer PLGA has gained wide acceptance in craniofacial surgery as an osteofixation material (Eppley and Sadove 1995a, Habal *et al.* 1999, Eppley *et al.* 1997). PLGA miniscrews have been developed and applied successfully in the treatment of craniofacial syndromes and in trauma cases (Waris *et al.* 1994, Serlo *et al.* 1998, Serlo *et al.* 2000, Serlo *et al.* 2001).

To develop a successful bone fixation device that can also release agents such as antibiotics it is necessary to consider mechanical, drug release, and biocompatibility properties. When the biomechanical properties of osteofixation devices are assessed, the fixation to the bone is of utmost importance. Pull-out tests of implanted screws have long been performed to evaluate holding power (Koranyi *et al.* 1970). Biocompatibility refers to how a material elicits an appropriate host response in a specific application (Vert *et al.* 1992) so that the implant material does not lead to any problems and can function properly. Tissue reactions to implanted material are both numerous and complex. The term “biocompatibility” also describes aspects of such interactions (Hunt *et al.* 1993, Vince *et al.* 1991). The most important aspect of biocompatibility testing is the analysis of the local tissue response (Vince *et al.* 1991).

Bone infection (osteomyelitis) may complicate surgical procedures and trauma. Osteomyelitis is commonly caused by the bacteria *S. aureus* or Pseudomonas spp., e.g. *P. aeruginosa* (Osterman *et al.* 1993). Osteomyelitis is still a serious problem for the patient as well as a difficult treatment predicament for the surgeon (Serlo *et al.* 2000). Untreated and unresolved, acute osteomyelitis may progress to chronic osteomyelitis with consequent bone destruction, deformity, sinus formation and other complications. Treatment of osteomyelitis is

based on a systematic approach, starting from the accurate identification of the pathogen and its sensitivity to antimicrobial treatment. The treatment keystones are radical surgical debridement of the infected tissues, obliteration of the dead space, adequate soft tissue coverage and a minimum of 4-6 weeks of intravenous antimicrobial therapy (Becker *et al.* 1994, Calhoun *et al.* 1997, Nie *et al.* 1998, Koort *et al.* 2005). Dead bone and soft tissue form good environments for bacteria. As infected bone has a compromised blood circulation even iv-administered antibiotics may not achieve adequate therapeutic levels without detrimental systemic effects (Becker *et al.* 1994, Shinto *et al.* 1992). Furthermore, bacteria on implant surface form biofilms and are in part protected by the extracellular polymeric matrix. Developing a local delivery system of antibiotics is an attractive treatment mode.

Several types of delivery system for local antibiotic delivery have been used, including use of bone cement (Carlsson *et al.* 1978, Nijhof *et al.* 2000), polymethylmethacrylate (PMMA) and bioresorbable carriers such as polylactide-co-glycolide (Humphrey *et al.* 1998) and hydroxyapatite ceramic (Shinto *et al.* 1992). PMMA beads impregnated with gentamicin have been successfully used for prophylaxis and treatment of bone infections since 1970s (Jacob *et al.* 1991, Ostermann *et al.* 1993, Becker *et al.* 1994). Having the antibiotic released, the non-absorbable carrier system may get itself colonized and infected by bacteria (Ashammakhi *et al.* 2005a). Because PMMA is a biostable polymer, second surgery is needed to remove the beads. This may lead to further damage to the soft tissue (Overbeck *et al.* 1995, Jacobs *et al*. 1991) and pose additional risks of infection (Becker *et al.* 1994). A bioresorbable carrier for the antibiotic would obviate the need for this additional surgical procedure and may also potentially reduce the duration and cost of hospitalization (Jacob *et al.* 1991). Antibiotic releasing osteofixation devices may reduce the risk of surgical infection, especially in trauma cases which may be contaminated and where bone infection may supervene even with careful surgical prophylactic measures (Habal 2001). The use of PLA, PGA, and PLGA matrices for controlled release of therapeutic agents has been explored (Nie *et al.* 1998, Calhoun *et al.* 1997, Overbeck *et al.* 1995, Gümüsderelioglü *et al.* 2000, Kitchell *et al.* 1985, Jain 2000, Viitanen *et al.* 2006). Their biocompatibility, mechanical properties, degradation rates, and resorbability through biological processes make them an attractive option because they do not need removal (Humphrey *et al.* 1998). Polymer matrices degrade in body fluids and progressively release the contained antibiotic. Polymer selection

affects their biodegradation rate and, therefore, a system can be designed for short or long drug-release periods (Gümüsderelioglü *et al.* 2000).

In the current study, ciprofloxacin was selected as the antibiotic of choice because of its activity against *S. aureus* and Pseudomonas spp., e.g. *P. aeruginosa* (Teupe *et al.* 1992). Ciprofloxacin has been shown to have a greater penetration through non-infected cortical bone, from PGA carriers, than has been reported for gentamycin released from PMMA beads (Overbeck *et al.* 1995). Released ciprofloxacin from sterilized manufactured PLGA implants was found to be bacteriologically bioactive (Niemelä *et al.* 2006).

The main disadvantage of bioresorbable screws is the time needed for their insertion. The application of bioresorbable screws includes drilling, tapping, insertion of screws and tightening them using a screwdriver. Tacks, which can be inserted by mechanical pressure, allow a considerable saving of operation time, depending on the number to be inserted. In this study pull-out forces of plain and ciprofloxacin-releasing PLGA 80/20 tacks were measured. Tacks were inserted with a recently developed inserter instrument (Crossbow), which at first was developed for installation of SR bioresorbable arrows into meniscus tissue (Albrecht-Olsen *et al.* 1993). In this method, tapping, and tightening of a screw is replaced by a rapid one-go shot of a tack into a drill hole. Tacks seemed to have better pull-out forces than screws. Serlo *et al.* (2002) used SR-P(L/DL)LA (70/30) tacks in the cranioplasty in human. With tacks and plates, stable and secure fixation was obtained. The cosmetic result was excellent one year after surgery.

In the present study the biocompatibility of the plain and ciprofloxacin-containing SR-PLGA 80/20 screws was good, as they elicited only a mild inflammatory tissue reaction when implanted in rabbit cranial bone (paper III and IV). No remarkable differences were seen in the degradation processes of these two types of screws during the 1.5 years follow-up time. Bioresorbable ciprofloxacin-releasing PLGA 80/20 screws can function as local delivery systems, besides their primary function in osteofixation. This should allow the achievement of high local antibiotic levels at the site of implantation in the bone. Ciprofloxacin-releasing SR-PLGA (80/20) screws have lower pull-out strength than corresponding plain SR-PLGA (80/20) screws and they can be used along with conventional SR-PLGA miniscrews for bone fixation and antibiotic delivery (paper II). Plain and ciprofloxacin-releasing tacks seemed to have better pull-out forces than corresponding screws. Because of the quick insertion of tacks, they will offer a good alternative for screws in osteofixation in the future.

2 Review of literature

2.1 Metal cranial osteofixation devices

Surgical reconstruction of the cranial skeleton has become a routine procedure for most craniofacial surgeons. Pioneered by Paul Tessier in the 1970s, this type of repair has been used in the treatment of a variety of disorders ranging from congenital malformations to tumor ablation and reconstruction. Initially, most large reconstructive procedures used stainless steel wire to stabilize the surrounding bone. In the 1980s, miniplate and microplate systems were developed for cranial bone stabilization. These systems offered the advantages of ease of application and improved rigid fixation (Stelnicki *et al.* 1998, Eppley *et al.* 1993, Beals *et al.* 1987). Classically, internal fixation of bones has been performed using metallic devices. Through advances in materials and manufacturing techniques, adequate fixation has become achievable with smaller and smaller plating systems (Tatum *et al.* 1997). Metals that are commonly used include titanium and its alloys (titanium-aluminium-vanadium), vitallium (cobalt-chromium-molybdenum alloy) and stainless steel (Eppley *et al.* 1993, Sun *et al.* 1997, Rubin *et al.* 1997, Mathy *et al.* 2002). Pure titanium has provided superb biocompatibility and strength (Tatum *et al*. 1997). Although many manufacturers purport that their implants are composed of pure titanium, trace amounts of other metals such as aluminium and nickel have been found in surrounding tissues (Eppley 1997c).

Plate and screw stabilization provides three-dimensional position control of surgical osteotomies or bone fragments. It has gained increased acceptance and application in orthognathic, trauma, and congenital facial reconstruction. This clinical experience has made the surgeon aware of biologic and clinical implications of rigid fixation apart from its ability to maintain bone position (Yaremchuk 1994). Although plate fixation is presumably safe in the adult patient, their safety in the skull of a developing child with growing skeleton is in question (Stelnicki *et al.* 1998).

2.1.1 Stress shielding

Rigid metallic fixation prevents the rapid proliferation of periosteal callus and deprives the bone of the normal stress pattern because of the large difference in

stiffness between cortical bone (E=10-20 GPa) and metals (E=100-200 GPa). The stiffer the osteosynthesis, the more frequent are delayed unions or nonunions (Vert *et al.* 1984). The effects of stress shielding are well-known in long bones. “Every change in the form and function of the bone or of its function alone is followed by certain definite changes in the bone internal architecture, and equally definite alteration in its external conformation, in accordance with mathematical laws.” (Wolff 1892). Bone growth responds to the need for new bone according to the stress that is placed on it. Conversely, decreasing the load would lead to bone resorption (Kennady *et al.* 1989a). Structural changes, including cortical thinning, porotical transforming, have been noted in long bones where rigid fixation plates have been placed (Kennady *et al.* 1989a, Kennady *et al.* 1989b, Paavolainen *et al.* 1978). The decrease in calcium content has also been noted (Paavolainen *et al.* 1978). Weakening of the bone leads to the increased risk of bone and implant fractures. In experimental studies, stress shielding has been shown to occur in grafted mandibular bone, and bone resorption has been reported clinically in association with use of rigid plate fixation (Kennady *et al.* 1989 a, b).

2.1.2 Infections

Metallic implants may also harbor infection which is difficult to eradicate without implant removal. The coagulase-negative staphylococci, particularly *Staphylococcus epidermidis*, cause more infections of medical devices than any other category of micro-organisms. These gram-positive bacteria are the leading cause of infections of orthopaedic devices (Christensen *et al.* 1994). The pathogenesis of such infections has been correlated with the ability of some strains to produce large amounts of extracellular material termed slime. Several polysaccharide components have been described as chemical markers of slime produced by *Staphylococcus epidermidis*. Hexosamine have been indicated as the main component of an antigen which may be active in colonization by *S. epidermidis* (Baldassarri *et al.* 1996). Some investigations have indicated that matrix proteins absorbed on the implant surface can support the adhesion of *Staphylococcus epidermidis* strains to various polymers, while others could not detect any increment in the rates of coagulase negative staphylococci adhesion to either silicone tubing or Teflon catheters. Conflicting data also exist regarding the possible contribution of surface hydrophobicity to the ability of coagulase negative staphylococci to infect biomaterial implants (Baldassarri *et al.* 1997). Upon contact, bodily fluids immediately coat all surfaces, including medical

devices, with a layer of host materials - primarily serum proteins and platelets - in a process known as surface conditioning. Once bound to the surface, these host materials can serve as the “signal” for receptor-specific bacterial binding via an adhesion-mediated attachment. Bacterial colonization begins with exposure of the device to bacteria and bacterial attachment to the surface. The colonization proceeds as bacteria produce slime (biofilm), which stabilizes the cell-to-cell and cell-to-surface associations allowing the bacteria to accumulate on the medical device (Christensen *et al.* 1994, Christensen *et al.* 1982). The biofilm protects the bacteria from the host defense mechanisms and from the action of antibiotics (Chang *et al.* 1992, Chang *et al.* 1991, Costerton *et al.* 1987, Gristina *et al.* 1985) and may alter the subsequent interaction of other bacteria (Chang *et al.* 1992, Chang *et al.* 1991). The enhanced antibiotic resistance of biofilm bacteria can lead to chronic bacterial infections (Hoyle and Costerton 1991). Many approaches have been explored to enhance the resistance of biomaterials to bacterial adhesion. It would seem to be advantageous to promote bactericidal, bacteriostatic, or phagocytic activity at the implant surfaces, and to develop biomaterials that minimize the initial adherence of bacteria (Gristina *et al.* 1985). In addition, there have been many approaches to treat these infections. Removal of the implant is the only method that assures a cure of the infection. Prevention of these infections is thus important (Chang *et al.* 1992).

It has been estimated that the use of local antimicrobial prophylaxis may carry less risk of inducing resistant strains than the current practice of routine systemic administration of antibiotics for 24-48 h after implant surgery (Mäkinen *et al.* 2005). Local antimicrobial prophylaxis is based on the controlled release of the antibiotic aimed to achieve adequate local bactericidal tissue concentrations for a prolonged time without significant systemic exposure (Holtom and Patzakis 2003). With metal implants, one option is to use thin biodegradable polymer coatings impregnated with antibiotics as a local drug delivery system (Gollwitzer *et al.* 2003, Lucke *et al.* 2003). The use of bioresorbable orthopedic implants provides a possibility for direct impregnation of antimicrobial agents within the polymer matrix of the implant (Mäkinen *et al.* 2005).

2.1.3 Corrosion

In vivo corrosion of the metal implants is a major potential disadvantage. Corrosion can cause reduction of the mechanical properties, shortening of the implant life time and harmful reactions in the host body both locally and

systemically. A number of investigations have demonstrated that metal ions are released from metallic implants as a result of corrosion (Sun *et al.* 1997, Williams 1972). These metal ions can be found in blood or serum, urine, regional lymph nodes, bone tissue adjacent to implants (Rubin *et al.* 1997, Sun *et al.* 1997), spleen (Tracana *et al.* 1995), liver and lungs (Eppley 1997c). It has been shown that Ti and Co ions can inhibit specific cellular functions such as alkaline phosphatase activity, extracellular calcification/ bone formation and bone-specific gene expression. The effect of metal ions on osteoblasts has been investigated. Results showed that metal ions may alter osteoblast behavior even at subtoxic concentrations (Sun *et al.* 1997).

The clinical importance of degradation of metal implants is evidenced by corrosion and wear products found in tissues surrounding the implant, which may ultimately result in a cascade of events involving inflammation and leading to periprosthetic bone loss. Furthermore, many authors have reported increased concentrations of local and systemic metal in association with metal implants. There also is a low but finite prevalence of corrosion-related fracture of the implant (Jacobs *et al.* 1998).

2.1.4 Pseudomigration and growth restriction

In craniomaxillofacial surgery the pseudomigration of metals can be a problem, especially in the growing skulls of children. The cranial growth takes place by the laying of new bone on the outer surface. An osteosynthesis plate becomes invaginated by appositional bone growth and intracranial resorption after a few weeks; the plate passively migrates into the cranium. Implants can be found on the inner side of the skull, or even against the dura (Fearon *et al.* 1995, Papay *et al.* 1995, Hönig *et al.* 1995, Yu *et al.* 1996, Mathy *et al.* 2002). The osteosynthesis plate may migrate also as a result of subimplant resorption at the interface between plate and subimplant bed. This resorption is caused by impairment of the nutrient supply to the subimplant tissue and is greater when the plate is flush with the bone (Hönig *et al.* 1995). Stelnicki *et al*. (1998) showed in minipigs that both titanium microplates and stainless steel wires can migrate intracranially. None of the plates or wires penetrated the dura, but several pieces of hardware from each group migrated far enough to rest directly on dural surface. In a retrospective review, Goldberg *et al.* (1995) noted that internalization of microfixation had occurred in 14 of 27 paediatric patients. Statistically significant factors for microplate translocation included longer plates and plates placed in the temporal

region. Specific complications related to translocation of microplates were not found in any patient. Direct effects of translocated microplates and screws on the underlying brain and dura remain unclear (Goldberg *et al.* 1995). Vanderkolk *et al*. showed in a CT-based study that microplates migrate intracranially in infant human patients (Stelnicki *et al.* 1998). Rovati *et al*. (1997) have reported a case where steel wire was included in the frontal sinus and was in contact with the dura mater causing pneumococcal meningitis seven years after implantation (Rovati *et al.* 1997).

There is a potential risk of damage to the brain upon traumatic implant dislodgement (Tatum *et al.* 1997). Hence, it is recommended that all metals used in infant crania should be removed as early as possible (within three months postoperatively, Hönig *et al.* 1995). Removal operations are associated with potential complications and extra costs, for obvious reasons, as the second operation is usually technically more difficult, consumes more time, and requires more facilities (Papay *et al.* 1995).

Thus, one potential drawback of rigid internal fixation is the need for an additional procedure to remove the plates and screws (Orringer *et al.* 1998). In paediatric craniomaxilofacial surgery, metals may lead to growth disturbances of the skull bones. It is not possible to use metallic osteosynthesis devices in infants under the age of six months because of the softness of the skull (Serlo *et al.* 2001). Resnick *et al*. (1990) noted a significant reduction in growth following plate and screw fixation across the coronal suture in rabbit models (Resnick *et al.* 1990). Wong *et al.* (1991) found in rabbit model that coronal suture plating and frontal bone plating resulted in local growth restriction. In a cat model a restriction of growth was noted after osteotomy and fixation with miniplates that crossed the coronal suture (Lin *et al.* 1991). A study with monkeys showed that osteotomy and fixation in a growing primate skull results in clearly visible and measurable changes in the subsequent skull growth and morphology, even when suture lines are not crossed (Yaremchuk *et al.* 1994). Yaremchuck *et al*. noted that the more complex the fixation, the greater the magnitude of growth changes are. Hence, it is advised that the least amount of fixation needed to provide three-dimensional stability should be used (Yaremchuck 1994).

2.1.5 Postoperative imaging

Postoperative imaging may be necessary to evaluate the status of both hard and soft tissues. Radio-opacity of metals can lead to obliteration of the view of tissues

lying behind them, interfering with adequate radiological evaluation such as CT scanning (Fiala *et al.* 1993, Fiala *et al.* 1994, Eppley *et al.* 1993) and MRI (Fiala *et al.* 1993, Fiala *et al.*1994). This is a disadvantage especially in the field of craniomaxillofacial surgery, where neuroimaging is needed. This interference is of particular concern in growing infants and children in whom serial long-term CT scanning may be necessary to monitor brain growth after reconstruction (Eppley *et al.* 1993). Current metallic compositions of fixation systems are strongly nonmagnetic and do not produce ferromagnetic forces. Thus, complications associated with metallic implants in MRI examination do not appear to be a significant concern (Eppley *et al.* 1993, Habal 1991). The artifacts seen in CT (Fiala *et al.* 1993, Fiala *et al.* 1994, Eppley *et al.* 1993, Anastakis *et al.* 1996) and MR scans associated with titanium implants were less than those associated with stainless steel and vitallium implants (Fiala *et al.* 1993, Fiala *et al.* 1994, Eppley *et al.* 1993). Problems associated with imaging techniques and plating systems are avoided when absorbable miniplates can be used (Habal 1991). Peltoniemi *at el*. showed that radiolucent SR-PLLA plates did not interfere with CT (Peltoniemi *et al.* 1997).

2.1.6 Palpability, visibility, hyper/cold sensitivity

Metal implants may also be associated with cold sensitivity in the facial skeleton, which is especially important consideration in cold climates (Yaremchuck 1994, Eppley and Prevel 1997a, Tatum *et al.* 1997). Visibility and palpability of plates in prominent areas can also cause problem (Tatum *et al.* 1997, Rubin *et al.* 1996, Yaremchuck 1994). Hypersensitivity reaction resulting in sequelae such as pain and overlaying dermatitis has been reported with Vitallium and stainless steel and suspected to also occur with titanium (Rubin *et al.* 1996).

2.2 Bioresorbable osteofixation devices

Soon after synthetic polymers were invented, about 60 years ago, the medical profession realized that this new class of materials may have potential for a variety of therapeutic and technical uses. Since then, the list of polymers evaluated with respect to the concept of biomaterials has grown rapidly (Vert *et al.* 1992, Waris *et al.* 2004b).

Research has been focused on a class of aliphatic polymers called poly-α-hydroxy acids (Vainionpää *et al.* 1989). This class has been under intensive

research in the development of osteosynthesis devices since the 1960s. Devices made of polyglycolide (PGA) or polylactide (PLA) are the strongest ones in this class (Ashammakhi *et al.* 1997). Poly(α-hydroxy-acids) constitute a particular class of polyesters whose repeating units, $[-O-CO-CHR-]_n$, are derived from α-hydroxy-acids, HO-CHR-COOH (Vert *et al.* 1992, Ashammakhi *et al.* 1997). Among the whole family, members composed of constitutive repeating units with R=H (glycolic acid) or $R=CH_3$ (lactic acid) are known to be bioresorbable (Vert *et al.* 1992). Polymers are produced by using ring-opening polymerization of cyclic glycolide and lactide diesters (Vert *et al.* 1984).

Poly(α-hydroxy-acid) polymers such as polylactides (PLAs) and polyglycolides (PGAs) undergo a two-phase degradation in the body. Phase I is primarily physical in nature. During this phase water molecules hydrolytically attack the chemical bonds of the polymer, resulting in random chain scission of the polymer molecules by cleavage of their ester groups. This process requires an aqueous environment, although enzymatic and cellular activities may in certain circumstances accelerate the process (Pietrzak *et al.* 1997, Eppley *et al.* 1994, Bos *et al.* 1991). In the process, overall molecular weight is reduced (Pietrzak *et al.* 1997). Initial breakdown of the polymer molecules occurs first in the amorphous areas of the material, which are more loosely packed than the denser crystalline regions. They are, therefore, more easily penetrated and attacked by body fluids (Eppley *et al.* 1994, Bos *et al.* 1991). Phase II involves the physiological response of the body whereby macrophages phagocytoze the fragments and metabolize them to substances such as water and carbon dioxide (Pietrzak *et al.* 1997).

Over the years, research has shown that PLA and PGA are suitable polymers for resorbable implantable devices (Montag *et al.* 1997, Pistner *et al.* 1993). The PLA polymers degrade very slowly due to their tightly packed semicrystalline structure and resultant hydrophobicity (Bos *et al.* 1991, Epple *et al.* 1994, Montag *et al.* 1997). Pure PLA implants may also cause a foreign body reaction over time, occasionally requiring extraction (Montag *et al.* 1997). Conversely, PGA polymers have an increased rate of resorption due to their more amorphous character and increased hydrophilicity (Eppley *et al.* 1994, Montag *et al.* 1997). This also results in embrittlement of the polymer and loss of tensile strength (Montag *et al.* 1997). In PLA-PGA copolymer compounds, the hydrolytic chain scission of each blend component is significantly modified by the presence of the second component (Eppley *et al.* 1994).

Bioresorbable implants are less stiff than bone, and they eliminate potential stress shielding associated with long term use of metallic implants. Bioresorbable

implants do not interfere with therapeutic or imaging usage of radiation, and make the revision procedures unnecessary (Habal *et al.* 1999). Self-reinforcment increases the strength of the device considerably, but still enables molding of the SR plates at room temperature. In contrast, nonreinforced plates need to be heated before shaping. To achieve the same mechanical strength they need to be bigger in size. This may increase the risk of clinically significant foreign body reaction (Bergsma *et al.* 1993). If PLGA implants are heated in excess of their T_g, their flexural modulus falls by 20%. The PLGA plates need two minutes of cooling down to regain 50% of their stiffness. After one week (*in vitro*), the mechanical properties of heated and nonheated plates are identical (Pietrzak *et al.* 1997b). The SR technique has increased the bending strength of devices several fold compared with their initial strength values (Törmälä *et al.* 1988). Internationally patented SR technique entails sintering of a polymer material to yield a composite in which matrix and reinforcing elements are of the same polymer (Törmälä *et al.* 1990). Bioresorbable devices maintain the required fixation, decompose gradually and the stresses are transferred gradually to the healing bone, which avoids clinically significant stress shielding (Rokkanen 1990).

Bioresorbable devices are radiolucent and they do not interfere with current imaging techniques, e.g. CT or MRI. Because bioresorbable devices can be considered tissue equivalent, they do not interfere with postoperative radiotherapy (Rozema *et al.* 1990). Postoperative radiation does not significantly affect the mechanical properties of the bioresorbable implants (Hofmann *et al.* 1996).

2.2.1 Polyglycolic acid (PGA)

PGA is manufactured by ring opening of the dimer (glycolide) hence the name polyglycolide. Both names, polyglycolic acid and polyglycolide, are used interchangeably (Ashammakhi *et al.* 1997). Glycolide, the cyclic dimer condensation product, is formed by dehydrating glycolic acid. PGA of high molecular weight (20,000 to 145,000) is a hard, tough, crystalline polymer melting at about 224-228 ^{o}C, with a T_g of 36^{o}C (Ashammakhi *et al.* 1997, Vainionpää *et al.* 1989, Suuronen *et al.* 1993). Unlike closely related polymers such as PLA, PGA is insoluble in most of the common polymer solvents (Vainionpää *et al.* 1989, Suuronen *et al.* 1999). SR-PGA shows the best initial strength properties equaling those of stainless surgical steel (330 MPa). Its mechanical properties are lost within six weeks and PGA is completely resorbed from the tissues in less than one year (Suuronen *et al.* 2000). PGA can be spun

into fibers which increase the strength properties of the polymer because of high preferred molecular orientation of the polymer in the fiber (Ashammakhi *et al.* 1997).

The degradation of PGA is a two-stage process. First, water diffuses into the amorphous regions of the polymer leading to random hydrolytic chain scission of the ester bonds. When most of the amorphous regions are degraded, hydrolytic attack continues on the crystalline domains (Holland *et al.* 1986). Small debris particles produced during degradation trigger phagocytosis reaction, by which macrophages, giant cells, and other leukocytes digest polymer debris particles. Finally, the foreign material disappears totally. *In vivo*, PGA is degraded into glycolic acid monomers by hydrolysis (Rokkanen 1998). When exposed to glycolate oxidase the glycolic acid molecules are transformed into glyoxylate which reacts with glycine transaminase, thus producing glycine. Glycine can be used in the protein synthesis or in the synthesis of serine which can be employed in the tricarboxylic acid cycle after transformation into pyruvate yielding energy, CO_2 and water. Glycolic acid is partially excreted in urine (Ashammakhi *et al.* 1997, Rokkanen 1998). Cellular enzymes affect the rate of the degradation (Williams and Mort 1977).

2.2.2 Polylactic acid (PLA)

Another important polymer used for manufacturing surgical devices is the polymer of lactic (α-hydroxycarboxylic) acid, PLA (Serlo *et al.* 2001). PLA is a pale-colored semicrystalline polymer (Suuronen *et al.* 1999), which has two enantiomeric isomers, the L and D isomers (Suuronen *et al.* 2000, Serlo *et al.* 2001). L-PLA is an optically active stereoregular form and D,L-PLA is an optically inactive racemic form. L-PLA is semicrystalline in nature due to high regularity of its polymer chain while D,L-PLA is an amorphous polymer because of irregularities in its polymer chain structure. Hence, the use of D,L-PLA enables a more homogeneous dispersion of the drug in the polymer matrix than the L-PLA (Jain 2000).

These isomers can be copolymerized in different ratios. Racemic PDLLA contains 50% of both enantiomers (50L:50D) and shows only modest mechanical strength. PLLA has the best strength properties (bending strength up to 240 MPa, Suuronen *et al.* 2000). The resorption time of PLLA is long. During degradation, it forms crystals, which take considerable time to resorb (approximately 5 to 7 years). The addition of the D isomer into the PLA polymer chain makes it more

amorphous, with faster degradation. Various degradation rates of the copolymer can be achieved by changing the percentages of L and D components (Suuronen *et al.* 1999).

The use of copolymers of L-lactic acid and D-lactic acid (e.g. 70L:30DL polymer) instead of pure PLLA has become more common. Mechanical strength is retained for 3 to 4 months. The biodegradation time of 70L:30DL is estimated to be 2 to 3 years. The degradation of P(L/LD)LA takes place in two phases, and the local tissue tolerance is, therefore, not exceeded during degradation (Suronen *et al.* 2000).

PLA with plastic properties was introduced as early as 1955. Later, surgical applications of materials made of either poly-L/D-lactide (PDLA) or poly-L-lactide (PLLA) were tested in the 1960s and 1970s. PLLA was initially more commonly used in clinical applications, but it is becoming replaced increasingly by more rapidly degrading PLDLA and PLGA copolymers (Ashammakhi *et al.* 2005b).

Poly-DL-lactic acid has a melting point (T_m) of about 60^{o}C and no detectable glass transition temperature (Vert *et al.* 1984). Poly-L-lactic acid has a T_m of about 174-184^{o}C with a T_g of 57^{o}C (Vainionpää *et al.* 1989, Serlo *et al.* 2001, Suuronen *et al.* 1999).

The degradation of P(D,L)LA occurs in two stages. Random hydrolytic chain scission of the ester bonds occurs first and is accompanied by a linear loss in molecular weight. The second stage begins at an average molecular weight of 15,000, at which phase also weight loss and total loss of tensile strength occur, along with an increase in chain breakdown (Holland *et al.* 1986). PLA undergoes hydrolytic de-esterification into lactic acid and then to pyruvate, which becomes incorporated into the tricarboxylic acid cycle to produce carbon dioxide and water (Serlo *et al.* 2001, Rokkanen 1998).

2.2.3 Polylactic/glycolic acid (PLGA)

Poly(lactide-co-glycolide) (PLGA), a bioresorbable aliphatic polyester, has been well documented for its excellent biodegradability, biocompatibility and non-toxicity properties (Loo *et al.* 2004). PGA is highly crystalline because it lacks the methyl side groups of the PLA. Lactic acid is more hydrophobic than glycolic acid and hence lactide-rich PLGA copolymers are less hydrophilic, absorb less water, and subsequently degrade more slowly (Jain 2000). The copolymer

PLLA/PGA is substantially amorphous, retains 70% of its initial strength for 6 to 8 weeks, and degrades within 1 year (Suuronen *et al.* 2000).

In PLGA copolymer compounds, the hydrolytic chain scission of each component is significantly modified by the presence of the second component (Eppley *et al.* 1994). The mechanical strength, swelling behavior, capacity to undergo hydrolysis, and subsequently the biodegradation rate are directly influenced by the crystallinity of the PLGA polymer. The resultant crystallinity of the PLGA copolymer is dependent on the type and the molar ratio of the individual monomer components in the copolymer chain. PLGA polymers containing lactic and glycolic acids in 50:50 ratio are hydrolyzed much faster than those containing a higher proportion of either of the two monomers. The degree of crystallinity and the T_m of the polymers are directly related to the molecular weight of the polymer (Jain 2000). The effect of copolymerisation is to slow the rate of PGA degradation while increasing the rate of PLA degradation. PLA-PGA copolymers can, therefore, have a varying range of degradation rates, depending on their compositional ratios. Resorption of the PGA increases the material porosity, thus facilitating diffusion of fluids and hydrolysis of the PLA (Eppley *et al.* 1994). The T_g of the PLGA copolymers are above the physiological temperature of 37^{o}C and hence they are glassy in nature. Thus, they have a fairly rigid chain structure which gives them significant mechanical strength to be formulated as drug delivery devices (Jain 2000).

Although PLGA is insoluble in water, it is hydrolytically unstable and is degraded by hydrolysis of its ester bonds. Through this hydrolytic attack, random chain scission occurs, causing it to degrade into lactic and glycolic acids (Loo *et al.* 2004). A three phase mechanism for the biodegradation of PLGA has been proposed. The first stage involves random chain scission so that the molecular weight of PLGA decreases significantly, but no appreciable weight loss or soluble monomer products are formed. In the second stage, a decrease in molecular weight is accompanied by a rapid loss of mass. Soluble oligomeric fragments and monomer products are formed in this stage. In the final stage soluble monomer products are formed from soluble oligomeric fragments. This phase is attained when the polymer is completely soluble. The rate of hydrolytic degradation of PLGA is, therefore, dependent on its physical properties, such as its molecular weight, degree of crystallinity and glass transition temperature (Raghuvanshi *et al.* 1993). The role of enzymes in any PLGA biodegradation is unclear. Most of the literature indicates that the PLGA biodegradation does not involve any enzymatic activity and occurs purely through hydrolysis. However, some

investigators have suggested an enzymatic role in PLGA breakdown based upon the difference in the *in vitro* and *in vivo* degradation rates (Jain 2000).

2.2.4 The cavalcade of bioresorbable implant testing

PGA and PLA materials

Higgins reported in 1954 the spinning of PGA and in 1967 Schmitt and Polistina obtained a patent on PGA sutures (Vainionpää *et al.* 1989). This led in 1970 to the first commercial synthetic biodegradable suture (Dexon®) developed by the Cyanamid Corporation using polyglycolic acid (Vainionpää *et al.* 1989, Frazza and Schmitt, 1971). In 1966, Kulkarni *et al*. published the first results of tests with polylactic acid (PLA) sutures (Ewers *et al.* 1991, Vainionpää *et al.* 1989, Vert *et al.* 1984, Vert *et al.* 1992, Suuronen *et al.* 1999). Kulkarni *et al*. introduced the manufacture of bioresorbable PLA sutures and films (Serlo et al. 2001). It was shown that PLA can be used as fibers, films or coatings. On the basis of these experiments Cutright *et al*. (1971) tested PLA sutures for internal fixation of midline fractures of the mandible in rhesus monkeys. No detrimental effects were observed and the degradation did not interfere with osseous union and healing. In 1972 Cutright and Hunsuck published the results of treating the floor of the orbit fractures in monkeys with 1.5 mm thick PLA plates. It was noted that the absorption was mediated by giant cells and phagocytes. The degradation was not complete after 38 weeks. The material showed very good biocompatibility (Vert *et al.* 1984, Ewers *et al.* 1991). Getter *et al*. (1972) used PLA plates and screws to stabilize mandibular fractures in six beagles. There were no rejection reactions and a plate or screw was no longer visible or could not be palpated after 24 weeks. After 40 weeks, fracture sites were indistinguishable from the adjacent bone. Roed-Petersen (1974) used PGA sutures (Dexon®) to fix mandibular fractures in two young patients. Interfragmentary fixation was provided for six weeks. Healing was uneventful. One year postoperatively, radiographs showed that the bur holes at the sites of the sutures had filled in. The results were successful and encouraging (Suuronen *et al.* 1993, Ewers *et al.* 1991, Vainionpää *et al.* 1989).

Williams studied the enzymic hydrolysis of PLLA and found that several enzymes increase the degradation rate of PLLA in aqueous medium (Williams 1981). Under the conditions employed, pronase, proteinase K and bromelain all had significant effects, with a smaller response to esterase and entirely negative

results with lactate dehydrogenase (Vainionpää *et al.* 1989). Vet *et al*. and Christel *et al*. demonstrated the slow degradation rate of high molecular weight PLLA and the possibilities to increase its degradation rate by stereocopolymerization and by copolymerization (Vainionpää *et al.* 1989).

In 1986 Gerlach *et al*. reported good results in fixation of mandibular fractures in beagle dogs with PLLA plates and screws (Ewers *et al.* 1991). Bos *et al*. (1987) treated ten patients with unstable zygomatic fractures with PLLA plates and screws. The results showed that this fixation system gives good stability over a sufficiently long period to enable undisturbed fracture healing. In 1989, Bos *et al*. (1989a) reported that PLLA plates and screws have good mechanical properties in mandibular fractures in sheep. In the same year Bos *et al*. also used PLLA plates and screws in fixation of mandibular fractures in dogs. All fractures healed without complications (Bos *et al.* 1989b). Eitenmüller *et al*. studied the fixation of osteotomy of the beagle radius with PLLA plates and screws. Bone healing was achieved in all animals after twelve weeks. Histopathological examination showed good tissue compatibility (Vainionpää *et al.* 1989). Illi *et al*. used PLA implants for six pediatric patients in correction of cranial deformities. After a follow-up time of 2-20 months, no problems in healing were observed (Illi *et al.* 1989).

Rozema *et al*. (1990) used PLLA implants for repair of blow-out type orbital floor defects in goats. No inflammation or rejection of the PLLA implant was seen during 78 weeks follow-up. New bone covered fully the PLLA plate. The following year PLLA implants were implanted subcutaneously in the backs of rats to study tissue reactions. Except for the early and final parts of the implant period, no acute or chronic inflammatory reaction was observed. It was estimated that more than three years will be required for total resorption of PLLA (Bos *et al.* 1991).

In 1991 Rozema *et al*. tested PLLA plates and screws in ten patients with zygomatic fractures. After 18 months plates and screws were still palpable indicating slow absorption. In this study it became apparent that the rate of degradation of plates was greater in the loaded bone than in unstressed bone (Ewers *et al.* 1991). Pakkanen *et al*. (1993) used SR-PLA tacks and PLA sutures in fixation in endoscopic brow lift. The results with this fixation method were promising.

Päivärinta *et al*. (1993) tested PGA and PLA screws in fixation of femoral osteotomy in rabbits. The occurrence of phagocytic mononuclear macrophages and foreign-body giant cells was highest in the PGA-fixed 12 week specimens.

The giant cells seemed to adhere to the implant surface at an early stage, whereas the ultimate digestion and clearing of the decomposing polymeric material later on were performed by macrophages invading the implant body. No PGA was left in the 36-week specimens, while the gross geometry of the PLA screws was still intact at 48 weeks. The inflammatory response to polymers was quite mild. Antikainen *et al*. (1994) used SR-PLLA and titanium miniplates to fix coronal sutures in newborn rabbits. After a follow-up period of six months both SR-PLLA and titanium plates caused similar asymmetry of the neurocranium. No obvious inflammatory or foreign body reactions were found. Ruuskanen *et al.* (1999) evaluated the histological behavior of SR-PGA membranes in subcutis of the rabbits. SR-PGA was found to be biocompatible. It caused no complications during the follow-up time (20 weeks). Ashammakhi *et al*. studied osteogenic potential of polyglycolide material by implanting SR-PGA membranes around the femora of rats. SR-PGA membranes were tolerated by bone and stimulated new bone formation (Ashammakhi *et al.* 1994).

PLLA plates and screws were used in fixation of zygomatic fractures in ten human patients. After 3.3-5.7 years eight patients were re-operated because of swelling at the site of implantation. The swelling area was removed and investigated histologically. Crystal-like PLLA material internalized by various cells was seen. It was noted that after 5.7 years of implantation, particles were still not fully resorbed (Bergsma *et al.* 1995).

Thaller *et al*. (1995a) tested PGA plates and screws in fixation of zygomatic osteotomies in rhesus monkeys. PGA implants provided satisfactory fixation in fractures. The ultimate fate of PGA plates and screws in osteotomies was studied histologically in rabbits. It was noted that PGA provokes a considerable foreign body reaction (Thaller *et al.* 1995b). Thaller *et al*. also compared PGA and titanium screws in fixation of zygomatic bone grafts in rabbits. Bone grafts secured with the polyglycolic acid screws demonstrated significantly less resorption than those maintained in position by traditional methods (Thaller *et al.* 1996). Van der Elst *et al*. (1995) used PLLA rods in fixation of fractures of femoral bones of young pigs. PLLA and stainless steel induced a similar tissue reaction during the three months of implantation time.

In 1997 Partio *et al.* treated nine patients with femoral fracture involving the physeal plate with SR-PGA and SR-PLLA screws. They concluded that these resorbable screws were suitable for the fixation of distal femoral fractures in adolescents. Jukkala-Partio *et al.* (1997) also tested SR-PLLA screws in subcapital femoral neck osteotomies in sheep. The fixation was compared to the

fixation with metallic screws. The results showed that there were no statistically significant differences in the load-carrying capacity between bones fixed with SR-PLLA screws and those fixed with metallic screws. SR-PLLA screws were found to be strong enough to support this demanding fixation of weight-bearing bones. Koskikare *et al.* (1996, 1997a) tested intraosseous SR-PLLA plates in fixation of osteotomies of distal femur in rabbits. These implants were considered to be suitable for the fixation of osteotomies. They did not diminish the amount of trabecular bone when compared to similar plates on the bone.

Nordström *et al*. (1998) tested SR-PLLA and SR-PGA pins in rats. Both seemed to induce an osteostimulatory response around their borders after implantation into cancellous bone. Reaction patterns were different for these two absorbable polyesters. During 48 weeks the response to the SR-PGA and SR-PLLA implant gradually faded. The inflammatory reaction to these polymers was quite mild.

Bloss *et al.* (1998) tested SR-PLLA pins and screws in fixation of distal radial fractures (n=224). The use of these implants showed good results. Suuronen *et al*. tested SR-PLLA implants in fixation of sheep mandibular osteotomies. The foreign-body reaction was mainly mild, and the osteotomies were well united. After five years *in vivo*, the material was almost completely resorbed, but small particles of polymer could still be detected at the implantation site (Suuronen *et al.* 1998).

Haers *et al*. used SR-P(L/D)LA (85/15) plates and screws in ten human patients bimaxillary surgery and simultaneous genioplasty. No clinical complications were encountered, jaws were clinically stable and there was no clinical evidence of foreign body reactions (Haers and Sailer, 1998, Haers and Suuronen, 1998).

Peltoniemi *et al*. compared SR-PLLA plates and titanium miniscrews with titanium plates on the craniotomy lines in growing sheep. All the SR-PLLA-plated craniotomy lines healed by 20 weeks, whereas none of the titanium plated ones consolidated during the follow-up time of one year (Peltoniemi *et al.* 1997, 1998).

Kumta and Leung (1998) used PLLA pins successfully in the management of hand fractures. They used pins in large osteochondral and intra-articular shear fractures of the metacarpus and proximal phalanx.

Kallela *et al.* (1999) compared SR-PLA (70L/30DL) screws and stainless steel screws in fixation of mandibular osteotomies in sheep. It was concluded that the fixation properties and biocompatibility of SR-PLA screws during the bone-

healing period following osteotomies and fractures in the maxillofacial region are promising for the manufacture of osteosynthesis screws.

Mäkelä *et al.* (1999) compared SR-PLLA and steel wires in fixation of sternotomy in rabbits. No differences in the healing of the sternotomies were observed between these materials. Mäkelä *et al.* (2002) also compared strength retention properties of SR-PLLA, polyglyconate (Maxon) and polydioxanone (PDS) sutures *in vitro*. SR-PLLA sutures had the most prolonged strength retention *in vitro*, but the lowest elongation (elasticity). Compared with straight sutures, knots had lower tensile strength and elongation values.

Serlo *et al*. used SR-PLA plates and wires in addition to titanium in 15 patients (14 children and one adult) in cranioplasties. No complications were observed during an eight year follow-up (Serlo *et al.* 2001).

Turvey *et al*. used SR-PL(D/L)LA (70/30) plates and screws to stabilize maxillary and mandibular osteotomies in 70 human patients. Three patients experienced problems that resulted in immediate loosening of the bone screws. The remaining 67 experienced no problems in follow-up time (6 to 24 months, Turvey *et al.* 2002).

PLGA materials

In 1974 Cutright *et al*. published a study in which the bioresorbability of the polymers and copolymers of PLA and PGA was investigated. This study confirmed the previous results with regard to absorbability. It was noted that absorption time depended on the ratio of PGA to PLA. It was noted that PGA degraded most slowly. Those results were not consistent with those of Törmälä *et al*. in 1987, according to which PLA is degraded more slowly than PGA (Vainionpää *et al.* 1989, Ewers *et al.* 1991).

Copolymerization of a small amount of lactide with glycolide (PGA/PLLA) led to the development of the second commercial synthetic bioresorbable suture (Vicryl®) on which Wasserman and Versfelt obtained a patent in 1974 (Vainionpää *et al.* 1989).

In 1977, Miller *et al*. studied, in rats, the difference in rate of degradation between pure polymers of PLA, PGA and various ratios of copolymer PLGA (75/25, 50/50, 25/75). Half-life of the polymers and copolymers decreased from five months for 100% PGA to one week with 50/50 copolymer and increased to 6.1 months for 100% PLA. It was concluded that the control of degradation rate of the implant could best be attained by varying the composition of PLA and PGA

between 75% and 100% PLA along with a corresponding 25% to 0% PGA (Miller *et al.* 1977).

Hollinger (1983) tested PLGA (50/50) copolymer in experimentally created osseous defects in the tibias of rats. Bone healing rate was assessed. The treated site was compared with untreated bony lesions. The implant sites displayed an accelerated rate of healing. No adverse host tissue responses were observed histologically. After positive results in animal experiments, two groups of patients with ankle fractures were treated in Helsinki in 1984 (Rokkanen *et al.* 1985). One group had metal implants and the second group had PGA/PLA fiber-reinforced implants. There were no differences in the results of treatment (Ewers *et al.* 1991).

The effects of fixation composed of resorbable polymers on craniofacial development were investigated in an animal model. PLGA (82/18) plates and screws were implanted across the coronal suture of immature rabbits. The study indicated that a large plate size (4-hole plate, 35 mm long, 6 mm wide and 1.5 mm thick) and slow resorption properties of polymer fixation can have a similar restrictive effect on bone growth as that of metal fixation (Eppley *et al.* 1992). To find out whether thinner plates will permit normal growth, the testing was continued by Eppley and Sadove in 1994. Smaller (4-hole, 1 mm thick, 16.5 mm long and 3.5 mm wide) PLGA (82/18) (LactoSorb®) plates were implanted across the coronal suture of immature rabbits. These resorbable plates permitted symmetrical frontal bone development, unaffected growth across the coronal suture, and a histologically normal underlying suture. These changes appeared to be the result of elongation of the fixation plate across the growth site (Eppley *et al.* 1994).

Eppley *et al*. compared PLGA 82/18 (LactoSorb®) and titanium implants in fixation of bilateral parietal bone grafts in rabbits. In one year's follow up time the resorbable plate demonstrated fixation stability similar to that of metal implants. No adverse local inflammatory reactions were seen as the polymer composite progressed to complete degradation by one year (Eppley and Sadove 1995a).

PLGA 82/18 (LactoSorb®) plates and titanium microscrews were used in 20 infants with calvarial deformities for skeletal fixation after osteotomies and repositioning. The plates were also studied in immature rabbits. No complications were reported after 12 postoperative months (Eppley and Sadove, 1995b). PLGA 82/18 implants were used in the fixation of zygomatic fractures in 27 patients. There were no implant related complications (Enislidis *et al.* 1998). Montag *et al*.

used LactoSorb system in 35 pediatric craniofacial patients in 1996. The LactoSorb system was found to be a reliable fixation system. No fixation-related complications were encountered (Montag *et al.* 1997). Pensler (1997) used PLGA 82/12 plating system in twenty patients in craniofacial procedures. PLGA plating system offered a significant advance over the metallic plates and screws. A major disadvantage of the system was that it was necessary to tap the screw holes. Tapping increases operation time.

Thirty facial fractures excluding mandible fractures were treated with PLGA (82/18) (LactoSorb®) plates and screws. PLGA 82/18 implants were proven to be safe and effective for use in midfacial skeleton (Eppley *et al.* 1997a). Parietal craniotomies of mature rabbit skull were repositioned and fixed with PLGA 82/18 plates and screws. No acute or chronic inflammatory cells were seen. The copolymer exhibited complete resorption within about one year. No contraindications were found for the use of LactoSorb implants in clinical work (Eppley *et al.* 1997b). Goldstein *et al*. used PLGA (82/18) (LactoSorb®) plates and screws for the correction of craniofacial deformities in eight pediatric patients in 1996. The study demonstrated the efficacy of these bioabsorbable implants. Excellent results were achieved (Goldstein *et al.* 1997).

Tatum *et al*. reported (1997) case reports of four maxillofacial trauma patients who underwent open reduction and internal fixation using absorbable plates and screws (Lactosorb 82/12). CT scan six months after repair showed adequate reduction of fractures and osseous healing. Kumar *et al*. applied LactoSorb® system in 22 pediatric patients undergoing craniofacial reconstruction in 1996. All patients except one showed satisfactory wound healing with no sign of infection or local inflammation. The plates provided satisfactory fixation and were not visible through the skin (Kumar *et al.* 1997). Weisberger *et al*. used PLGA (82/18) implants in 105 craniofacial reconstructions, 45 maxillofacial traumas, craniotomy flap repairs and in five cases involving reconstruction of the laryngotrachea. Implants were shown to be well tolerated by soft tissues and bone, and allow for unimpeded growth through eventual resorption after providing skeletal support for a long enough time to allow osseous healing (Weisberger *et al.* 1997).

Rinehart *et al*. used LactoSorb® system in seven pediatric patients in fixation of skull fractures. It was found that this system provided the most flexibility and security in three dimensional calvarial reconstruction (Rinehart *et al.* 1998).

Tharanon *et al*. used PLGA (82/18) plates and screws to stabilize the osteotomized calvarial bone in 33 pediatric patients. One patient had a

postoperative wound infection, and LactoSorb plates were palpable postoperatively in four patients. The LactoSorb® plating system provided adequate rigidity for stabilizing the osteotomized calvarial bone during surgery and maintained adequate rigidity after surgery during the bone healing period before absorption (Tharanon *et al.* 1998).

Westermark used PLGA (82/18) implants in fixation of 20 sagittal split osteotomy of the mandible in human patients. Implants offered reliable stability during the period of bony healing with no adverse tissue reactions in two years' follow-up (Westermark, 1999). PLGA 82/18 loses strength in approximately three months and absorbs completely in approximately one year (Habal *et al.* 1999). Edwards *et al.* (2000) fixed mandibular osteotomies with PLGA screws and plates. There were no postoperative infections or segmental instability up to 6 months after surgery. The study showed that PLGA implants are a viable alternative for fixation of anterior horizontal osteotomies of the mandible.

Self-reinforced PLGA 80/20 plates and screws were used in fixation osteotomies in 165 patients (161 children and four adults) in a multicenter study. It was found that the devices were easy to handle and apply and provided stable fixation. Postoperative complications occurred in 12 cases, comprising mainly of infections, bone resorption and delayed wound healing. It was shown that PLGA 80/20 implants can be used safely in corrective cranioplasties (Ashammakhi *et al.* 2004).

Waris *et al.* (2005) implanted SR-PLGA (80/20) screws transphyseally across the distal growth plate of the femur in immature rabbits. It was shown that the screws caused growth retardation 6 weeks postoperatively, after which the normal growth tendency was recovered. The duration of temporary growth retardation correlated with that of strength retention of the SR-PLGA 80/20 copolymer. These findings suggest that SR-PLGA 80/20 screws can be applied in transphyseal bone fixation. The use of bioresorbable screws for temporary epiphyseodesis seems attractive but requires further study.

2.2.5 Disadvantages

Bioresorbable fixation devices have been routinely clinically tested since 1983. The general acceptance of these devices has been hampered by the complications arising from early and late cellular reactions to the degradation products (Santavirta *et al.* 1990). The adverse host response, which was also observed with highly bioinert materials, was limited with the use of bioresorbable materials as

long as the amount of degraded particles did not exceed the clearance capacity of the body (Enislidis *et al.* 1998). Clinical complications are almost invariably associated with the homopolymers, as opposed to the copolymers. Such complications include pronounced fibrous encapsulation (PLLA), sterile sinus formation 8-16 weeks after operation (PGA), and bone resorption (PGA) (Pietrzak *et al.* 1997). The incidence of delayed transient sterile inflammatory reactions to PLLA has been reported to be high when bulky non-reinforced PLA plates have been placed on bone surfaces. Bergsma *et al.* reported foreign body reactions (subcutaneous swelling) that developed several years after the implantation of PLLA plates for fixation of zygomatic fractures (Peltoniemi *et al.* 1997, Bergsma *et al.* 1995).

A major drawback in the use of pure PGA has been the occurrence of adverse reactions in up to 60% of cases, in the form of swellings or fistulas draining sterile liquid containing polymer remnants. Osteolysis may also occur following the use of PGA implants (Suuronen *et al.* 2000). Local barriers and impaired clearance were believed to easily overwhelm the host clearing systems leading to exuberant, progressive, histiolytic reaction due to increased osmotic pressure. The same effect was observed when degradation speed changed due to infection or when the morphology of polymers was dominated by crystalline components. Bioresorbable materials need to be constructed from materials having as few crystalline and as many amorphous components as possible. The amorphous state is safe for *in vivo* implantation because it can be degraded easily. PLGA 82/18 (LactoSorb®) implants are mainly constituted of amorphous material, which is easily degradable in the human organism (Enislidis *et al.* 1998). Copolymers rather than homopolymers are preferred for clinical application and they are thought to diminish risks of inflammatory reactions. Copolymeric devices made of amorphous PLDLA or PLGA have not caused clinically significant foreign body reactions. However, it should still be noted that the smallest possible amount of polymer should be used, to ensure tissue absorption (Ashammakhi *et al.* 2005b).

It is now possible to produce relatively small yet strong miniplates employing the self-reinforcing technique. However, it would be an advantage to have even smaller microplates and microscrews, as in many cases plate thickness is often a problem where soft tissue cover is thin, with the risk of palpability, extrusion, or sinus formation. In CMF surgery, the increase in the clinical application of SR devices has been relatively gradual and no such complications have yet been reported in short- and long-term follow-up studies. Complications are mainly

associated with nonreinforced bioresorbable devices. In the case of nonreinforced implants, large implants have been used to compensate for their brittleness and low strength (Ashammakhi *et al.* 2005b). Eppley *et al.* (1992) showed in their study with rabbits that a large plate size and slow resorption properties of polymer fixation can have a similar restrictive effect on bone growth as that of metal fixation. In 1994 Eppley showed that elongation of the fixation plate appears after initiation of degradation when plated across a growth site in rabbits. This does not restrict the growth of the skull. Waris *et al.* (Waris *et al.* 2005) showed that bioresorbable SR-PLGA 80/20 screws cause temporary growth retardation in rabbit femur when implanted transphyseally across the distal growth plate of the femur.

Bioresorbable fixation in craniofacial surgery provides secure fixation while eliminating much of the concern over intracranial migration of metallic plates and screws. One limitation is the need for tapping the drill hole before screw insertion. Macropore Inc. described P(L/D)LA 70/30 tacks (Cohen *et al.* 2001). Our group introduced bioresorbable tacks that can be applied using a special instrument, a tack-shooter. For faster insertion of implants, an inserter instrument (Crossbow) was first developed for arthroscopic arrow installation into meniscal tissue (Törmälä *et al.* 2000). Use of tacks can lead to a reduction in operative time because the tacks do not require tapping or tightening. We have found that operation time can be reduced by 10-15% when using bioresorbable tacks instead of screws, after having passed the early phase of learning curve, during which the time was somewhat prolonged (Spanio *et al.* 2002). A summary table (1) of the pros and cons of the bioresorbable implants is provided below.

Table 1. Summary of the advantages and disadvantages of bioresorbable implants.

Advantages	Disadvantages
Bioresorbability	Foreign body reactions
No need to implant removal operation	(considering mainly homopolymers)
Costs for re-operations are avoided	Fistulas
No stress shielding in the long term	Swelling
Allows growth of immature skeleton	Local pain and tenderness
Better cosmetic results in the long term	
Biocompatibility	Longer operation time
Well tolerated by tissues	Bioresorbable screws need pretapping
Radiolucency	Weaker than metal implants
Do not interfere with diagnostic imaging or use of radiation	SR-method has improved this situation
Fixation reliability	Learning curve
Stronger implants with SR-method	Takes time to learn to handle implants
Moldable at room temperature	Same with all new implants
No implant migration	
Multifunctionality	Multifunctionality
Can be designed with different molecular ratios	Additives may compromise strength
Can be used as drug delivery systems	Incorporation of biological factors may be difficult due to denaturation and/or lack of controlled release

2.3 Multifunctional osteofixation devices

Recently, multifunctional (MF) devices have been developed to combine mechanical, pharmacologic and biological functions. These novel implants can have three different functions, namely osteofixation, infection prophylaxis, and osteoconduction. The current study concentrates on multifunctional implants which can have two functions, namely osteofixation and infection prophylaxis.

Bacterial adhesion to implanted materials and the capability of many microorganisms to form biofilms on foreign bodies are well-established steps in the pathogenesis of implant-associated infections. In a biofilm, bacteria are protected from the host immune defense and exhibit a marked but reversible increase in antibiotic resistance (Gollwitzer *et al.* 2003). There is a high demand for novel prevention techniques against biomaterial-related infections. Although the administration of systemic prophylactic antibiotics has been found to be the most important single factor in the prevention of implant infections (Court-Brown 1990, Mini *et al.* 1997), it has been associated with the increasing prevalence of

resistant strains such as MRSA (Eggimann and Pittet 2001, Harbarth *et al.* 2000). The use of local antimicrobial prophylaxis may carry a lower risk of inducing resistant strains than the current practice of routine systemic administration of antibiotics for 24-48 h after implant surgery (Mäkinen et al. 2005). Local antimicrobial prophylaxis is based on the controlled release of the antibiotics aimed to achieve adequate local bactericidal tissue concentrations for a prolonged time without significant systemic exposure (Holtom and Patzakis 2003). In metal implants, one option is to use thin bioresorbable polymer coatings as a local drug delivery system (Gollwitzer *et al.* 2003, Lucke *et al.* 2003). The use of bioresorbable orthopedic implants provides a possibility for direct impregnation of antimicrobial agents within the polymer matrix of the implant (Mäkinen *et al.* 2005).

Developing a drug-releasing implant with reliable mechanical properties that can be used for bone fixation is challenging. An additive in the implant matrix usually has an unfavorable effect on the mechanical properties of the final product. The other challenging issue has been the appropriate control of drug release from the implant in a way that avoids falls below therapeutic levels (may lead to development of resistant bacterial strains) or above toxic local or systemic levels (Ashammakhi *et al.* 2005a).

Bioresorbable devices are being increasingly used in bone surgery, e.g. in craniomaxillofacial surgery, trauma, orthopedics and hand surgery. Among the most commonly used bioresorbable polymers that are used to manufacture such implants are PLGA and P(L/DL)A (Törmälä *et al.* 1998, Ashammakhi *et al.* 2004). The use of such bioresorbable polymeric materials as drug releasing systems has also been explored (Nie *et al.* 1998, Calhoun *et al.* 1997, Tielinen *et al.* 1998, Gümüsderelioglü *et al.* 2000, Teupe *et al.* 1992, Leinonen *et al.* 2002, Ramchandani *et al.* 1998, Viitanen *et al.* 2006). In the form of rods, PGA was used to release ciprofloxacin and studied in femora in rabbits and demonstrated good antibiotic penetration to bone tissue (Overbeck *et al.* 1995). Vancomycin carried by PLA or PLGA was also studied successfully in an experimental rabbit osteomyelitis model (Calhoun *et al.* 1997). PLGA implants releasing ciprofloxacin were tested by Ramchandani *et al.* (1998) and were shown useful in the treatment of deep skeletal infections. The coating of K-wires with PDLLA-gentamycin or PDLLA-teicoplanin has also been explored (Gollwitzer *et al.* 2003). Lucke *et al.* (2003) showed that PDLLA and 10% gentamicin coated K-wires reduced implant related osteomyelitis when tested in tibiae in rats. Our group developed ciprofloxacin releasing bone fixation bioabsorbable devices.

Bioresorbable polymers (PLGA 80/20 or PLDLA 70/30) were used as the matrix, ciprofloxacin as the antibiotic and bioactive glass 93/13 as the osteoconductive agent (Ashammakhi *et al.* 2003, 2005b).

2.3.1 Function 1. Osteofixation

Bioresorbable surgical devices offer certain advantages over metallic ones (Rokkanen 1998). They maintain the fixation, decompose gradually, and stresses are transferred gradually to the healing bone so that no stress shielding occurs (Rokkanen 1990). An implant removal operation is not needed. This reduces the total cost when compared with metallic devices (Rokkanen *et al.* 1999, Ashammakhi *et al.* 2001).

Early attempts to develop bioresorbable devices were based on the use of implants which were manufactured by traditional melt-molding techniques (Törmälä 1992). These bioresorbable implants did not have high enough mechanical strength values compared to metallic ones (Daniels *et al.* 1990, Törmälä 1992). The strength retention properties of polymeric devices can be improved by increasing the bulk of polymer in the device or by using a polymer with a higher molecular weight ("as-polymerized"). One approach involves the using of elements such as fibers, fibrils or oriented polymer chains to reinforce the matrix of the device. These reinforcing elements may be produced of material different from that of the matrix of the device, such as carbon fibers or other polymers. To produce stronger materials, more and more reinforcing elements are needed, and chemical agents called adhesion promoters are also needed. The degree of reinforcement, however, may be limited because of a limit to the amount of adhesion promoters that can be used, as those normally used are toxic (Ashammakhi *et al.* 2005 b).

Self-reinforcement

In the late 1970s Törmälä *et al*. concluded that the high demands of strength of fixation materials can be fulfilled best by developing reinforced, bioresorbable composites (Törmälä 1992) and developed the self-reinforcement method. Self-reinforced absorbable composites are polymeric materials where reinforcing elements and matrix material have the same chemical element composition (Törmälä 1992). When the self-reinforcing method is used, adhesion promoters are not needed (Ashammakhi *et al.* 2005b). The more advanced self-reinforcing

technique, partial fibrillation by orientational drawing, has further strengthened the SR implants significantly (Ashammakhi *et al.* 1997). Self-reinforced resorbable composites fulfill the physical demands of secure fixation materials and devices because they have a ductile deformation mode, high initial strength, appropriate elastic modulus and they lose their strength gradually and are totally absorbable (Rokkanen 1990).

The SR technique was first applied in pure PGA and PLLA. Animal studies showed that bone fixation with them was as strong as, or stronger than, similar fixation with metal devices (Suuronen *et al.* 1999). So far, various self-reinforced osteofixation devices have been produced, such as rods, pins, screws, plates, and tacks. They have proved to be successful (Ashammakhi *et al.* 2004, Saikku-Bäckström *et al.* 1999, Koskikare *et al.* 1997b). The first applications involved SR-PLLA, with SR-PLDLA and SR-PLGA devices being used nowadays (Ashammakhi *et al.* 2004).

Adding another function by incorporating antimicrobial agents or growth factors to bioresorbable materials may impair their mechanical strength. *In vitro* immersion tests have confirmed that the shear strength of the current antibiotic-containing screws decreased by a half within six weeks. This limits clinical applications of the screws (Mäkinen *et al.* 2005). Although, there have been difficulties in combining drugs to absorbable polymers without compromising their mechanical properties, SR high strength implants with drug-releasing behavior have been developed (Ashammakhi *et al*. 2005a).

2.3.2 Function 2. Osteoconduction

To develop further reliable biocompatible bioresorbable fixation devices, development of the next generation was envisaged. To address the problem of replacement of the bioresorbable screw tracks with fibrous tissue, osteoconductive agent was added. Bioactive glass (BG) 93/13 was included to confer the osteoconductive function (Ashammakhi *et al.* 2003). BG has been shown to be a promising bone substitute material in experimental bone defects. BG was first introduced by Hench *et al*. in 1971. BGs are silicate glasses containing sodium, calcium, and phosphate as the main components (Turunen *et al.* 1995). BGs have been reported to be bone bonding and osteoconductive (Aho *et al.* 1993). BG has shown to be useful in the treatment of fresh bone defects adjacent to dental implants. BG coating seemed to improve osseointegration in

the defect area (Turunen *et al.* 1998). It can be used as a filler in bone defects (Heikkilä *et al.* 1995).

PLGA has been also shown to possess osteoconductive properties, permitting gradual replacement of the implant by bone. Loading this polymer with osteogenic cells may also confer osteoinductive properties (Dean *et al.* 1998). Ruuttila *et al*. showed that SR-PLA 70 plates coated with BG are capable of inducing a proliferative response of human primary osteoblasts, and appears to support the development of mature osteoblast phenotype (Ruuttila *et al.* 2006).

2.3.3 Function 3. Infection resistance

Despite modern advances in the surgical and antimicrobial armamentarium, the treatment of bone and implant-related infection remains a formidable challenge for the clinician for a variety of anatomic, vascular, and immunological reasons. Anatomic and vascular alterations make it very difficult to deliver antibiotics to the affected area of bone at a sufficiently high concentration solely by the intravenous or oral route (Nie *et al.* 1998). Treatment of osteomyelitis is based on a systematic approach, starting from the accurate identification of the pathogen and its sensitivity to antimicrobial treatment. The treatment key-stones are radical surgical debridement of the infected tissues, obliteration of the dead space, adequate soft tissue coverage and a minimum of 4-6 weeks of intravenous antimicrobial therapy (Lew *et al.* 2004, Lin *et al.* 1992).

The presence of foreign materials leads to an increased risk of bacterial colonization and infection because bacteria prefer colonization on nonviable materials and tissues. Once the surface of the implant is colonized, attached bacteria form a biofilm. Biofilm protects them from attack by the body's immune-inflammatory defense mechanisms, including phagocytic cells, and antibodies. Bacteria residing in such protective biofilm are difficult to eradicate. The prevention of bacterial adhesion to and growth on prosthetic surfaces remains the primary strategy of infection prophylaxis because once a surface biofilm has formed, the enclosed bacteria are demonstrably resistant to antibiotics and host defenses (Tollefson *et al.* 1987). By the present strategies in prosthetic surgery, the rate of infection has been significantly reduced. Ultraclean operation rooms, improved designs of prosthetic devices, and standardized surgical techniques with a shortening of operation times lowered the incidence of infection. Perioperative administration of antibiotics 20-40 min prior to surgery has become a routine

procedure in prophylaxis. Failure to use antibiotics is estimated to result in a sevenfold increase in the rate of infection (Lucke *et al.* 2005).

Successful treatment of chronic osteomyelitis requires sustained high concentrations of antibiotics locally within the infected bone (Jacob *et al.* 1989). The infection of the bone leads to circulatory disorder with associate bone necrosis. Necrotic tissues provide a culture medium for bacteria that produce a fibrous glycocalyx material, which together with necrotic bone becomes a so-called biofilm that cannot be overcome by the body's immune systems or with antibiotics (Overbeck *et al.* 1995). Even with the use of larger doses of antibiotics, implant-related infections are often difficult to treat without the ultimate removal of the implant (Ashammakhi *et al.* 2005a). By local application of antibiotics systemic side effects of the antibiotic can be avoided and higher local drug levels can be achieved without the risk of systemic toxicity. The technique of delivery must guarantee a rapid release of the antibiotic from the carrier and local drug levels well above the minimal inhibitory concentration (MIC) of relevant microorganisms need to be achieved. The drug release must be restricted for a limited period of time to prevent development of resistant bacterial strains. Bactericidal antibiotics should be favored over bacteriostatic (Lucke *et al.* 2005).

Fluoroquinolones have long been considered the drugs of choice for chronic osteomyelitis because of their favorable penetration in poorly vascularized sites of infection, their advantageous bactericidal effect on all the probable pathogens of chronic osteomyelitis, and the lack of serious adverse reactions (Lew *et al.* 2004). Holtom *et al.* studied the inhibitory effects of the quinolone antibiotics (trovafloxacin, ciprofloxacin and levofloxacin) on osteoblastic cells *in vitro*. As many antibiotics commonly used in bone infections (vancomycin, cefazolin (Edin et al. 1996), rifampicin and gentamicin (Isefuku *et al.* 2001, Isefuku *et al.* 2003), quinolones had an inhibitory effect on cell growth at high concentrations (Holtom *et al.* 2000) and they may delay bone healing (Perry *et al.* 2003).

Local antibiotic delivery systems

Several types of delivery system for local antibiotic delivery have been used, including the use of bone cement (Carlsson *et al.* 1978, Nijhof *et al.* 2000), polymethylmethacrylate (PMMA), hydroxyapatite ceramic (Shinto *et al.* 1992) and bioresorbable carriers such as PLGA (Humprey *et al.* 1998). McKee *et al.* (2002) showed that tobramycin-impregnated bone graft substitute (calcium

sulfate α-hemihydrate) was effective in eradicating bone infection when used in the treatment of infected bony defects and nonunions in humans. Shinto *et al.* (1992) showed in rats that antibiotic impregnated porous calcium hydroxyapatite ceramic may be a useful method for the treatment of chronic osteomyelitis. Local antibiotic therapy with gentamycin-impregnated PMMA bead chains is effective for the treatment of chronic osteomyelitis when used as an adjunct to definitive surgical care (Jacob *et al.* 1989). Ostermann *et al*. compared PMMA-tobramycin beads combined with systemic antibiotic prophylaxis against systemic antibiotic prophylaxis in compound fractures in 700 patients. The difference in the incidence of infection was statistically significant. Prophylactic use of antibiotic-laden PMMA beads in addition to systemic antibiotics was of benefit in preventing infectious complications in compound fractures, particularly in Type IIIB open fractures (Ostermann *et al.* 1993). Moehring *et al.* (2000) tested tobramycin-releasing PMMA beads in open fractures. Antibiotic beads seemed to be useful in preventing infections in open fractures. The PMMA beads have qualified as the standard adjuvant local therapy. The beads commonly are implanted temporarily for 1-2 weeks then the beads have to be removed in a second operation. A radiographic control for removal of the beads is often necessary (Overbeck *et al.* 1995). The prophylactic use of antibiotic-impregnated bone cement has also been applied to prevent infection. A good example is the use of gentamicin-releasing beads which are currently in clinical use (Ashammakhi *et al.* 2005a).

Bioresorbable local antibiotic delivery system

The main advantage of using a bioresorbable system is that a second surgical procedure for removal of solid material is not needed (Lucke *et al.* 2005). Implant removal procedures can be more challenging than primary surgical operations, and they are associated with more complications and costs. Thus, strategies to reduce implant related infections are worth developing. One of such strategies is to try to interfere with bacterial colonization of the implant and with subsequent development of infection (Ashammakhi *et al.* 2005a). PLA and PGA have been used for many years as surgical sutures because they are bioresorbable, biocompatible, and physically strong (Kitchell *et al.* 1985). The use of PLA, PGA and PLGA matrices for the controlled release of therapeutic agents has been extensively studied. The drugs can be incorporated in either a dissolved or dispersed form within the polymer matrices which degrade in biological fluids

with a progressive release of immobilized drug. Polymer selection affects their biodegradation rate and, therefore, a system can be designed for short or long release periods (Gümüsderelioglü *et al.* 2000).

PLGA is a widely investigated bioresorbable polymer and it has been extensively used in several biomaterials applications as well as drug delivery systems (Ramchandani *et al.* 1998). PLGA systems have been developed for delivery of active compounds such as proteins and pharmaceutical products at controlled release rates. Drug delivery devices are characterized, not only by their chemical composition, but by their physical features such as size, shape, density, and porosity. The latter properties are the results of manufacturing procedures. The mechanism of drug release from PLGA compressed matrices is a combination of diffusion and erosion. As drug on the surface of the device diffuses away, exposed polymer hydrolyzes and a greater matrix surface area is exposed. Drug may also diffuse through the polymer to the surface. PLGA polymers may be prepared in any molar ratio of lactic to glycolic acids; the chosen proportion is an important factor in the system design. The *in vivo* polymer degradation rate depends strongly on the mole ratio of the monomers: polymers prepared in a 50:50 proportion are hydrolyzed much faster than those with a higher proportion of either monomer. The single most important factor in the design of the PLGA controlled release system is the percentage of drug loading. This factor is based on the solubility of the drug (Kitchell *et al.* 1985).

Testing of bioresorbable local antibiotic delivery systems

Ramchandani *et al.* (1998) studied concentrations of ciprofloxacin released from ciprofloxacin-PLGA (50:50) implants in rabbits. They concluded that the local delivery of ciprofloxacin using PLGA 50:50 as carrier may be useful in the treatment of deep skeletal infections. Sustained drug levels, greater than MIC of ciprofloxacin up to 70 mm from the site of implantation, were detected for a period of six weeks. Wang *et al*. investigated cefazolin- and gentamicin-releasing PLGA 50/50 implants for a long-term drug release *in vitro*. The results suggested that the bioresorbable beads released high concentrations of antibiotic (well above minimum inhibitory concentration) *in vitro* for the period of time needed to treat bone infection, i.e. 2-4 weeks. This would enable their use as the first line choice for patients with osteomyelitis and various infections as well as for the prophylaxis of these infections (Wang *et al.* 2004).

Mäkinen *et al*. evaluated the efficacy of a ciprofloxacin-containing PLGA screws in the prevention of biomaterial-related infection due to *Staphylococcus aureus* in a rabbit model. Although antibiotic-PLGA screws were contaminated with *S. aureus* before implantation, all cultures were negative in follow-up. The study confirmed the *in vivo* efficacy of bioresorbable antibiotic containing bone screws in the prevention of biomaterial-related infection (Mäkinen *et al.* 2005).

Niemelä *et al.* (2006) compared ciprofloxacin-PLGA, PLGA and titanium screws in preventing bacterial attachment and biofilm formation *in vitro*. Ciprofloxacin-PLGA implants were superior. After 21 days incubation in *S. epidermidis* suspension, no biofilm was observed on 93-100% of PLGA-ciprofloxacin implants and on 74-93% of titanium implants (Niemelä *et al.* 2006). In the same study PLGA-ciprofloxacin implants showed clear bacterial growth inhibition on agar plates, while titanium and plain PLGA implants did not show any inhibition.

Calhoun *et al.* (1997) evaluated vancomycin-releasing PLDLA-PGA implants in rabbits' tibial localized osteomyelitis developed with *Staphylococcus aureus*. The study indicated that bioresorbable antibiotic beads may be more effective than parenteral antibiotics after debridement surgery for the management of chronic localized osteomyelitis. Bioresorbable antibiotic beads do not require removal surgery.

Jacob *et al.* (1989) evaluated the efficacy of bioresorbable PDLLA-co-GA microspheres containing ampicillin for the local treatment of experimental staphylococcal osteomyelitis. It was concluded that this bioresorbable antibiotic system demonstrated the potential efficacy in treatment of osteomyelitis as an adjunct to definitive surgical care.

Lin *et al.* (1992) studied the ciprofloxacin concentrations in bone released from PLA implants in rabbits and its efficacy in the treatment of induced osteomyelitis. Implants demonstrated therapeutic local concentration and low systemic levels over an eight week interval. Using the chronic osteomyelitis rabbit model, a comparative series demonstrated the therapeutic efficacy of antibiotic-PLA in the treatment of chronic osteomyelitis.

Nie *et al.* (1998) tested ofloxacin-releasing P(D/L)LGA implants to treat induced Pseudomonas aeruginosa osteomyelitis in rabbits. Implants were suitable vehicles for the delivery of high local concentrations of ofloxacin. These concentrations resulted in eradication of the bacterial pathogen in this rabbit model.

Lucke *et al*. (2005) compared PDLLA coated, uncoated and Gentamicin-PDLLA- coated K-wires in the treatment of induced osteomyelitis in rat tibias. Half of the animals also received a single shot of gentamicin 30 min prior to surgery. Implant-related osteomyelitis could be prevented by prophylaxis of systemically applied gentamycin in 15% of animals. Onset of infection could be prevented in 90 % of animals treated with gentamicin-coated K-wires, and in 80 % of the animals that were treated with a combination of local and systemic application. The local application from PDLLA-coated implants might support systemic prophylaxis in preventing implant-associated osteomyelitis.

Teupe *et al.* (1992) used PLLA cylinders carrying 6% by weight ciprofloxacin for *in vitro* investigation of a slow-release antibiotic deposit. The concentrations of ciprofloxacin released were initially very high (up to 180mg/l) but they decreased rapidly within the first five days (4.2-22.5 mg/l). The high amounts of ciprofloxacin released at the beginning could be useful in filling up the natural bone defect and exceeding the demanded MIC at the same time.

Veiranto *et al.* (2002) studied *in vitro* self-reinforced ciprofloxacin-releasing P(D/L)LLA 70/30 screws. The screws gradually released ciprofloxacin and at the same time had sufficient mechanical strength at least 12 weeks at the level, which ensures their fixation properties.

Wei *et al.* (1991) tested kanamycin-releasing PL(D/L)LA implants in rabbit femur. In all bone tissues around the implant, the concentration of antibiotic exceeded the MIC for common causative organisms of osteomyelitis for six weeks. The implant seemed to be clinically useful as a drug delivery system for treating chronic osteomyelitis.

Andreopoulus *et al.* (1996) measured *in vitro* antibiotic concentrations released from PLA implants. Release was controlled by the drug diffusion and the matrix degradation, the latter being the most critical factor. The obtained concentration levels were above MIC against the major causative bacteria of osteomyelitis.

Overbeck *et al.* (1995) tested the penetration depth of ciprofloxacin into bone cortex and marrow from ciprofloxacin-releasing PGA implants in rabbits. After four weeks of implantation, bactericidal levels were measured up to a penetration depth of nearly 10 mm. The study showed a significantly greater penetration of ciprofloxacin into bone than has been reported for gentamycin cement beads. Koort *et al.* (2005) has shown that ciprofloxacin and bioglass containing PDLLA 50/50 implants are efficient in the treatment of localized osteomyelitis due to *Staphylococcus aureus*.

2.4 Economic viewpoints

Considering the economic viewpoints, the high acquisition costs of the resorbable implants partially outweighed the benefit of avoiding all removal procedures (Böstman 1994). Juutilainen *et al.* (1997) compared the economic costs of ankle fractures treated with bioresorbable or metallic implants. It was showed that resorbable fixation devices are more economical than metallic implants in operative treatment of ankle fractures. Costs can also be reduced by using one large bioresorbable panel plate that can be cut into several small plates. By using bioresorbable tacks and their application device (tack-shooter) costs can be further reduced due to reductions made in operative time and reduction in the risk of operation time-related complications. Multifunctional osteofixation devices that contain antibiotics may also contribute to long-term cost-effectiveness by reducing the rate of infections. However, this issue has to be addressed in a future clinical study (Ashammakhi *et al.* 2005 b).

3 Purpose of the present study

The primary purpose of the present research work was to evaluate the suitability of self-reinforced polylactide/glycolide (PLGA 80/20) implants for bone fixation in plain and ciprofloxacin-releasing configuration. Particular attention was paid to evaluation of tissue reactions to implants, bone tissue concentration of ciprofloxacin released from implants and mechanical properties of implants. The specific objectives were as follows:

1. To assess the tissue reactions of multifunctional ciprofloxacin-releasing SR-PLGA 80/20 screws in the cranial bones of rabbits (paper IV).
2. To assess the tissue reactions caused by plain non-antibiotic SR-PLGA 80/20 screws in rabbit cranial bone (paper III).
3. To measure the bone tissue concentrations of ciprofloxacin released from SR-PLGA 80/20 screws implanted in the cranial bone of rabbits (paper V).
4. To compare the pull-out forces of ciprofloxacin-releasing and plain SR-PLGA 80/20 miniscrews in human cadaver parietal bones (paper II).
5. To compare the pull-out forces of ciprofloxacin-releasing and plain SR-PLGA (80/20) tacks in human cadaver parietal bones (paper VI).
6. To compare the pull-out forces of PLGA tacks and screws in human cadaver parietal bones (paper I).

4 Materials and methods

4.1 *In vivo* experimental studies

4.1.1 Rabbits

In studies 3 (paper III), 4 (paper IV) and 5 (paper V), adult male New Zealand white rabbits, weighing 2.8 to 3.3 kg were used. The rabbits were treated according to laws governing experimental animals. The investigation was approved by the Committee on Animal Experimentation of the University of Oulu.

4.1.2 Surgical technique

The animals were anaesthetized with an intramuscular injection of medetomide hydrochloride (Domitor®, Orion, Espoo, Finland) 300 μg/kg and ketamine hydrochloride (Ketalar®, Parke-Davis, Solna, Sweden) 20 mg/kg. Cefuroxime (Lifurox®, Eli Lilly, Firenze, Italy) 60 mg was given intravenously as infection prophylaxis at the beginning of the procedure. The operation area was shaved and washed with chlorhexidine 5 mg/ml (Klorhexol®, Leiras. Turku, Finland).

A longitudinal midline skin incision was made over the sagittal suture. The periosteum was incised in a cross fashion and it was raised off the bone. Holes were drilled in the bone, one on each side of the sagittal suture (5 mm posterior to the coronal suture and 5 mm lateral to the sagittal suture). Holes were made with electric drill under continuous flowing saline to minimize heat insult to the bone. In 3rd experiment (paper III) bioresorbable and titanium screws were applied. In 4th study (paper IV) ciprofloxacin-releasing screws were applied. Periosteum flaps were turned back over the screws and skin was closed in layers, subcutis with Vicryl® 5-0 (Ethicon, Norderstedt, Germany) and skin with Dermalon® 4-0 (Davis & Geck, Sherwood Medical, St. Louis, USA).

4.1.3 Follow-up

The animals were divided into seven follow-up groups. In 3rd study (Paper III) there were three rabbits and in 4th study (Paper IV) four rabbits in each group. Follow-up periods were 2, 4, 8, 16, 24, 54 and 72 weeks. The animals were killed

with overdose of pentobarbital (Mebunat®, Orion, Espoo, Finland). Parietal bone blocks of 2 x 2 cm including the implant in their center were taken for histological examination and in experiment 5 for concentration measurements. Titanium screws had to be removed, because our microtomy had no capacity to cut titanium.

4.1.4 Examination methods

The specimens were stored in 40 % ethanol, mounted in methylmethacrylate, cut with microtome to 8 µm thick sections and stained using modified Masson-Goldner trichrome staining method. The results were analyzed using a light microscope at x16, x40, x100, x250, x400 and x1000 magnifications, using polarizing and ordinary light. For the observation, the area around the screw was divided to three smaller areas: A, B and C, which contained a total of 18 areas of interest (Fig. 1). The number of macrophages, giant cells, and active osteoblasts (seen as cuboidal cells) was assessed and fibrous tissue layers were calculated and the degradation rate of bioabsorbable screw was analyzed. Three slides were prepared from each screw. Each screw was, practically, examined three times in all those 18 areas. The slides were first checked by two observators (pathologist and author). In the second round the cells and fibroblast layers were counted area by area (18 areas per slide, three slides from each screw). In 3rd study there were three and in 4th study four rabbits in each follow-up group.

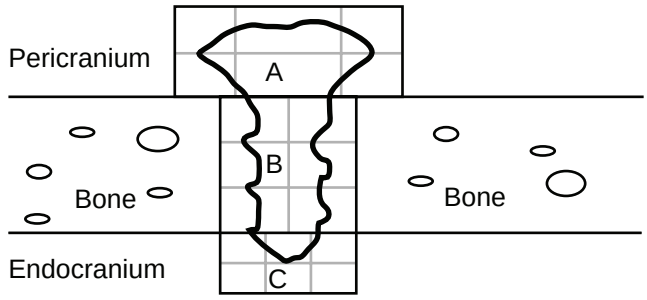

Fig. 1. Examination areas divided into three zones containing a total of 18 areas of interest.

For the measurement of bone ciprofloxacin concentration, ciprofloxacin was extracted from finely ground rabbit bone with a solution containing methanol and buffer. The quantitation procedure was made using HPLC-FLD (high performance liquid chromatography – fluorescence detection) and internal standard method. Ofloxacin was used as an internal standard.

About 400 mg of rabbit bone was finely ground with a homogenisator (Mikro-Dismembrator S, B. Brown). 100-400 mg of finely ground bone was weighed to a 15 ml polypropylene test tube. 75 µl of internal standard solution (1,0 µg/ml in methanol), 150 µl of methanol, 200 µl of water and 425 µl of 7 % perchloric acid in water (*v*/*v*) were added. The mixture was shaken vigorously for 10 min to achieve a suspension. The suspension was centrifuged 10 min at 4000 rpm and the solution was transferred to a microcentrifuge tube. The solution was centrifuged at 14000 rpm for 5 min and filtered with 0.45 µm membrane filter. Finally 20 µl of sample was injected into the HPLC-column. Standard samples and quality control samples were handled identically, but instead of methanol certain amounts of solutions containing ciprofloxacin in methanol and methanol were added. All water used was deionised reverse osmosis water (Milli-RO 30 Plus and Milli-Q Synthesis A10, Millipore corp.)

HPLC-FLD analysis was carried out using Waters 2695 Separations Module, Waters 2475 multi λ Fluorescence detector and Millennium^{32} version 4.0 software. The column used was a Nova-Pak C_8 150 x 3.9 mm i.d. 60 Å column (Waters Co.). The mobile phase consists of 9 % acetonitrile and 91 % of buffer. The buffer contains 10 mM sodium dihydrogen phosphate (aq) and 20 mM tetrabutyl ammonium hydrogen sulphate (aq), pH 2.5. The pH was adjusted with 2 M NaOH (aq). The buffer was filtered before use through a 0.45 µm HV filter (Millipore Corporation, Bedford, MA). The flow rate of mobile phase was 1.0 ml/min. The column oven was set to temperature 25 ^{o}C and the autosampler to 20 ^{o}C. The excitation wavelength was 290 nm and the emission wavelength 470 nm. The retention time of ciprofloxacin was about 5.2 min and internal standard about 3.3 min. All water used was deionised reverse osmosis water (Milli-RO 30 Plus and Milli-Q Synthesis A10, Millipore corp.)

The concentrations were calculated with Millennium^{32} version 4.0 software. The standard curve was generated using weighted (1/x) linear regression.

4.2 Implants for *in vivo* experimental studies

4.2.1 Ciprofloxacin-releasing self-reinforced miniscrews

The investigated prototypes of the antibiotic-containing self-reinforced miniscrews consist of two components, a bioresorbable matrix polymer and antibiotic (8 wt%). The bioresorbable matrix polymer in antibiotic-containing (and plain) miniscrews was commercial PuraSorb®PLG (Purac Biochem bv., Gorinchem, Netherlands), which is a semicrystalline bioresorbable synthetic PLGA 80/20. The inherent viscosity of the PLGA 80/20 was 6.28 dl/g (0.1%, chloroform, 25 °C). The antibiotic was ciprofloxacin ($C_{17}H_{18}FN_3O_3$), which is a synthetic fluoroquinolone.

The miniscrews were machined from self-reinforced rods. The geometry of the miniscrews was the same as that of commercial BioSorbPDX® 1.5 Screws (Bionx Implants, Ltd., Tampere, Finland). The length of the screws was 4.0 mm, the thread diameter was 1.5 mm and the core diameter was 1.0 mm (Fig. 11). The finished miniscrews were gamma-sterilized before the pull-out tests.

4.2.2 SR-PLGA 80/20 screws (BioSorbPDX®)

The BioSorbPDX® screws (Fig. 3) were manufactured from PLGA (molar composition: 80L/20G) by Bionx Implants Ltd., Tampere, Finland. The total length of the screws was 4 mm. The length of the part that sinks to the bone was 3 mm. The outer diameter of the thread was 1.5 mm and the inner diameter is 1.0 mm. Screw holes were drilled to the bone with the drill bit of 1.1 mm in diameter with an electric drill. Screw holes were tapped with the manual screw tap. Screws were driven into the tapped holes and tightened using a screwdriver. Drill bits, taps and screws were all delivered by Bionx Implants Ltd., Tampere, Finland.

4.2.3 Titanium screws

Titanium screws were obtained from Stratek Medical AG (Obersdorf, Switzerland). The total length of the screws was 2.0 mm. The outer diameter of the thread was 1.2 mm and the core diameter 0.9 mm. Screw holes were drilled in the bone with a drill bit of 1.0 mm, with an electric drill. Screws were driven into the holes and tightened using a screwdriver. Drill bits, screws and screwdrivers were all obtained from Stratek Medical AG (Obersdorf, Switzerland).

4.3 Implants for biomechanical studies

4.3.1 Antibiotic-releasing self-reinforced tacks

The tacks were developed and manufactured from PLGA (80L/20G) by Conmed Linvatec Ltd., Tampere, Finland. They were T-shaped and have barbs on the external surface of their shaft (Fig. 12). The inner diameter and barbs/rounds of the tack were designed to be thicker than plain tacks. This change in the design was made to increase the pullout force of antibiotic-releasing implants because the administration of antibiotics may decrease the strength of the biomaterial (paper II). Upon application, the barbs anchor the tack into drill-holes in the bone and prevent its extraction. The total length of the tacks was 6 mm. The length of the part that sinks into the bone was 5 mm. Thread's outer diameter was 2 mm. The inner diameter was 1.3 mm in plain and 1.5 mm in ciprofloxacin-releasing tacks. The diameter of the drill bit used with tacks was 1.5 mm (Bionx Implants Ltd., Tampere, Finland). The antibiotic was ciprofloxacin ($C_{17}H_{18}FN_3O_3$), which is a synthetic fluoroquinolone.

4.3.2 Self-reinforced polylactide/glycolide (SR-PLGA) 80/20 tacks (BioSorbPDX®), tack-applicator gun and tack application

The tacks (BioSorbPDX®) were developed and manufactured from PLGA (80L/20G) by Bionx Implants Ltd., Tampere, Finland. They were T-shaped and have barbs on the external surface of their shaft (Fig. 3). Upon application, the barbs anchor the tack into drill-holes in the bone and prevent its extraction. The total length of the tacks was 4 mm. The length of the part that sinks into the bone was 3 mm. The thread's outer diameter was 1.5 mm and inner diameter 1.0 mm. The diameter of the drill bit used with tacks was 1.2 mm (Bionx Implants Ltd., Tampere, Finland).

In order to accelerate and simplify the tack application into drill-holes in the bone, the tack-applicator gun (Bionx Implants Ltd., Tampere, Finland) has been developed. The applicator gun (Fig. 2) comprises a housing, hand grip, cocking lever, piston, magazine, magazine slot, cannula, cannula-releasing knob and trigger. It is used by pressing first the cocking lever (1). The piston (2) is then pulled back to its rearmost position. The magazine (3), loaded with tacks (up to six), is pressed into the magazine room (4) and pushed in completely so that the tip of the first tack is facing the cannula (5). The piston (2) is then pushed lightly

forward until it locks. The tip of the cannula is pressed firmly against the bone drill hole (through hole in the bioresorbable plate), and the tacks are shot by pulling the trigger of the gun (7). The next tack can be loaded in the firing position by raising the magazine up one step after cocking and again pulling the piston to its rearmost position. The cannula can be released (and changed to another) by pulling the cannula-releasing knob (6) downwards.

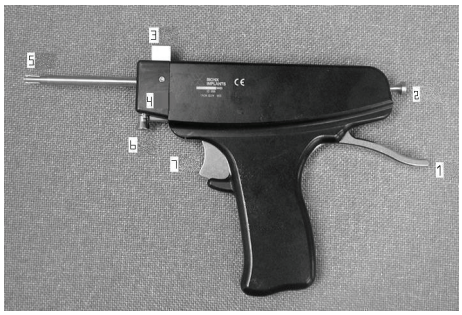

Fig. 2. Tack-applicator gun.

4.3.3 PLGA 82/18 (LactoSorb®) screws

The LactoSorb® screws (Fig. 3) were manufactured from PLGA (82L/18G) by Biomet Inc., Warsaw, USA. The total length of the screw was 4 mm. The length of the part that sinks to the bone was 3 mm. The outer diameter of the thread was 1.5 mm and the inner diameter was 1.0 mm. Screw holes were drilled to the bone with the LactoSorb® drill bit (diameter 1.1 mm). Screw holes were tapped with the manual 1.5 mm adjustable stop bone tap (LactoSorb Inc.). Screws were driven into the tapped holes and tightened using LactoSorb® screw driver.

Fig. 3. From left to right: SR-PLGA 80/20 tack, screw and PLGA 82/18 (LactoSorb®) screw.

4.4 Biomechanical studies

4.4.1 Human cadaver parietal bones

In this study human cadaver cranial bones were used. Five boat-shaped parietal bone pieces (ca. 6 cm x 20 cm) were collected from five human cadavers with the permission of The Provincial Government of Oulu, Finland, according to Finnish law. All cadavers were male. They had no reported disorders/diseases related to bone. Their ages varied from 47 to 75 years in 1st experiment, from 44 to 75 years in 2nd experiment and from 29-77 years in 6th experiment. After collection, the bones were stored in a freezer at –25 °C. Just before testing, the bones were left to warm up at room temperature and the periosteum was removed. Thickness of the bone varied from 4 to 7 mm in 1st experiment (paper I), from 5 to 8 mm in 2nd experiment (paper II) and from 6 to 7 mm in 6th experiment (paper VI). The thickness of the cortex varied from 1.5 to 2.2 mm in 1st (paper I) and 2nd (paper II) experiment and from 1.5 mm to 2.0 mm in 6th (paper VI) experiment. To minimize the differences occurring from the different quality of the bone, the same amount of the different implants were pulled from the same area of the bone.

4.4.2 Mechanical tests

The maximum uniaxial tensile force needed to produce failure in the fixation in bone is defined as holding power. Pull-out tests measure holding power against tension applied along the longitudinal axis of the implant. Although uniaxial pull-out tests are not completely adequate for testing the fixation properties of orthopedic implants, they provide an accurate method of evaluation of one of the parameters of holding power and provide a method for comparing different implants (Koranyi *et al.* 1970). Screw-holding strength as measured by pull-out tests is a standard criterion for evaluating the efficacy of different screw types (Vangsness *et al.* 1981). The holding power of a screw is not only a mechanical property of the screw but is determined by the shearing strength of the material into which it is inserted (Thompson *et al.* 1997). To measure the force needed for tack and screw extraction from the bone a tensile testing machine (Lloyd Instruments LR30 K, Lloyd Instruments, UK) was used. All the implants were applied so that the head of the implant was held on a metallic (aluminum) jig. The jig was kept in close proximity to the bone surface. This application simulated the

application of bioresorbable screw/tack and plate. The jig had two holes on its edges and the wire was woven through them. After implant application the bone was fixed between two parallel plates so that only the jig and the wire were seen from a slot of the upper plate. The heads of the wire were then clamped by the jaws of the tensile testing machine. The jig was pulled up until the screw/tack withdrew from the bone. The testing speed was 10 mm/min. BioSorb® and LactoSorb® screws have a slightly different shape of head (angle of the head of the LactoSorb® screw 55, and the BioSorb® screw 45 degrees). We made a special jig that fits the angle of the screw-head for each type of screw. The BioSorb® screws and tacks were pulled with the same jig. The wire was changed after every ten pull-out measurements. The results were saved on computer and analyzed.

Fig. 4. Pull-out testing system.

4.5 Statistical evaluation

In the first study (paper I), the mean of the measurements taken from the same bone was calculated. Means were analyzed using ANOVA. The results are expressed as means and standard deviations (SDs). In study 2 (paper II) and 6 (paper VI) means, standard deviations (SDs), and 95 % confidence intervals (CIs) of the pull-out forces of the implants were calculated (SPSS version 10.0 for Windows 2001). Student's t-test was used for statistical evaluation. Differences were considered statistically significant when $p < 0.001$. The bone tissue ciprofloxacin concentrations were calculated with Millennium[32] version 4.0 software. The standard curve was generated using weighted (1/x) linear regression.

5 Results

5.1 *In vivo* experimental studies (histology and ciprofloxacin bone tissue concentrations)

Postoperative recovery of all rabbits was uneventful. Macroscopically, the operative area healed well in all animals. No macroscopic signs of wound infection or other disturbances of wound healing were observed. The screws remained in their original implantation sites. A slight change in the color of the bioresorbable screws was seen and was considered as a sign of biodegradation.

5.1.1 Ciprofloxacin-releasing SR-PLGA 80/20 screws

After *two weeks* postoperatively, no changes in the dimensions of the bioresorbable screws were observed. PLGA material was clearly seen and it had polarizing activity. Many macrophages were seen around the screw heads and a few in areas near to the screw heads but corresponding to screw shafts. Some fibroblast proliferation was apparent as there were 2-3 layers of fibroblasts around the screw heads but none had yet formed between the screws and bone in areas corresponding to shaft and tip areas (Table 4). The number of the giant cells was highest (Table 2) near the heads of the screws (area A). Active (cuboidal) osteoblasts were seen at the edges of the bone next to the screw threads (area B) (Table 3).

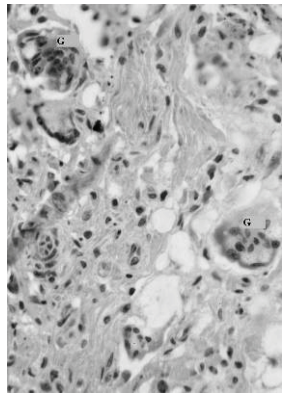

Fig. 5. Giants cells (G) in the area A near the head of the screw. Follow-up time was two weeks.

After *four weeks*, the morphology of the bioresorbable screws had not changed. PLGA material was clearly seen using polarized light. The fibrous tissue surrounding the screws was thicker than before (more than three layers of fibroblasts) and started to appear also around the screw tips (areas A and C) (Fig.1, Table 4). Macrophages were seen in area A (around the screw heads) and their number started to increase in area B (around the shaft of the screws, Table 1). Giant cells were seen around the heads of screws in lower number than before (area A). The highest number of active osteoblasts was seen at this time point around the screw shafts (area B, Table 3).

After *eight weeks*, there were no visible changes in the dimensions of the PLGA screws. Polarizing biomaterial was clearly seen. More fibrous tissue surrounding screw heads and tips (3-4 and 2-3 fibroblast layers, respectively) which was more evident and thicker than before. Fibrous tissue had also appeared in the shaft area between the bone and screw bodies (Table 4). The number of giant cells had decreased further. Macrophages started to form a line around the edges of the screws but their number was the same as at four weeks (Table 2). The number of active osteoblasts had decreased as compared to the 4-week follow-up group (Table 3).

After *16 weeks*, fragmentation of the heads of the PLGA screws had started. The fibrous tissue was compact in nature, comprised more fibroblast layers now than earlier and had completely surrounded the screws (all A, B and C areas). Polarization of the screw material had decreased remarkably. Although the number of active (cuboidal) osteoblasts had decreased further, they were still seen around the shaft of the screw (area B). The number of macrophages and a few giant cells that were also seen around the edges of the screws had decreased and some macrophages were seen inside the screw matrices (Table 2).

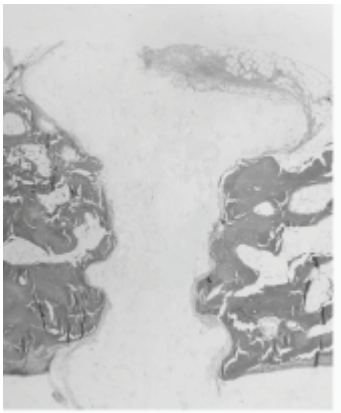

Fig. 6. Sixteen weeks follow-up. The fragmentation of the heads of the PLGA screws had started.

After *24 weeks*, a change had occurred in the morphology of the PLGA screws so that their heads were now fragmented. The PLGA material still remaining no longer had polarizing activity. The fibrous tissue capsule was compact in nature and its thickness reached maximum thickness of fibroblast layers (*n*=9) around the screw heads (Table 4). Active osteoblasts were still seen around the shafts of some screws (Table 3). Although the total number of macrophages has decreased, more macrophages were seen inside the matrix of the head of the screws in area A. No giant cells were seen.

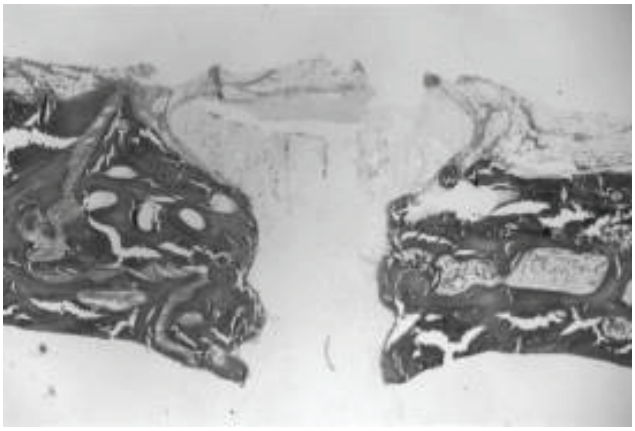

Fig. 7. Twenty-four weeks follow-up.

After *one year*, there was still some PLGA material left. The void of the PLGA screws was filled with adipose tissue, fibrous tissue and "foamy macrophages", which had intracellular PLGA particles. Capsular fibroblast layers had become thicker in areas B and C but they had, however, decreased to six layers in area A corresponding to the screw-heads. PLGA material was also seen extracellularly. A fibrous tissue capsule surrounded the remnants of the PLGA screws.

After *1½ years*, the amount of PLGA material remaining had decreased remarkably. Nearly all of the remnants of PLGA material which could still be seen, were inside "foamy macrophages". The number of macrophages had decreased (Table 2). No giant cells were visible. The amount of adipose and loose fibrous tissue had increased at the site that had been occupied by the PLGA screws. However, the number of fibroblast layers in the capsule had decreased in all examined areas (A, B and C). No active osteoblasts were seen in any in the areas examined (Table 3).

Table 2. Means and standard deviations of numbers of macrophages (MP) and giant cells (GC) seen at different follow-up periods. A, B and C indicates the examination areas in histological sections. A is the area around the head of the screw. B is the area around the shaft of the screw. C is the area below the tip of the screw.

Area	Cell type	Follow-up (weeks)						
		2	4	8	16	24	54	72
A	MP	134.7±45.2	64.5±18.5	63.0±11.6	32.0±15.1	13.5±6.4	120.0±42.4	12.0±4.3
	GC	5.5±1.4	4.0±1.7	2.0±0.3	1.0±1.4	0.0	0.0	0.0
B	MP	3.3±2.3	7.5±0.7	12.5±4.5	16.0±3.8	24.0±7.1	59.0±40.5	15.0±5.9
	GC	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C	MP	0.0	0.0	17.0±1.6	9.5±2.5	0.0	0.0	0.0
	GC	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3. Means and standard deviations of numbers of active (cuboidal) osteoblasts seen at different follow-up periods. A, B and C indicate examination areas in histological sections where A is the area around the head of the screw, B is the area around the shaft of the screw, and C is the area below the tip of the screw.

Area	Follow-up (weeks)						
	2 weeks	4 weeks	8 weeks	16 weeks	24 weeks	54 weeks	72 weeks
A	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	9.0±7.5	44.5±15.4	23.5±11.6	10.5±3.8	13.0±8.3	0.0	0.0
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 4. Means and standard deviations of numbers of fibrous tissue layers seen at different follow-up periods. A, B and C indicate observation areas in histological sections. A is the area around the head of the screw. B is the area around the shaft of the screw. C is the area below the tip of the screw.

Area	Follow-up (weeks)						
	2 weeks	4 weeks	8 weeks	16 weeks	24 weeks	54 weeks	72 weeks
A	2.3±0.8	3.1±2.2	3.8±3.2	5.0±5.1	9.0±4.0	6.2±5.7	3.0±2.6
B	0.0	0.0	1.0±2.3	2.2±2.8	2.8±3.3	3.4±3.9	1.3±1.2
C	0.0	2.0±0.7	2.8±2.2	3.1±2.8	2.0±1.6	5.0±3.2	1.5±1.3

5.1.2 SR-PLGA 80/20 screws and titanium screws

After *2 weeks*, no changes in the dimensions of the bioresorbable screws were observed (Fig. 8). PLGA material was clearly visible and had a polarizing activity. Slight fibroblast proliferation, microvascularisation and osteoblast activity were seen at the edge of PLGA screws (Tables 6 and 7). Many macrophages were seen near the head of the PLGA screws (Table 5). All these reactions were also evoked by the titanium screws, but they were less evident than those induced by the PLGA screws.

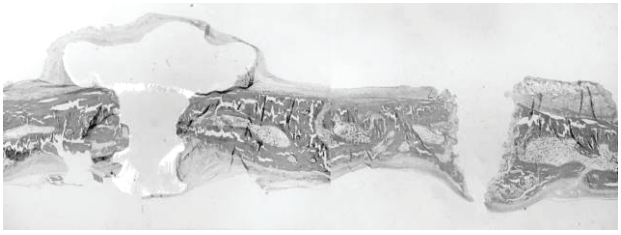

Fig. 8. Two weeks follow-up. On the left, SR-PLGA screw and on the right the void of the titanium screw. PLGA material is clearly visible and has a light polarizing activity.

After *4 weeks*, the morphology of the bioresorbable screws had not changed. PLGA material was clearly visible using polarized light. A prominent fibrous tissue surrounded the PLGA screw, but only a thin fibrous tissue layer was seen around the titanium screws. Microvascularisation was seen at the edges of both types of screw. Macrophages were seen in areas A and B (Table 5). Giant cells were also seen around the heads of both types of screw, with more around the PLGA screws. Osteoblastic activity was evident around the PLGA screw shaft in area B (Table 6).

After *8 weeks*, there were no visible changes in the dimensions of the PLGA screws. Polarizing biomaterial was clearly seen in the sections. Compact fibrous tissue surrounded the PLGA screws (Table 7). Fibrous tissue formation was less prominent around the titanium screws. Macrophages and giant cells were seen around both types of screw (Table 5). Osteoblastic activity was no longer as prominent as it had been at four weeks (Table 6).

After *16 weeks*, the heads of the PLGA screws had undergone fragmentation. Fibrous tissue surrounded the particles derived from the fragmented heads of the PLGA screws. Some polarizing material remained. Fibrous tissue capsule surrounded both types of screws (Table 7). Capsule was more prominent around the PLGA screws. Active (cuboidal) osteoblasts were still evident, but in lower numbers than at eight weeks (Table 6). Only a few small new bone islets were seen at the edges of the screws. Some giant cells were also seen. The number of the macrophages had decreased (Table 5).

After *24 weeks*, a significant change in the morphology of the PLGA screws had occurred (Fig. 9). The PLGA material had no polarizing activity left. A compact fibrous tissue capsule surrounded all the PLGA screws (Table 7), but only thin capsules were visible around the titanium screws. Giant cells and macrophages were seen mostly in area A, around the head of the PLGA screw (Table 5). Active osteoblasts were still in evidence around the shafts of the PLGA screws (area B) (Table 6).

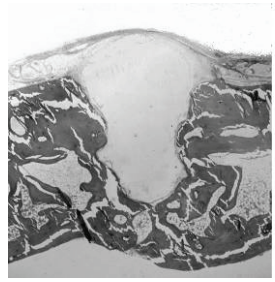

Fig. 9. Twenty-four weeks follow-up. A significant change in the morphology of the PLGA screws had occurred.

After *one year*, some PLGA material still remained (Fig. 10, left). The bed of the PLGA screws was replaced by adipose tissue, fibrous tissue and “foamy macrophages”, which contained intracellular PLGA particles. PLGA material was also observed extracellularly. Fibrous tissue capsule surrounded the remnants of

the PLGA screws (Table 7). New bone islets were seen above the heads of the PLGA screws. Titanium screws were also surrounded by fibrous tissue.

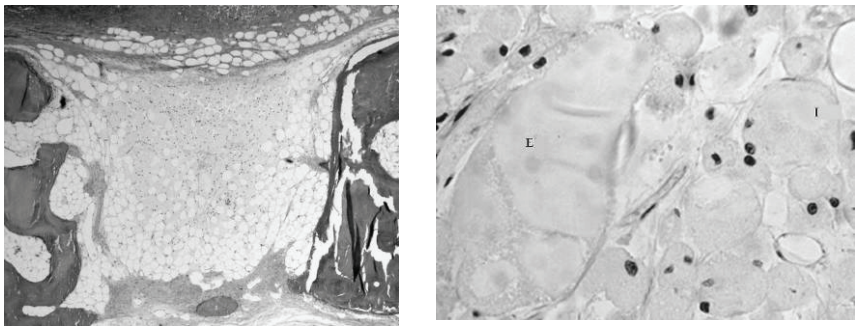

Fig. 10. Left: one year follow-up. Biomaterial is seen as dark spots in the middle of the implant bed. Right: In a close-up view PLGA material was seen both extracellularly (E) and intracellularly (I).

After *1.5 years*, the amount of PLGA material remaining had decreased markedly. Nearly all the visible remnants of the PLGA material were inside "foamy macrophages". The number of macrophages had already decreased (Table 5). No giant cells were observed. The amount of adipose and fibrous tissue had increased at the sites where the absorbed PLGA screws had been located. There were no evidence of active osteoblasts in any of the examined areas.

Table 5. Average number of macrophages (MP) and giant cells (GC) seen at different follow-up periods around the SR-PLGA screws. A, B and C indicate areas examined histologically. A is the area around the head of the screw. B is the area around the shaft of the screw. C is the area below the tip of the screw.

Area	Cell type	Follow-up (weeks)						
		2 weeks	4 weeks	8 weeks	16 weeks	24 weeks	54 weeks	72 weeks
A	MP	+++	++	++	+	++	-	-
	GC	0	3.7	4.0	2.7	1.7	0	0
B	MP	++	+	+	-	-	++	+
	GC	-	-	-	-	-	-	-
C	MP	+	-	+	-	-	-	-
	GC	-	-	-	-	-	-	-

- = 0, + = 1-20, ++ = 20-60, +++ = > 60

Table 6. Average number of active (cuboidal) osteoblasts seen at different follow-up periods around the SR-PLGA screws. A, B and C indicate the areas that were histologically examined. A is the area around the head of the screw. B is the area around the shaft of the screw. C is the area below the tip of the screw.

Area	Follow-up (weeks)						
	2 weeks	4 weeks	8 weeks	16 weeks	24 weeks	54 weeks	72 weeks
A	+	+	+	-	-	-	-
B	++	++	+	+	+	-	-
C	+	-	-	-	-	-	-

- = 0, + = 1-20, ++ = 20-60, +++ = >60

Table 7. Average number of fibrous tissue layers seen at different follow-up periods in the case of SR-PLGA screws. A, B and C indicate areas of interest subjected to histological examination. A is the area around the head of the screw. B is the area around the shaft of the screw. C is the area below the tip of the screw.

Area	Follow-up (weeks)						
	2 weeks	4 weeks	8 weeks	16 weeks	24 weeks	54 weeks	72 weeks
A	5.6±1.6	6.6±1.2	6.3±1.2	18.7±3.2	19.6±2.7	14±3.3	13.6±2.4
B	0	0	0	2±1.2	2.6±1.2	2.6±1.3	0
C	0	0.6±0.6	0	2±1.1	5.6±1.3	4.6±2.1	0

5.2 Biomechanical properties (pull-out strength)

5.2.1 Ciprofloxacin-releasing SR-PLGA 80/20 screws

The pull-out forces of the ciprofloxacin-containing and plain screws were 66.8 ± 4.9 N and 96.3 ± 9.3 N ($p<0.001$), respectively. The most common cause of failure was screw-shaft breakage (60% of the ciprofloxacin-containing and 52% of the plain screws). Scanning electron microscopy (SEM) showed that the fibrillar strip-like microstructure of plain SR-PLGA miniscrews turns into a coarse uni-axial platelet-like morphology in antibiotic SR-PLGA miniscrews as a result of the addition of ciprofloxacin.

Table 8. Pull-out strength in Newtons (N) of plain SR-PLGA screws (control) and ciprofloxacin-releasing SR-PLGA screws (AB) presented as means, standard deviations (SDs and 95 % confidence intervals (CIs).

Implant	Pullout strength (N)		95% CI	
	Mean	SD	Lower	Upper
AB screw	66.8	4.9	60.8	72.9
control screw	96.3	9.3	84.7	107.9

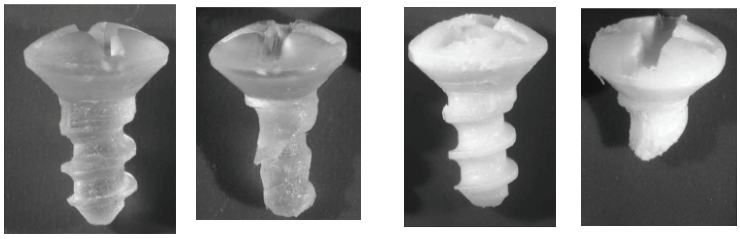

Fig. 11. The screw-threads of plain (the two panels to the left) and the screw-shaft of antibiotic-containing (the two panels to the right) SR-PLGA (80/20) miniscrews broke in the pull-out test as shown in the figures before and after testing.

5.2.2 SR-PLGA 80/20 tacks and ciprofloxacin-releasing SR-PLGA 80/20 tacks (length 6.0 mm and diameter 2.0 mm)

The pull-out forces of the ciprofloxacin-releasing and plain tacks were 147.0 ± 5.94 and 141.4 ± 8.97 N respectively (p=0.14). The cause of failure in all cases was barb breakage.

Table 9. Pull-out strength in Newtons (N) of plain SR-PLGA tacks (control) and ciprofloxacin-releasing SR-PLGA tacks (C) presented as means, standard deviations (SDs) and 95% confidence intervals (CIs).

Implant	Pullout strength (N)		95 % CI	
	Mean	SD	Lower	Upper
AB tack	147.0	5.94	141.8	152.2
Control	141.4	8.97	133.6	149.2

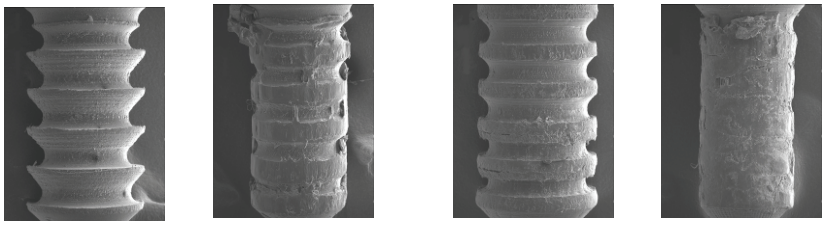

Fig. 12. The barbs of plain (the two panels to the left) and antibiotic-containing (the two panels to the right) SR-PLGA (80/20) tacks broke in the pull-out test as shown in the figures before and after measurement.

5.2.3 SR-PLGA 80/20 tacks, PLGA 82/18 screws (LactoSorb®) and SR-PLGA 80/20 screws (BioSorb®)

The pull-out force of the SR-PLGA tacks was 115.9 ± 8.3 N, that of Lactosorb® screws was 112.9 ± 12.1 N and that of Biosorb® screws was 110.4 ± 8.9 N (p=0.69). The most common reason for failure in the case of the tacks was barb breakage (55.1%); thread breakage in the case of BioSorb® screws (66 %) and stem split in the case of Lactosorb® screws (56 %).

Table 10. Pull-out strength (in Newtons, N) of SR-PLGA tacks and screws (BioSorb®), and PLGA (LactoSorb®) screws presented as means, standard deviations (SDs and 95 % confidence intervals (95 % CI).

Implant	Pull-out strength (N)		95% CI	
	Mean	SD	Lower	Upper
Tack	115.9	8.3	105.6	126.2
BioSorb®	110.4	8.9	99.4	121.4
Lactosorb®	112.9	12.1	97.9	128.0

5.2.4 Ciprofloxacin concentrations in bone tissue

The level of ciprofloxacin in the bone tissue was moderate in the first two weeks (mean 4.4 μg/g). After four weeks the release level started to rise, reaching a mean of 14.1 μg/g and remained at high levels up to eight weeks (mean 7.6 μg/g). At 16 weeks (mean 0.09 μg/g) and 24 weeks (mean 0.04 μg/g) drug levels were very low. After one year, levels rose again slightly to a mean of 1.3 μg/g and fell again, being 0.8 μg/g (mean)1.5 years after implantation. (Fig 13.)

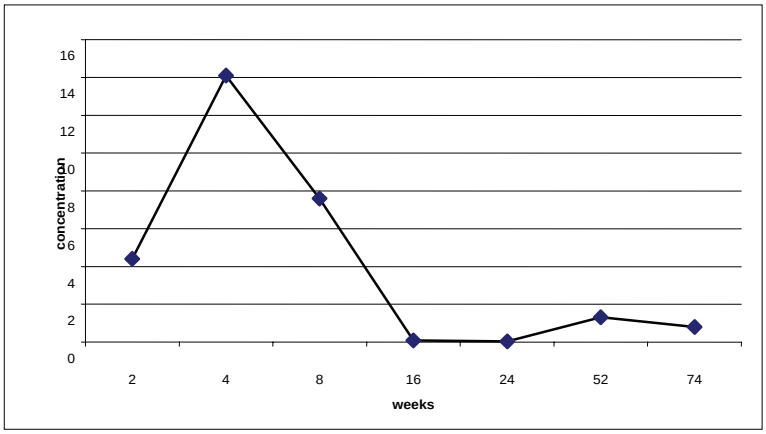

Fig. 13. Concentration of ciprofloxacin as a function of time after implantation of ciprofloxacin-releasing SR-PLGA screw in rabbit calvarial bone. Results are expressed as µg of drug/g of bone.

6 Discussion

Among the most commonly used bioresorbable polymers that are used to manufacture such implants are polylactide-co-glycolide (PLGA) and poly-L/DL-lactide (P(L/DL)A) (Törmälä *et al.* 1998, Ashammakhi *et al.* 2004). Recently, the copolymer PLGA has gained wide acceptance in craniofacial surgery as osteofixation material (Eppley & Sadove, 1995a, Habal *et al.* 1999, Eppley *et al.* 1997a). Several studies have demonstrated the biocompatibility of polyglycolide (PGA) and polylactide (PLA) and their copolymer (PLGA) (Ashammakhi *et al.* 2001, Nordström *et al.* 1998, Peltoniemi *et al.*1999). The use of bioresorbable polymeric materials as drug releasing systems has also been explored (Nie *et al.* 1998, Calhoun *et al.* 1997, Gümüsderelioglü *et al.* 2000, Teupe *et al.* 1992, Leinonen *et al.* 2002, Ramchandani *et al.* 1998). Administration of drugs using bioresorbable PLGA polymers has generated immense interest due to their excellent biocompatibility and biodegradability. They are easy to formulate into drug carrying devices and have been approved by the FDA for drug delivery use (Jain 2000).

PLGA copolymers have gained wide acceptance in craniofacial surgery as osteofixation material due to their excellent biocompatibility and biodegradability. They are easy to formulate into drug carrying devices and have been approved by the FDA for drug delivery use.

Any polymeric device must have the following tissue characteristics to be of clinical use: biocompatibility, sufficient biomechanical strength to permit bone healing, complete material resorption, and elimination of polymeric residues and metabolic products by a physiologic excretory method (Eppley and Sadove 1995a). Assessment of the applicability of osteofixation devices and resorption characteristics of particular polymers or copolymers as well as its fixation effectiveness at a specific bone site are of utmost importance prior to clinical use (Eppley and Sadove 1995a). In this study tissue reactions of SR-PLGA 80/20 and ciprofloxacin-releasing SR-PLGA 80/20 screws were evaluated, bone concentrations of ciprofloxacin released were measured in the cranial bones of rabbits and pull-out forces of plain SR-PLGA 80/20 screws and tacks and corresponding ciprofloxacin-releasing screws and tacks were evaluated in human cadaver parietal bones.

The presence of implanted material within a body causes an inflammatory response. The inflammatory response around the implant is often characterized by

the presence of inflammatory cells such as macrophages, lymphocytes, and polymorphonuclear leucocytes. These cells, and the mediators they produce, are important in determining the overall biocompatibility of an implanted device. The reactions that occur between the tissue and material are both numerous and very complex. Four aspects of this interaction can be identified. These comprise the initial interfacial reactions (dominated by macromolecular adsorption onto the material surface), the effect of the implant on the surrounding tissue, the effect of the tissue on the material, and the systemic response to the implanted material. These four phenomena are collectively referred to by the term biocompatibility (Hunt *et al.* 1993). As the majority of biomaterials require surgical implantation, it is convenient and relevant to consider biocompatibility in the light of the mechanisms of the normal wound-healing response, and the influence of the presence of an implanted biomaterial on this process (Vince *et al.* 1991).

After surgical implantation of biomaterials, the normal initial inflammatory response is followed by the formation of blood vessel-rich lose connective tissue or granulation tissue, which encapsulates the implant and soon develops to a denser connective tissue capsule (Habal *et al.* 1999, Pietrzak *et al.* 1997, Therin *et al.* 1994). In the current study a compact fibrous tissue capsule surrounded the screws already after four weeks. The capsule matured over time so that the number of fibroblast layers increased to reach a maximum by 24 weeks around the screw heads (both screws) and by 24 weeks (plain screws) and 54 weeks (ciprofloxacin-releasing screws) around the screw shafts and tip areas. There was also a maturation process seen as the organization of the first relatively loose capsule turning to be more compact than loose over time. By 54 and 72 weeks, the capsules surrounding the heads of screws were thinner than before, comprising lower numbers of fibroblast layers. This can be explained by the fact that the foreign material has degraded and was resorbed in the examined area. In areas corresponding to the shaft of the screws, a similar decrease in the thickness of the fibrous tissue layer was observed after 54 weeks and was seen at 72 weeks. With both types of PLGA screws (plain and ciprofloxacin containing) the capsule was thicker and it formed faster in area A (screw head) than in areas B (screw shaft) and C (screw tip). This may be related to more extensive polymer degradation from the screw heads leading to macrophage reactions and then to a prominent repair in the fibrous tissue scar formation.

The fibrous tissue capsule seemed to be much thinner in the case of titanium screws than in the case of plain or ciprofloxacin-releasing PLGA screws in all follow-up periods. This may in part be due to the artifact caused by the removal

of the titanium screws before preparing the histological sections, which may lead to distortion of the real capsule. On the other hand, the body may respond to the reduction of the mass and volume of the bioabsorbable implants and the resulting debris formed during the degradation process by filling in the space formerly occupied by the implant with fibrous tissue or bone (in case of intraosseous implants, Habal *et al.* 1999). It is known that inert materials produce thinner capsules than reactive materials. The surface texture of the implant can also influence the tissue responses (Ashammakhi 1996).

After surgical implantation of biomaterials, the initial inflammatory response is followed by the formation of loose connective tissue or granulation tissue, which encapsulates the implant and soon develops to a denser connective tissue capsule. In the current study a compact fibrous tissue capsule surrounded the screws already after four weeks. The capsule matured over time.

Macrophages have been demonstrated to play an important role in the resorption process of polymeric materials (Lam *et al.* 1993). These cells, and the mediators they produce, are important in determining the overall biocompatibility of an implanted device (Hunt *et al.* 1991). In the current study the highest number of macrophages was seen at 2-8 weeks postoperatively. Most of them were seen around the heads of the PLGA screws (area A). This may be due to the fact that small PLGA particles become detached from the surface of the implant, during the implantation process, and elicit a macrophage reaction. In the case of plain and ciprofloxacin-releasing screws similar numbers of macrophages were seen at two weeks but in the case of ciprofloxacin-releasing screws, less at four and eight weeks in area A (screw head). The highest number of giant cells was seen at an early stage (two weeks), after which their number started to decrease, probably taking a lesser role in the host reaction. The ultimate digestion and clearing of the decomposing polymeric material later on is probably more dependent on the macrophages that have invaded the implant matrices. At 16 and 24 weeks no difference in the inflammatory reaction profiles could be discerned when plain and ciprofloxacin PLGA screws were compared. In the case of ciprofloxacin-releasing screws, after one year, the amount of macrophages increased again. The reason for this increase is unclear and needs further research, but it is noteworthy that at this same time, concentrations of ciprofloxacin increased slightly after the 16 and 24 week low values. By comparison, the number of macrophages at a corresponding time point with plain SR-PLGA screws was not as great. One

explanation can be the increased infiltration to clear out the material debris left in the extracellular space, as most of this small debris is formed in the period between 6 and 12 months. This is also supported by the observation with plain PLGA miniscrews screws studied by authors.

> *Macrophages have been demonstrated to play an important role in the resorption process of polymeric materials. In the current study the highest number of macrophages was seen at 2-8 weeks postoperatively.*

In earlier reports on closely-related material (LactoSorb PLGA (82L/18G) screws), Eppley and Sadove (1995) note that after one year of implantation in cranial bones of rabbits, there was no evidence of PLGA polymer. This has also been mentioned in other studies (Habal *et al.* 1999, Pietrzak *et al.* 1997). Observations in the current study, however, indicate that some PLGA material still remains one year after implantation. This was seen with both plain and ciprofloxacin-releasing SR-PLGA 80/20 screws. The methods with which a resorbable polymer is processed to fabricate the implant, including terminal sterilization, can affect its degradation rate (Habal *et al.* 1999). Self-reinforcing increases the strength of polymeric devices so that they can be sterilized by gamma irradiation instead of ethylene oxide (commonly used to sterilize such implants). Gamma irradiation leads to faster degradation by breaking down the long polymer chains. PLGA (80/20 and 82/18) is substantially amorphous and has a degradation profile intermediate between the PLA and PGA (Peltoniemi *et al.* 2002). Degradation proceeds faster in the amorphous areas of the implant leading to an increase in the relative concentration of polymer crystals, which are more resistant to degradation. They remain in the tissue for long periods of time accompanied with inflammatory cells (Peltoniemi *et al.* 2002). An amorphous absorbable copolymer will typically degrade evenly and uniformly, eliminating the potential adverse reactions. Polymer microstructure, which refers to the relative proportion and the size of the crystalline and amorphous domains of the material, is one of the most influential properties affecting both degradation of and physiological response to absorbable polymers (Habal *et al.* 1999). Implantation site, mechanical stresses and size of the implant could also influence implant degradation rate.

Although modern medicine incorporates many operative and antibiotic-involving conservative modes of therapy, the treatment of the bone infection (osteomyelitis) after surgical procedures and trauma continues to be a serious problem (Overbeck *et al.* 1995). The combination of surgical removal of necrotic

tissue and the administration of antibiotics are the primary methods of treatment of chronic osteomyelitis (Wei *et al.* 1991, Overbeck *et al.* 1995). Achievement and maintenance of adequate therapeutic levels of antibiotic in bone without detrimental systemic effects is often difficult since infected bone has a compromised blood circulation (Becker *et al.* 1994, Shinto *et al.* 1992). Necrotic bone provides a surface for bacterial attachment, which may lead to biofilm formation creating a space for bacteria protected from the body's natural defense. For eradicating bacteria encased in such a biofilm, antibiotics must be used in doses which in serum would lead to concentrations many times higher than the minimum bactericidal concentration. As it is difficult to deliver antibiotics to the target site by the intravenous route, local administration is considered to be essential (Wei *et al.* 1991).

> *The treatment of osteomyelitis after surgical procedures and trauma continues to be a serious problem. Achievement and maintenance of adequate therapeutic levels of antibiotic in bone without detrimental systemic effects is often difficult because infected bone has a compromised blood circulation. As it is difficult to deliver antibiotics to the target site by the intravenous route, local administration is considered to be essential.*

The implantation of antibiotic-containing cement beads (polymethyl methacrylate or PMMA beads) has been proved to be effective in the delivery of high concentrations of antibiotics to a local area (Overbeck *et al.* 1995, Becker *et al.* 1994, Jacob *et al.* 1991, Ostermann *et al.* 1993). Because of some of the disadvantages of the antibiotic-containing cement beads discussed above, the development of bioresorbable drug carrier systems represents a potential major advance (Overbeck *et al.* 1995). As the diversity of biomaterials increases, the aim is to produce interactive rather than passive biomaterials that act on the environment in which they are placed in (Hunt *et al.* 1995). The use of bioresorbable polymeric materials (PLA, PGA, PLGA) as drug-releasing systems has been under exploration (Nie *et al.* 1998, Calhoun *et al.* 1997, Gümüsderelioglü *et al.* 2000, Teupe *et al.* 1992, Leinonen et al. 2002, Ramchandani *et al.* 1998).

In the present study ciprofloxacin-releasing polylactide/polyglycolide 80/20 (SR-PLGA) screws were proven to be biocompatible and to possess sufficient strength to allow clinical use. The concentrations of ciprofloxacin were measured in the cranial bones of rabbits. The minimum inhibitory concentration (MIC) of ciprofloxacin against the most common germ associated with implant-related

infections, *Staphylococcus aureus*, is 0.1-1.0 µg/g (Overbeck *et al.* 1995). Ciprofloxacin concentration at two weeks follow-up was 4.4 ± 4.2 µg/g, 14.1 ± 2.7 µg/g at 4 weeks, and 7.6 ± 4.5 µg/g at 8 weeks. The therapeutic level was sustained for least the first eight weeks after implantation since the concentrations of ciprofloxacin released was at two, four, and eight weeks higher than the MIC of ciprofloxacin. The same kind of *in vivo* results have been published earlier by Overbeck *et al.* (Overbeck *et al.* 1995) who used PGA cylinders in rabbit femur and by Ramchandani *et al.* (Ramchandani *et al.* 1998) who used instead PLGA (50/50) cylinders in rabbit femur. Both had follow-up times up to six weeks. Ramchandani *et al.* (1998) also showed that drug levels, greater than the MIC of ciprofloxacin, were measured up to 70 mm at the site of implantation. In the present study, measurement of drug concentration was performed in the bone tissue in an area at a distance of 5 mm in all directions from the screw hole. The sizes of bone blocks were equal, ca. 1 cm^2, but small differences and heterogeneity might naturally occur in biological samples.

The use of bioresorbable polymeric materials (PLA, PGA, PLGA) as drug releasing systems has been under exploration. In this study ciprofloxacin-releasing PLGA implants were used. The therapeutic ciprofloxacin level was sustained at least for the first eight weeks after implantation since the concentrations of ciprofloxacin released was at two, four and eight weeks higher than the MIC of ciprofloxacin.

Concentrations of ciprofloxacin were very low at 16 weeks (0.09 ±0.04 µg/g) and at 24 weeks (0.04 ± 0.02 µg/g). The slightly higher concentrations measured at one year (1.3 ± 1.0 µg/g) was surprising because in *in vitro* studies all ciprofloxacin had been released in six months (Veiranto *et al.* 2002). This may be due to the sample preparation technique. The bioresorbable ciprofloxacin-releasing screw was removed from the block with an electric drill (diameter 2 mm). After one year and 1.5 years follow-up times the screw bed could hardly be recognized and the screw itself had softened. Softening makes removal of the screw difficult and remnants of the material itself might have been left in the blocks, thus causing higher (artefactual) concentrations in the measurements. In histological study, only small fragments of biomaterial were seen in the samples after one year follow-up. The concentration of ciprofloxacin in the surrounding bone tissue at 1.5 years was lower again (0.8 ± 0.6 µg/g), which was expected since in histological samples almost all biomaterial had been absorbed by that time.

Various antibiotics can be used to prevent or treat bone infections. Many factors have to be considered in the planning of the antibiotic to be used in multifunctional drug releasing implants. It should cover the causative or potential infectious agents and it should have appropriate bone penetration to achieve effective concentration in the surrounding bone tissue. The antibiotic should be suitable for processing into the implant without unwanted interactions with the polymer and it should be releasable from the implant. The antibiotic should be approved for clinical use and should preferably be of low cost (Ashammakhi *et al.* 2005a). Ciprofloxacin covers common bone infection causing bacteria such as *S. aureus*, *S. epidermis* and *P. aeruginosa*. It has a minimum inhibitory concentration (MIC) of 0.1-1.0 µg/g against *Staphylococcus aureus*. It was found to have good penetration to bone tissue including compact cortical bone so that acceptable levels were achieved in the immediate vicinity of the implant (Overbeck *et al.* 1995). It has a high melting temperature, thus, it can be processed under conditions used in melt extrusion manufacturing techniques which were used for development of the multifunctional implants used in the present work. However, other methods can theoretically be used such as injection molding. It is, however, a challenge to produce mechanically reliable devices from such antibiotic spiked materials. Non-self-reinforced, e.g. injection molded P(L/DL)LA 70:30 fixation pins have, however, initial shear strength of 152 MPa (Ashammakhi *et al.* 2005a).

Ciprofloxacin-releasing implants were found to inhibit bacterial proliferation in an average area of 34 mm in diameter around them, which can be expected to be adequate for killing bacteria in the area surrounding the implant when it is applied in tissue. It was also found that ciprofloxacin-releasing PLGA was superior to titanium in preventing *S. epidermidis* attachment and biofilm formation *in vitro*, whereas pure PLGA was poorer than titanium in this respect (Niemelä *et al.* 2006). This would warrant future precautions when using plain polymeric implants for their possible vulnerability to colonization. Although there are, so far, no clinical reports on the increased incidence of infections upon the use of polymeric bioresorbable implants as compared to metals, the study raises questions in this regard. In the study by Niemelä *et al*. (2006), no bacterial growth was found in 57.5 % of the examined areas on ciprofloxacin-releasing PLGA implants on the first day, whereas less than a fifth of the surface area of the plain PLGA and titanium implants was negative. During the whole study period, the mean bacterial count was lowest with the ciprofloxacin PLGA specimens. Biofilm

was not seen in any of the incubation periods in 93 %–100 % of the ciprofloxacin PLGA specimens or it surrounded some only slightly.

Adding antibiotic may compromise the strength of the resulting screws. It was shown in this study that the ciprofloxacin-containing SR-PLGA screws had lower pullout strength (66.8 ± 4.9 N) than the corresponding plain conventional SR-PLGA screws (96.3 ± 9.3 N). In our first experiment the pull-out force of the SR-PLGA tacks was 115.9 ± 8.3 N, that of Lactosorb® screws 112.9 ± 12.1 N and that of Biosorb® screws 110.4 ± 8.9 N (p=0.69). Scanning electron microscopy (SEM) showed that the fibrillar strip-like microstructure of plain SR-PLGA miniscrews turns into a coarse uni-axial platelet-like morphology in antibiotic SR-PLGA miniscrews as a result of the addition of ciprofloxacin. It is recommended that ciprofloxacin-releasing screws should only be applied in nonload-bearing or slightly load-bearing applications. *In vitro* studies of ciprofloxacin-releasing implants have shown that the screws retain their mechanical properties at a level that ensures their fixation properties at least for nine weeks (SR-PLGA rods, Ø 3mm) (Veiranto *et al.* 2004). In this study, new material patches made into tacks proved that this problem with pullout strength can be avoided as the average strength of ciprofloxacin PLGA was 147 MPa and that of the corresponding control tacks was 141 MPa.

Ciprofloxacin release properties were satisfactory (above therapeutic level and below toxic level with good high burst peak). Hence, these devices can be used either for prophylaxis (high early shot) or for treatment (prolonged maintained release) of bone infections. Ciprofloxacin has shown to have inhibitory effects on osteoblastic cells *in vitro* (at concentration 40 µg/ml) (Holtom *et al.* 2000). The potentially detrimental level that can adversely affect osteoblast-like cell growth is over 20 µg/ml (Miclau *et al.* 1998). In *in vitro* tests all loaded ciprofloxacin was released from the studied screws after 23 weeks. During that time, the concentration of ciprofloxacin released per day remained in the range of 0.6-11.6 µg/ml after the start-up peak. *In vitro* the maximum concentration value of released ciprofloxacin was recorded in the 8th week (Veiranto *et al.* 2004). It has also been shown that experimental fractures in rat femur exposed to ciprofloxacin disclose impaired fracture healing (Perry *et al.* 2003). The authors suggest that administration of quinolones during early fracture repair may compromise fracture healing in humans. This observation should be considered when ciprofloxacin screws are used in clinical trials and careful clinical and radiological evaluation and follow up of these fractures should be performed.

In the present study the biocompatibility of the SR-PLGA (80L/20G) and ciprofloxacin-releasing SR-PLGA screws was good as they elicited only a mild inflammatory tissue reaction. They seemed not to interfere with osteoblastic activity. Adding antibiotic may compromise the strength of the resulting screws. It is recommended that ciprofloxacin-releasing screws should only be applied in nonload-bearing or slightly load-bearing applications.

Clinical complications of bioresorbable implants are mainly associated with homopolymers (PLLA, PGA). These include pronounced fibrous encapsulation (PLLA), sterile sinus formation (PGA) and bone osteolysis (PGA) (Pietrzak *et al.* 1997). Polyglycolide-polylactide copolymers can have different rates of degradation depending on their compositional ratios (Eppley and Sadove 1995a, Pietrzak *et al.* 1997, Kitchell *et al.* 1985, Eppley *et al.* 1997b). Amorphous, hydrophilic PGA polymers have an accelerated degradation rate when compared to more crystalline PLLA polymers (Eppley *et al.* 1997b). It has been suggested that PLGA 82L/18G (LactoSorb) has intermediate absorption characteristics, which are well tolerated by the body. These include degradation, slow enough not to overwhelm the body's local ability to clear the degradation products, yet fast enough to demonstrate clearance after approximately one year and lack of crystallinity (Pietrzak *et al.* 1997). SR-PLGA 80L/20G screws have a molar composition close to that of Lactosorb screws (82L/18G). In the present study the biocompatibility of the SR-PLGA (80L/20G) and the ciprofloxacin-releasing SR-PLGA screws was good as they elicited only a mild inflammatory tissue reaction. They seemed not to interfere with osteoblastic activity.

Clinical studies are in progress with antibiotic implants in orthopedics and traumatology and expansion to other disciplines of surgery will take place soon. For the clinical use in USA the implants need the sales permission from FDA (Food and Drug Administration). First permission has been received for non-antibiotic implants. An application for antibiotic implants will be filed in near the future. The products also need the CE mark for marketing in EU countries. This mark has already been received for non-antibiotic implants.

7 Conclusions

With reference to the purpose of the present investigation, the conclusions are as follows:

1. The biocompatibility of ciprofloxacin-releasing SR-PLGA 80/20 screws was good. Only mild inflammatory reaction was noted. The macrophage and giant cell involvement was similar in ciprofloxacin-releasing and plain SR-PLGA screws. The highest number of them was seen at eight weeks follow-up time. Screws did not interfere with osteoblastic activity. A tiny amount of intracellular PLGA material still remained in the cranial bones of rabbits at the 1½ years' follow-up time. The fibrous tissue capsule grew in thickness during the follow-up time around both plain and ciprofloxacin-releasing screws.
2. The biocompatibility of the SR-PLGA 80/20 screws was good, as they elicited only a mild inflammatory tissue reaction and did not interfere with osteoblastic activity. Also in the case of plain SR-PLGA screws remnants of PLGA material were still seen in the cranial bones of rabbits 1½ years after implantation.
3. Ciprofloxacin-releasing SR-PLGA 80/20 screws released gradually the loaded antibiotic when implanted in rabbit cranial bone. This led to high local antibiotic levels at the site of implantation in bone up to eight weeks. After this, drug levels were very low although there was a slight increase in drug levels one year after implantation. After 1½ years the drug levels had again decreased.
4. Ciprofloxacin-releasing SR-PLGA 80/20 miniscrews have lower pull-out force than the corresponding plain screws. The addition of antibiotic led to a coarse platelet-like morphology of the implant and reduced the pull-out force.
5. Changing the shape of ciprofloxacin-releasing implants from a screw to a tack increased the pull-out force to levels similar to those measured for the plain implants.
6. Plain SR-PLGA 80/20 tacks have good holding power to human cadaver parietal bones: slightly better than the corresponding PLGA screws (BioSorbPDX® and LactoSorb®). Since the use of tacks does not require pretapping or tightening their use saves time and costs when applied in a clinical setting for osteofixation.

In these relatively extensive studies no features were found which would prevent the clinical use of SR-PLGA (80L/20G) copolymer in surgery.

References

Aho A, Heikkilä J, Aho H, Andersson Ö & Yli-Urpo A (1993) Morphology of osteogenesis in bioactive glass interface. Ann Chir Gyn 82: 145-154.

Albrecht-Olsen P, Kristensen G & Törmälä P (1993) Meniscus bucket handle fixation with an absorbable Biofix tack development of a new technique. KneeSurg Sports Traumatol Arthroscopy 1: 104-106.

Anastakis D, Antonyshyn O, Cooper P, Yaffe M, Bush K & Mawdsley G (1996) Computed tomography artefacts associated with craniofacial fixation devices: an experimental study. Ann Plast Surg 37(4): 349-355.

Andreopoulos A, Korakis T, Duonis E, Anastasiadis A, Tzivelekis P & Kanellakopoulou K (1996) In vitro release of new quinolones from biodegradable systems: A comparative study. J Biomat Appl 10: 338-347.

Antikainen T, Pernu H, Törmälä P, Kallioinen M, Waris T & Serlo W (1994) Development of the neurocranium after transsutural fixing by new, resorbable poly-L-lactide miniplates. A comparison to fixing with the common titanium miniplates. Acta Neurochir (Wien) 128: 26-31.

Ashammakhi N (1996) Neomembranes: a concept review with special references to self-reinforced polyglycolide membranes. J Biomed Mat Res [Appl Biomater.] 33: 297-303.

Ashammakhi N, Mäkelä E, Vihtonen K, Törmälä P, Waris T & Rokkanen P (1999) Osteogenic potential of self-reinforced polyglycolide membrane. SOT 22(3): 179-183.

Ashammakhi N, Peltoniemi H, Waris E, Suuronen R, Serlo W, Kellomäki M, Törmälä P & Waris T (2001) Developments in Craniomaxilofacial Surgery: Use of Self-Reinforced Bioabsorbable Osteofixation Devices. Plast Rec Surg 108(1): 167-180.

Ashammakhi N, Renier D, Arnaud E, Marchac D, Ninkovic M, Donaway D, Jones B, Serlo W, Laurikainen K, Törmälä P & Waris T (2004) Successful use of BioSorb osteofixation devices in 165 cranial and maxillofacial cases: a multicenter report. J Craniofac Surg 15(4): 692-701.

Ashammakhi N & Rokkanen P (1997) Absorbable polyglycolide devices in trauma and bone surgery. Biomat 18(1): 3-9.

Ashammakhi N, Veiranto M, Niemelä SM, Tiainen J, Leinonen S, Suokas E & Törmälä P (2005a) Development of Multifunctional Materials (MFM): Bioabsorbable Drug-Releasing Hard Tissue Fixation Screws. Mat Sci Forum 492-493: 195-200.

Ashammakhi N, Veiranto M, Viitanen P, Suokas E, Aro H, Koort J, Tiainen J, Leinonen S, Niemelä SM, Ikäheimo I, Koskela M, Syrjälä H, Waris T & Törmälä P (2003) Development of Multifunctional Materials (MFM): Bioabsorbable Osteoconductive Drug- Releasing Hard Tissue Fixation Screws. 4th Annual meeting of BRG, June 3, Helsinki, Finland.

Ashammakhi N, Waris T, Serlo W & Törmälä P (2005b) Self-Reinforced Bioabsorbable Devices for osteofixation of Craniofacial Bones. In: Yaszemski M (ed) Biomaterials in orthopedics: 169-184.

Baldassarri L, Donelli G, Gelosia A, Voglino M, Simpson A & Christenssen G (1996) Purification and haracterization of the Staphylococcal slime-associated antigen and its occurrence among Staphylococcus epidermidis clinical isolates. Infection and immunity 64(8): 3410-3415.

Baldassarri L, Donelli G, Gelosia A, Simpson A & Christenssen G (1997) Expression of slime interferes with in vitro detection of host protein receptors of Staphylococcus epidermidis. Infection and immunity 65(4): 1522-1526.

Beals SP & Munro IR (1987) The use of miniplates in craniomaxillofacial surgery. Plast Reconstr Surg 79(1): 33-38.

Becker P, Smith R, Williams R & Dutkowsky (1994) Comparison of Antibiotic Release from Polymethylmethacrylate Beads and Sponge Collagen. J Orthop Res 12(5): 737-741.

Bergsma J, Rozema F, Bos R, De Bruijn W (1993) Foreign body reactions to resorbable poly(L-lactide) bone plates and screws used for the fixation of unstable zygomatic fractures. J Oral Maxillofac Surg 51: 666-670.

Bergsma J, de Bruijn W, Rozema F, Bos R & Boering G (1995) Late degradation tissue response to poly(L-lactide) bone plates and screws. Biomat 1(16): 25-31.

Bloss B, Rogers T & Seligson D (1998) Bioabsorbable pins and screws for the fixation of distal radial fractures. Tech Orthop 13(2): 153-159.

Bos R, Rozema G & Leenslag J (1987) Resorbable poly(L-lactide) plates and screws for the fixation of zygomatic fractures. J OralMaxillofac Surg 45(9): 751-753.

Bos R, Rozema F, Boering G, Nijenhuis A, Pennings A & Jansen H (1989 a) Bone-plates and screws of bioabsorbable poly (L-lactide)—an animal pilot study. Br J Oral Maxillofac Surg 27(6): 467-476.

Bos R, Rozema F, Boering G, Nijenhuis A, Pennings A & Vervey A (1989 b) Bioabsorbable plates and screws for internal fixation of mandibular fractures. A study in six dogs. Int J OralMaxillofac Surg 18(6): 365-369.

Bos R, Rozema F, Boering G, Nijenhuis A, Pennings A, Vervey A, Nieuwenhuis P & Jansen H (1991) Degradation of and tissue reaction to biodegradable poly(L-lactide) for use as internal fixation of fractures: a study in rats. Biomat 12(1): 32-36.

Böstman O (1994) Economic considerations on avoiding implant removals after fracture fixation by using absorbable devices. Scand J Soc Med 22(1): 41-45.

Calhoun J & Mader J (1997) Treatment of osteomyelitis with a biodegradable antibiotic implant. Clin Orthop Rel Res 341: 206-214.

Carlsson Å, Josefsson G & Lindberg L (1978) Revision with gentamicin-impregnated cement for deep infections in total hip arthroplasties. J Bone Joint Surg Inc 60A(8): 1059-1064.

Chang C & Merritt K (1991) Effect of staphylococcus epidermidis on adherence of pseudomonas aeruginosa and proteus mirabilis to polymethyl methacrylate (PMMA) and gentamicin-containing PMMA. JOrthop Res 9(2): 284-288.

Chang C & Merritt K (1992) Microbial adherence on poly(methyl methacrylate) (PMMA) surfaces. J Biomed Mater Res 26: 197-207.

Christensen G, Baldassarri L & Simpson A (1994) Colonization of medical Devices by Coagulase-Negative staphylococci. In: Bisno A (ed) Infections Associated with Indwelling Medical Devices. 2nd edition.Washington DC: 45-72.

Christensen G, Simpson A, Bisno A & Beachey E (1982) Adherence of Slime-Producing Strains of Staphylococcus epidermidis to smooth surfaces. Inf Immunity 37(1): 318-326.

Cohen S, Holmes R, Amis P, Fitchner H & Shusterman M (2001) Tacks:A new technique for craniofacial fixation. J Craniofac Surg 12(6): 596-602.

Costerton W, Cheng KJ, Geesey G, Ladd T, Nickel C, Dasgupta M & Marrie T (1987) Bacterial biofilms in nature and disease. Ann Rev Microbiol 41: 435-64.

Court-Brown C (1990) Antibiotic prophylaxis in orthopaedic surgery. Scand J Infect Dis Suppl 70: 74-79.

Cutright D, Hunsuck E & Beasley J (1971) Fracture reduction using a biodegradable material, polylactic acid. J Oral Surg 29: 393-397.

Dean D, Topham N, Rimnac C, Mikos A, Goldberg D, Jepsen K, Redtfeldt R, Liu Q, Pennington D & Ratcheson (1998) Osseointegration of preformed polymethylmethacrylate craniofacial prostheses coated with bone marrow-impregnated poly (DL-lactic-co-glycolic acid) foam. Plast Reconstr Surger 104(3): 705-712.

Edin M, Miclau T, Lester G, Lindsey R &Dahners L (1996) Effect of cefazolin and vancomycin on osteoblasts in vitro. Clin Orthop 333: 245-51.

Edwards R, Kiely K & Eppley B (2000) Resorbable fixation techniques for genioplasty. J Oral Maxillofac Surg 58: 269-272.

Eggimann P & Pittet D (2001) Infection control in the ICU. Chest 120: 2059-2093.

Enislidis G, Pichorner S, Lambert F, Wagner A, Kainberger F, Kautzky M & Ewers R (1998) Fixation of zygomatic fractures with a new biodegradable copolymer osteosynthesis system. Int J Oral Maxillofac Surg 27:352-355.

Eppley B & Prevel C (1997 a) Nonmetallic fixation in traumatic midfacial fractures. J Craniofac Surg 8(2): 103-109.

Eppley B & Reilly M (1997 b) Degradation characteristics of PLLA-PGA bone fixation devices. J Craniofac Surg 8(2): 116-120.

Eppley B (1997c) Resorbable biotechnology for craniomaxillofacial surgery. J Craniofac Surg 8(2): 85-86.

Eppley B & Sadove M (1992) Effects of resorbable fixation on craniofacial skeletal growth: A pilot experimental study. J Craniofac Surg 3(4): 190-196.

Eppley B & Sadove M (1994) Effects of resorbable fixation on craniofacial skeletal growth: modifications in plate size. J Craniofac Surg 5(2): 110-114.

Eppley B & Sadove M (1995 a) A comparison of resorbable and metallic fixation in healing of calvarial bone grafts. Plast Reconstr Surg 96(2): 316-322.

Eppley B & Sadove M (1995 b) Resorbable coupling fixation in craniosynostosis surgery: experimental and clinical applications. J Craniofac Surg 6(6): 477-482.

Eppley B, Sparks C, Herman E, Edwards M, McCarty M & Sadove M (1993) Effects of skeletal fixation on craniofacial imaging. J Craniofac Surg 4(2): 67-73.

Ewers R & Lieb-Skowron J (1990) Bioabsorbable osteosynthesis materials. Facial Plast Surg 7(3): 206-213.

Fearon J, Munro I & Bruce D (1995) Observation on the use of rigid fixation for craniofacial deformities in infants and young children. Plast Reconstr Surg 95(4): 634-637.

Fiala T, Novelline R & Yaremchuk M (1993) Comparison of CT imaging artefacts from craniomaxillofacial internal fixation devices. Plast Reconstr Surg 92(7): 1227-1232.

Fiala T, Paige K, Davis T, Campbell T, Rosen B & Yaremchuk M (1994) Comparison of artifact from craniomaxillofacial internal fixation devices: magnetic resonance imaging. Plast Reconstr Surg 93(4): 725-731.

Frazza E & Schmitt E (1971) A new absorbable suture. J Biomed Mater Res Symp 1: 41-52.

Getter L, Cutright D, Bhaskar S & Augsburg J (1972) A biodegradable intraosseous appliance in the treatment of mandibular fractures. J Oral Surg 30: 344-348.

Goldberg D, Bartlett S, Yu J, Hunter J & Whitaker L (1995) Critical Review of microfixation in pediatric craniofacial Surgery. J Craniofac Surg 6(4): 301-307.

Gollwitzer H, Ibrahim K, Meyer H, Mittelmeier W, Busch R & Stemberger A (2003) Antibacterial poly(D,L-lactic acid) coating of medical implants using a biodegradable drug delivery technology. J Antimicrob Chemother 51: 585-91.

Goldstein J, Quereshy F & Cohen A (1997) Early experience with biodegradable fixation for congenital pediatric craniofacial surgery. J Craniofac Surg 8(2): 110-115.

Gristina A & Costerton W (1985) Bacterial Adherence to biomaterials and tissue. J bone Joint Surg 67-A(2): 264-273.

Gümüşderelioğlu M & Deniz G (2000) Sustained release of mitomycin-C from poly (DL-lactide)/poly (DL-lactide-co-glycolide) films. J Biomater Sci Polymer Edn 11(10): 1039-1050.

Habal M (1991) Editorial. J Craniofac Surg 2: 55.

Habal M & Pietrzak W (1999) Key points in the fixation of the craniofacial skeleton with absorbable biomaterial. J Craniofac Surg 10(6): 491-499.

Haers P & Sailer H (1999) Biodegradable self-reinforced poly-L/DL-lactide plates and screws in bimaxillary orthognathic surgery: short term skeletal stability and material related failures. J Cranio Maxillofac Surg 26: 363-372.

Haers P, Suuronen R, Lindqvist C & Sailer H (1998) Biodegradable polylactide plates and screws in orthognathic surgery: technical note. J Cranio Maxillofac Surg 26: 87-91.

Harbarth S, Samore M, Lichtenberg D & Carmeli Y (2000) Prolonged antibiotic prophylaxis after cardiovascular surgery and its effect on surgical site infections and antimicrobial resistance. 101(25): 2916-2921.

Hofmann G, Wagner F, Keller A and Feist H (1996) Postoperative irradiation treatment and bioresorbable implants in orthopaedic surgery: an experimental in vitro study. Biomaterials 17: 1149-1153.

Holland S, Tighe B & Gould P (1986) Polymers for biodegradable medical devices. The potential of polyesters as controlled macromolecular release systems. J Controlled Rel 4: 155-180.

Hollinger J (1983) Preliminary report on the osteogenic potential of a biodegradable copolymer of polylactide (PLA) and polyglycolide (PGA). J Biomed Mater Res 17: 71-82.

Holtom P & Patzakis M (2003) Newer methods of antimicrobial delivery for bone and joint infections. Instr Course Lect 52: 745-749.

Holtom P, Pavkovic S, Bravos P, Patzakis M & Shepherd M (2000) Inhibitory effects of the quinolone antibiotics trovafloxacin, ciprofloxacin, and levofloxacin on osteoblastic cells in vitro. J Orthop Res 18(5): 721-727.

Hoyle B & Costerton J (1991) Bacterial resistance to antibiotics: the role of biofilms. Prog Drug Res 37: 91-105.

Humphrey J, Mehta, Seaber A & Vail T (1998) Pharmacocinetics of a degradable drug delivery system in bone. Clin Orthop Rel Res 349: 218-224.

Hunt J, Vince D & Williams D (1993) Image analysis in the evaluation of biomaterials. J Biomed Eng 15: 39-45.

Hönig J, Merten H & Luhr HG (1995) Passive and active intracranial translocation of osteosynthesis plates in adolescent minipigs. J Craniofac Surg 6(4): 292-298.

Illi O, Sailer H & Stauffer U (1989) Résultats preliminaries de l'ostéosynthése biodegradable en chirurgie cranio-faciale chez l'enfant. Chir Pédiatr 30: 284-287.

Isefuku S, Joyner C & Simpson, A (2001) Toxic effect of rifampicin on human osteoblast-like cells. J Orthop Res 19(5): 950-954.

Isefuku S, Joyner C & Simpson A (2003) Gentamicin may have an adverse effect on osteogenesis. J Orthop Trauma 17(3): 212-216.

Jacob E, Setterström J, Bach D, Heath J, McNiesh M & Cierny G (1991) Evaluation of biodegradable ampicillin anhydrate microcapsules for local treatment of experimental staphylococcal osteomyelitis. Clin Orthop Rel Res 267: 237-244.

Jacobs J, Gilbert J & Urban R (1998) Current concepts review corrosion of metal orthopaedics implants. J Bone Joint Surg 80-A(2): 268-279.

Jain R (2000) The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials 21(23): 2475-2490.

Jukkala-Partio K, Laitinen O, Partio E, Vasenius J, Vainionpää S, Pohjonen T, Törmälä P & Rokkanen P (1997) Comparison of the fixation of subcapital femoral neck osteotomies with absorbable self-reinforced poly-L-lactide lag-screws or metallic screws in shep. J Orthop Res 15: 124-127.

Juutilainen T, Pätiälä H, Ruuskanen M & Rokkanen P (1997) Comparison of costs in ankle fractures treated with absorbable or metallic fixation devices. Arch Orthop Trauma Surg 116: 204-208.

Kallela I, Tulamo R, Hietanen J, Pohjonen T, Suuronen R & Lindqvist C (1999) Fixation of mandibular body osteotomies using biodegradable amorphous self-reinforced (70L:30DL) polylactide or metal lag screws:an experimental study in sheep. J Cranio Maxillofac Surg 27: 124-133.

Kennady M, Tucker M, Lester G & Buckley M (1989 a) Histomorphometric evaluation of stress shielding in mandibular continuity defects treated with rigid fixation plates and bone grafts. Int J Oral Maxillofac Surg 18: 170-174.

Kennady M, Tucker M, Lester G & Buckley M (1989 b) Stress shielding effect of rigid internal fixation plates on mandibular bone grafts. Int J Oral Maxillofac Surg 18: 307-310.

Kitchell J & Wise D (1985) Poly(lactic/glycolic acid) biodegradable drug-polymer matrix systems. Methods Enzymol 112: 436-448.

Koort J, Mäkinen T, Suokas E, Veiranto M, Jalava J, Knuuti J, Törmälä P & Aro H (2005) Efficacy of ciprofloxacin-releasing bioabsorbable osteoconductive bone defect filler for treatment of experimental osteomyelitis due to staphylococcuc aureus. Antimicrob.Agents Chemother 49(4): 1502-1508.

Koranyi E, Bowman MS, Knecht CD & Janssen M (1970) Holding power of orthopedic screws in bone. Clin Orthop Rel Res 72: 283-286.

Koskikare K, Hirvensalo E, Pätiälä H, Rokkanen P, Pohjonen T, Törmälä P & Lob G (1996) Intraosseous plating with absorbable self-reinforced poly-L-lactide plates in the fixation of distal femoral osteotomies on rabbits. J Biomed Mater Res 30: 417-421.

Koskikare K, Pihlajamäki H, Pätiälä H & Rokkanen P (1997a) Comparison of intra- and extraosseally placed self-reinforced poly-L-lactide plates in the fixation of distal femoral osteotomies in rabbits. Ann Chir Gyn 86: 261-268.

Koskikare K, Hirvensalo E, Pätiälä H, Rokkanen P, Pohjonen T, Törmälä P & Lob G (1997b) Fixation of osteotomies of the distal femur with absorbable, self-reinforced, poly-L-lactide plates. Arch Orthop Trauma Surg 116: 352-356.

Kumar A, Staffenberg D, Petronio J & Wood R (1997) Bioabsorbable plates and screws in pediatric craniofacial surgery: A review of 22 cases. J Craniofac Surg 8: 97.

Kumta S & Leung P (1998) The technique and indications for the use of biodegradable implants in fractures of the hand. Tech Orthop 13(2): 160-163.

Lam K, Schakenraad J, Esselbrugge H, Feijen J & Nieuwenhuis P (1993) The effect of phagocytosis of poly(L-lactic acid) fragments on cellular morphology and viability. J Biomed Mater Res 27: 1569-1577.

Länsman S, Serlo W, Linna O, Pohjonen T, Törmälä P, Waris T & Ashammakhi N (2002) Treatment of pectus excavatum with bioabsorbablepolylactide plates: preliminary results. J Pediatr surg 37(9): 1281-1286.

Leinonen S, Suokas E, Veiranto M, Törmälä P, Waris T & Ashammakhi N (2002) Holding power of bioabsorbable ciprofloxacin-containing self-reinforced poly-L/DL-lactide 70/30 bioactive glass 13 miniscrews in human cadaver bone. J Craniofac Surg 13(2): 212-218.

Leinonen S, Tiainen J, Kellomäki M, Suokas E, Ashammakhi N, Törmälä P & Waris T (2001) Holding power of bioabsorbable self-reinforced poly-L/LD-lactide 70/30 tacks and miniscrews in human cadaver metatarsal bone. J Craniofac Surg 14(2): 171-175.

Lew D & Waldvogel F (2004) Osteomyelitis. Lancet 364(24): 369-79.

Lin K, Bartlett S, Yaremchuk M, Grossman R, Udupa J & Whitaker L (1991) An experimental study on the effect of rigid fixation on the developing craniofacial skeleton. Plast Reconstr Surg 87(2): 229-35.

Lin S, File I, Shah A, Pizzutillo P & Tuan R (1992) A bioresorbable drug delivery system for the treatment of chronic osteomyelitis. Fourth World Biomaterials Congress, April 24-28, Berlin, Federal Republic of Germany.

Loo S, Ooi C, Boey Y (2004) Influence of electron-beam radiation on the hydrolytic degradation behaviour of poly(lactide-co-glycolide) (PLGA). Biomaterials 26(18): 3809-3817.

Lucke M, Schmidmaier G, Sadoni S, Wildemann B, Schiller R, Haas NP & Raschke (2003) Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats. Bone 32(5): 521-531.

Lucke M, Wildemann B, Sadoni S, Surke,C, Schiller R, Stemberger A, Raschke M, Haas N & Schmidmaier G (2005) Systemic versus local application of gentamicin in prophylaxis of implant-related osteomyelitis in a rat model. Bone 36(5): 770-778.

Mäkelä P, Ruuskanen M, Ashammakhi N, Kallioinen M, Pohjonen T, Serlo W, Törmälä P &Waris T (1999) Comparison of self-reinforced poly-L-lactide and steel wire in fixation of sternotomy in rabbits. Ann Chir Gyn 88: 318-321.

Mäkelä P, Pohjonen T, Törmälä P, Waris T & Ashammakhi N (2002) Strength retention properties of self-reinforced poly L-lactide (SR-PLLA) sutures compared with polyglyconate (Maxon®) and polydioxanone (PDS) sutures. An in vitro study. Biomaterials 23: 2587-2592.

Mäkinen T, Veiranto M, Knuuti J, Jalava J, Törmälä P & Aro H (2005) Efficacy of bioabsorbable antibiotic containing bone screw in the prevention of biomaterial-related infection due to Staphylococcus aureus. Bone 36: 292-299.

Mathy J, Warren S & Longaker M (2002) Discussion. J Craniofac Surg 13(2): 219-221.

McKee M, Wild L, Schemitsch E & Waddell J (2002) The use of an antibiotic-impregnated, osteoconductive, bioabsorbable bone substitute in the treatment of infected long bone defects: early results of a prospective trial. J Orthop Trauma 16(9): 622-627.

Miller R, Brady J & Cutright D (1977) Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. J Biomed Mater Res 11(5): 711-719.

Mini E, Nobili S & Periti P (1997) Methicillin-resistant staphylococci in clean surgery. Is there a role for prophylaxis? Drugs 54(6): 39-52.

Moehring D, Gravel C, Chapman M & Olson S (2000) Comparison of antibiotic beads and intravenous antiobiotics in open fractures. Clin Orthop Rel.Res 372: 254-261.

Montag M, Morales L & Daane S (1997) Bioabsorbables: Their use in pediatric craniofacial surgery. J Craniofac Surg 8(2): 100-102.

Nie L, Nicolau D, Tessier P, Kourea H, Browner B & Nightingale C (1998) Use of bioabsorbable polymer for the delivery of ofloxacin during experimental osteomyelitis treatment. J Orthop Res 16(1): 76-79.

Niemelä SM, Ikäheimo I, Koskela M, Veiranto M, Suokas E, Törmälä P, Waris T, Ashammakhi N & Syrjälä H (2006) Ciprofloxacin-releasing bioabsorbable polymer is superior to titanium in preventing Staphylococcus epidermidis attachment and biofilm formation in vitro. J Biomed Mater Res[B] Appl Biomater 76(1): 8-14.

Nijhof M, Dhert W, Fleer A, Vogely C & Verbout A (2000) Prophylaxis of implant-related staphylococcal infections using tobramycin-containing bone cement. J Biomed Mater Res 52(4): 754-761.

Nordström P, Pihlajamäki H, Toivonen T, Törmälä P & Rokkanen P (1998) Tissue response to polyglycolide and polylactide pins in cancellous bone. Arch Orthop Trauma Surg 117: 197-204.

Orringer J, Barcelona V & Buchman S (198) Reasons for removal of rigid internal fixation devices in craniofacial surgery. J Craniofac Surg 9(1): 40-44.

Osterman P, Henry S & Seligson D (1993) The role of local antibiotic therapy in the management of compound fractures. Clin Orthop Rel Res 295: 102-111.

Overbeck J, Winckler S, Meffert R, Törmälä P, Spiegel H & Brug E (1995) Penetration of ciprofloxacin into bone: a new Bioabsorbable Implant. J Investigat Surg 8: 155-162.

Paavolainen P, Karaharju E, Slätis P, Ahonen J & Holmström T (1978) Effect of rigid plate fixation on structure and mineral content of cortical bone. Clin Orthop Rel Res 136: 287-293.

Päivärinta U, Böstman O, Majola A, Toivonen T, Törmälä P & Rokkanen P (1993) Intraosseous cellular response to biodegradable fracture fixation screws made of polyglycolide or polylactide. Arch Orthop Trauma Surg 112: 71-74.

Pakkanen M, Salisbury A & Ersek R (1996) Biodegradable positive fixation for the endoscopic brow lift. Plast Reconstr Surg 98(6): 1087-1091.

Papay F, Hardy S, Morales L, Walker M & Enlow D (1995) “False” migration of rigid fixation appliances in pediatric craniofacial surgery. J Craniofac Surg 6(4): 309-313.

Partio E, Tuompo P, Hirvensalo E, Böstman O & Rokkanen P (1997) Totally absorbable fixation in the treatment of fractures of the distal femoral epiphyses. A prospective clinical study. Arch Orthop Trauma Surg 116(4): 213-216.

Peltoniemi H, Ahovuo J, Tulamo RM, Törmälä P 6 Waris T (1997) Biodegradable and titanium plating in experimental craniotomies: a radiographic follow-up study. J Craniofac Surg 8(6): 446-451.

Peltoniemi H, Ashammakhi N, Kontio R, Waris T, Salo A, Lindqvist C, Gratz K & Suuronen R (2002) The use of bioabsorbable osteofixation devices in craniomaxillofacial surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 94(1): 5-14.

Peltoniemi H, Tulamo R, Pihlajamäki H, Kallioinen M, Pohjonen T, Törmälä P, Rokkanen P & Waris T (1998) Consolidation of craniotomy lines after resorbable polylactide and titanium plating: a comparative experimental study in sheep. Plast reconstr Surg 101(1): 123-133.

Peltoniemi H, Tulamo R, Toivonen T, Hallikainen D, Törmälä P & Waris T (1999) Biodegradable semirigid plate and miniscrew fixation compared with rigid titanium fixation in experimental calvarial osteotomy. J Neurosurg 90(5): 910-917.

Pensler J (1997) Role of resorbable plates and screws in craniofacial surgery. J Craniofac Surg 8(2): 129-134.

Perry A, Prpa B, Rouse M, Piper K, Hanssen A, Steckelberg J & Patel R (2003) Levofloxacin and trovafloxacin inhibition of experimental fracture-healing. Clin Orthop Rel Res 414: 95-100.

Pietrzak W, Sarver D & Verstynen M (1997) Bioabsorbable polymer science for the practicing surgeon. J Craniofac Surg 8(2): 87-91.

Piettrzak W, Sarver D, Bianchini S & D'Alessio K (1997b) Effect of simulated intraoperative heating and shaping on mechanical properties of a bioabsorbable fracture plate material. J Biomed Mater Res (Appl Biomater) 38: 17-24.

Pistner H, Gutwald R, Ordung R, Reuther J & Mühling J (1993) Poly(l-lactide): a long-term degradation study in vivo. Biomaterials 14(9): 671-677.

Raghuvanshi R, Singh M & Talwar G (1993) Biodegradable delivery system for single step immunization with tetanus toxoid. Int J Pharm 93: R1-R5.

Ramchandani M & Robinson D (1998) In vitro and in vivo release of ciprofloxacin from PLGA 50:50 implants. J Control Rel 54: 167-175.

Resnick J, Kinney B & Kawamoto H (1990) The effect of rigid internal fixation on cranial growth. Ann Plast Surg 25(5): 372-374.

Rinehart G & Pittman T (1998) Growing skull fractures: Strategies for repair and reconstruction. J Craniofac Surg 9(1): 65-72.

Rokkanen P, Bostman O, Vainionpää S, Vihtonen K, Törmälä P, Laiho J, Kilpikari J & Tamminmaki M (1985) Biodegradable implants in fracture fixation: early results of treatment of fractures of the ankle. Lancet 1: 1422-1424.

Rokkanen P (1990) Absorbable implants in the fixation of fractures. Ann Chir Gyn 79: 117-122.

Rokkanen P (1998) Bioabsorbable fixation devices in orthopaedics and traumatology. Ann Chir Gyn 87: 13-20.

Rovati L, Pricca M, Caronni E, Granata G, Donati R & Gaini S (1990) A delayed complication with steel wire osteosynthesis. J Craniofac Surg 8(4): 323-25.

Rozema F, Bos R, Pennings A & Jansen H (1990) Poly(L-lactide) implants in repair of defects of the orbital flor: an animal study. J Oral Maxillofac Surg 48(12): 1305-1309.

Rozema F, Levendag P, Bos R, Boering G and Pennings A (1990) Influence of resorbable poly(L-lactide) bone plates and screws on the dose distributions of radiotherapy beams. Int J Oral Maxillofac Surg 19(6): 374-376.

Rubin P & Yaremchuk M (1997) Complications and toxicities of implantable biomaterials used in facial reconstructive and aesthetic surgery: a comprehensive review of the literature. Plast Reconstr Surg 1(5): 1336-1353.

Ruuskanen M, Ashammakhi N, Kallioinen M, Pohjonen T, Törmälä P & Waris T (1999) Evaluation of self-reinforced polyglycolide membrane implanted in the subcutis of rabbits. Ann Chir Gyn 88: 308-312.

Ruuttila P, Niiranen H, Kellomäki M, Törmälä P, Konttinen Y & Hukkanen M (2006) Characterization of human primary osteoblast response on bioactive glass (BaG 13-93)- coated poly-L,DL-lactide (SR-PLA70) surface in vitro. J Biomed Mater Res[B] Appl Biomater.78(1): 97-104.

Saikku-Bäckström A, Tulamo R, Pohjonen T, Törmälä P, Räihä J & Rokkanen P (1999) Material properties of absorbable self-reinforced fibrillated poly-96L/4D-lactide (SR-PLA96) rods; a study in vitro and in vivo. J Mater Sci Mater Med 10: 1-8.

Santavirta S, Konttinen YT, Saito T, Gronblad M, Partio E, Kemppinen P & Rokkanen P (1990) Immune response to polyglycolic acid implants. J Bone Joint Surg Br 72: 597-600.

Serlo W, Ashammakhi N, Törmälä P & Waris T (2000) A new technique for correction of trigonocephaly in an infant: application of an absorbable endocranial plate. Child Nerv Syst 16: 595-597.

Serlo W, Ashammakhi N, Törmälä P & Waris T (2002) A new technique for cranial bone osteofixation: use of bioabsorbable tacks and plates to fix parietal bone split grafts used for reconstruction of a posttraumatic frontal bone defect. J Craniofac Surg 13(2): 331-336.

Serlo W, Kaarela O, Peltoniemi H, Merikanto J, Ashammakhi N, Lassila K, Pohjonen T, Törmälä P & Waris T (2001) Use of self-reinforced polylactide osteosynthesis devices in craniofacial surgery: a long-term follow-up study. Scand J Plast Reconstr Hand Surg 35: 285-292.

Shinto Y, Uchida A, Korkusuz F, Araki N & Ono K (1992) Calcium hydroxypatite ceramic used as a delivery system for antibiotics. J Bone Joint Surg [Br] 74(B): 600-604.

Spanio S, Ashammakhi N (2002) Use of new bioabsorbable tacks and a tackshooter in cranial bone osteofixation saves operative time. J Craniofac Surg 13(5): 693-696, discussion 697.

Stelnicki E & Hoffman W (1998) Intracranial migration of microplates versus wires in neonatal pigs after frontal advancement. J Craniofac Surg 9(1): 60-64.

Sun L, Wataha J & Hanks C (1997) Effects of metal ions on osteoblast-like cell metabolism and differentiation. J Biomed Mater Res 34: 29-37.

Suuronen R (1993) Biodegradable fracture-fixation devices in maxillofacial surgery. Int J Oral Maxillofac Surg 22: 50-57.

Suuronen R, Pohjonen T, Hietanen J & Lindqvist C (1998) A 5-year in vitro and in vivo study of the biodegradation of polylactide plates. J Oral Maxillofac Surg 56: 604-614.

Suuronen R, Haers P, Lindqvist C & Sailer H (1999) Update on bioresorbable plates in maxillofacial surgery. Fac Plast Surg 15(1): 61-72.

Suuronen R, Kallela I & Lindqvist C (2000) Bioabsorbable plates and screws: current state of the art in facial fracture repair. J Cranio Maxillofac Trauma 6(1): 19-27.

Tatum S, Kellman R & Freije J (1997) Maxillofacial fixation with absorbable miniplates: computed tomographic follow-up. J Craniofac Surg 8(2): 135-140.

Teupe C, Meffert R, Winckler S, Ritzerfeldt W, Törmälä P & Brug E (1992) Ciprofloxacin-impregnated poly-L-lactic acid drug carrier. Arch Orthop Trauma Surg 112: 33-35.

Thaller S, Lee T & Tesluk H (1995 a) Polyglyconate fixation successfully stabilizes zygomatic osteotomies in a nonhuman primate. J Craniofac Surg 6(6): 459-464.

Thaller S, Moore C & Tesluk H (1995 b) Biodegradable polyglyconate plates and screws: a histological evaluation in a rabbit model. J Craniofac Surg 6(4): 282-287.

Thaller S, Moore C, Tesluk H & Holmes R (1996) Cranial bone grafting: biodegradable versus titanium fixation in a rabbit model. J Craniofac Surg 7(1): 54-59.

Tharanon W, Sinn D, Hobar C, Sklar F & Salomon J (1998) Surgical outcomes using bioabsorbable plating systems in pediatric craniofacial surgery. J Craniofac Surg 9(5): 441-444.

Thompson J, Benjamin J & Szivek J (1997) Pullout strengths of cannulated and noncannulated cancellous bone screws. Clin Orthop 341: 241-24.

Tielinen L, Manninen M, Puolakainen P, Pihlajamäki H, PohjonenT, Rautuvuori J & Törmälä P (1998) Polylactide pin with transforming growth factor β1 in delayed osteotomy fixation. Clin Orthop Rel Res 355: 312-322.

Tollefson D, Bandyk D, Kaebnick H, Saebrook G & Towne J (1987) Surface biofilm disruption. Enhanced recovery of microorganisms from vascular prostheses. Arch Surg 122: 38-43.

Törmälä P, Rokkanen P, Laiho J, Tamminmäki M & Vainionpää S (1988) Material for osteosynthesis devices. US Patent 4: 743257.

Törmälä P, Rokkanen P, Vainionpää S, Laiho J, Heponen V & Pohjonen T (1990) Surgical materials and devices. US Patent 4 968317.

Törmälä P (1992) Biodegradable self-reinforced composite materials; manufacturing structure and mechanical properties. Clinic Mater 10: 29-34.

Törmälä P, Karhi O, Koho P & Tamminmäki M (2000) A novel inserter intrument (Crossbow) for installation of self-reinforced bioabsorbable arrows into meniscus tissue. Knee Surg Sports Traumatol Arthrosc 8: 370-372.

Törmälä P, Pohjonen, T & Rokkanen P (1998) Bioabsorbable polymers: materials technology and surgical applications. Proc Instn Mech Engrs 212(H): 101-111.

Tracana R, Pereira M, Abreu A, Sousa J & Carvalho G (1995) Stainless steel corrosion products cause alterations on mouse spleen cellular populations. J Mater Sci Mater Med 6: 56-61.

Turunen T, Peltola J, Happonen RP & Yli-Urpo A (1995) Effect of bioactive glass granules and polytetrafluoroethylene membrane on repair of cortical bone defect. J Mater Sci Mater Med 6: 639-641.

Turvey T, Bell B, Tejera T & Proffit W (2002) The use of self-reinforced biodegradable bone plates and screws in orthognathic surgery. J Oral Maxillofac Surg 60: 59-65.

Vainionpää S, Rokkanen P & Törmälä P (1989) Surgical applications of biodegradable polymers in human tissues. Prog Polym Sci 14: 679-716.

van der Elst M, Dijkema A, Klein C, Patka P & Haarman H (1995) Tissue reaction on PLLA versus stainless steel interlocking nails for fracture fixation: an animal study. Biomater 16(2): 103-106.

Vangsness T, Carter D & Frankel V (1981) In vitro evaluation of the loosening characteristics of self-tapped and non-self-tapped cortical bone screws. Clin Orthop Rel Res 157: 279-286.

Veiranto M & Törmälä P (2002) In vitro mechanical and drug release properties of bioabsorbable ciprofloxacin containing and neat self-reinforced P(L/DL)LA 70/30 fixation screws. J Mater Sci Mater Med 13: 1259-1263.

Vert M, Christel P, Chabot F & Leray J (1984) Bioresorbable plastic materials for bone surgery. In: Hastings G (ed) Macromolecular Biomaterials. CRC press Inc, Boca Raton, Florida: 120-140.

Vert M, Li M, Spenlehauer G & Guerin P (1992) Bioresorbability and biocompatibility of aliphatic polyesters. J Mater Sci Mater Med 3: 432-446.

Viitanen P, Suokas E, Törmälä P, Ashammakhi N (2006) Release of diclofenac sodium from polylactide-co-glycolide 80/20 rods. J Mater Sci Mater Med 17: 1267-1274.

Vince D, Hunt J & Williams D (1991) Quantitative assessment of the tissue response to implanted biomaterials. Biomater 12: 731-736.

Wang G, Liu S, Ueng S & Chan EC (2004) The release of cefazolin and gentamicin from biodegradable PLA/PGA beads. Int J Pharmac 273(1-2): 203-212.

Waris T, Pohjonen T & Törmälä P (1994) Self-reinforced absorbable polylactide (SR-PLLA) plates in craniofacial surgery. A preliminary report on 14 patients. Eur J Plast Surg 17: 236-238.

Waris E, Ashammakhi N, Kaarela O, Raatikainen T & Vasenius J (2004) Use of bioabsorbable osteofixation devices in the hand. J Handsurg J British Soc Surg Hand 29(6): 590-598.

Waris E, Konttinen YT, Ashammakhi N, Suuronen R & Santavirta S (2004b) Bioabsorbable fixation devices in trauma and bone surgery: current clinical standing. Expert Rev Med Devices 1: 229-40.

Waris E, Ashammakhi N, Kelly C, Andrus L, Waris T & Jackson I (2005) Transphyseal bioabsorbable screws cause temporary growth retardation in rabbit femur. J Pediatr Orthop 25(3): 342-345.

Wei G, Kotoura Y, Oka M, Yamamuro T, Wada R, Hyon SH & Ikada Y (1991) A bioabsorbable delivery system for antibiotic treatment of osteomyelitis. J Bone Joint Surg 73B(2): 246-252.

Weisberger E & Eppley B (1997) Resorbable fixation plates in head and neck surgery. Laryngoskope 107: 716-719.

Westermark A (1999) Lactosorb resorbable osteosynthesis after sagittal split osteotomy of the mandible: a 2-year follow-up. J Craniofac Surg 10(6): 519-522.

Williams D (1972) Corrosion and corrosion prevention in orthopaedic implants. Proc R Sos Med 65(11): 1027.

Williams D & Mort E (1977) Enzyme-accelerated hydrolysis of polyglycolic acid. J Bioeng 1(3): 231-238.

Williams D (1981) Enzymic hydrolysis of polylactic acid. Eng Med 10: 5.

Wolff J (1892) In: Hirchwald A (ed) Das gasetz der transformation der knochen. Berlin. (An english translation of this monograph has been published by Spinger-Verlag in 1986.)

Wong L, Dufresne C, Richtsmeier J & Manson P (1991) The effect of rigid fixation on growth of the neurocranium. Plast Reconstr Surg 88(3): 395-403.

Yaremchuk M (1994) Experimental studies addressing rigid fixation in craniofacial surgery. Clin Plast Surg 21(4): 517-524.

Yaremchuk M, Fiala T, Barker F & Ragland R (1994) The effects of rigid fixation on craniofacial growth of rhesus monkeys. Plast Reconstr Surg 93(1): 1-10.

Yu J, Bartlett S, Goldberg D, Gannon F, Hunter J, Habecker P & Whitaker L (1996) An experimental study of the effects of craniofacial growth on the long-term positional stability of microfixation. J Craniofac Surg 7(1): 64-68.

Original publications

I Tiainen J, Leinonen S, Ilomäki J, Suokas E, Törmälä P, Waris T & Ashammakhi N (2002) Comparison of the Pull-out Forces of Bioabsorbable Polylactide/glycolide Screws (BioSorb and LactoSorb) and Tacks: a Study on the Stability of Fixation in Human Cadaver Parietal Skull Bones. J Craniofac Surg 13(4): 1-6.

II Tiainen J, Veiranto M, Suokas E, Törmälä P, Waris T, Ninkovic M & Ashammakhi N (2002) Bioabsorbable ciprofloxacin-containing and plain self-reinforced polylactide-polyglycolide 80/20 screws: pull-out strength properties in human cadaver parietal bones. J Craniofac Surg 13(3): 427-433.

III Tiainen J, Soini Y, Törmälä P, Waris T & Ashammakhi N (2004) Self-reinforced polylactide-polyglycolide 80/20 screws take more than 1½ years to resorb in rabbit cranial bone. J Biomed Mater Res [Appl Biomat] 70B(1): 49-55.

IV Tiainen J, Soini Y, Suokas E, Veiranto M, Törmälä P, Waris T & Ashammakhi N (2006) Tissue Reactions to Multifunctional Bioabsorbable Ciprofloxacin-Releasing Polylactide-Polyglycolide 80/20 Screws in Rabbits' Cranial Bone. J Mater Sci Mater Med 17(12): 1315-1322.

V Tiainen J, Veiranto M, Koort J, Suokas E, Kaarela O, Törmälä P, Waris T & Ashammakhi N (2006) Bone Tissue Concentrations of Ciprofloxacin Released from Biodegradable Screws Implanted in Rabbits Skull. Eur J Plast Surg (Accepted).

VI Tiainen J, Knuutila K, Veiranto M, Suokas E, Törmälä P, Kaarela O, Waris T & Ashammakhi N (2006) Multifunctional Bioabsorbable Ciprofloxacin-Releasing Polylactide-Polyglycolide 80/20 Tacks: Assessment of Pullout Strength in Human Cadaver Cranial Bone. (Manuscript).

Original publications are not included in the electronic version of the dissertation.

930. Keskiaho-Saukkonen, Katriina (2007) Prolyl 4-hydroxylase. Studies on collagen prolyl 4-hydroxylases and related enzymes using the green alga *Chlamydomonas reinhardtii* and two *Caenorhabditis* nematode species as model organisms

931. Sandelin, Pirkko (2007) Kertomuksia psyykkisestä väkivallasta terveydenhuollon työ- ja opiskeluyhteisöissä

932. Dahlbacka, Sebastian (2007) Optimal pH-management during operations requiring hypothermic circulatory arrest. An experimental study employing pH- and/or α-stat strategies during cardiopulmonary bypass

933. Kangasniemi, Mari (2007) Monoliittisesta trilogiseen tasa-arvoon. Tasa-arvo hoitotyön etiikan tutkimuksessa

934. Ronkainen, Johanna (2007) Costs in today's radiology. ABC analysis of typical situations in the transitional period

935. Vainionpää, Aki (2007) Bone adaptation to impact loading—Significance of loading intensity

936. Mousavinasab, Firoozeh (2007) Effects of lifestyle and genetic factors on the levels of serum adiponectin, a novel marker of the metabolic syndrome, in Finnish servicemen

937. Kääriäinen, Maria (2007) Potilasohjauksen laatu: hypoteettisen mallin kehittäminen

938. Itäranta, Petri (2007) Wnt4 and Wnt6 secreted growth and differentiation factors and neural crest in the control of kidney development

939. Hirsso, Päivi (2007) Alopecia; its prevalence and association with cardiovascular diseases, risk factors and quality of life—cross-sectional population-based studies

940. Tuohimäki, Carita (2007) The use of coercion in the Finnish civil psychiatric inpatients. A part of the Nordic project Paternalism and Autonomy

941. Karvonen, Juha T. (2007) Somatization in young adults. The Northern Finland 1966 Birth Cohort Study

942. Poutanen, Raija (2007) Boys and girls as health-promoting actors—determinants of oral health-related lifestyle among 11- to 12-year-old schoolchildren

943. Arpiainen, Satu (2007) Transcriptional regulation of the hepatic cytochrome *P450 2a5* gene

944. Päiväläinen-Jalonen, Satu (2007) Expression and stability of myelin-associated elements

Book orders:
OULU UNIVERSITY PRESS
P.O. Box 8200, FI-90014
University of Oulu, Finland

Distributed by
OULU UNIVERSITY LIBRARY
P.O. Box 7500, FI-90014
University of Oulu, Finland

UNIVERSITY OF OULU P.O. Box 7500 FI-90014 UNIVERSITY OF OULU FINLAND

ACTA UNIVERSITATIS OULUENSIS

ISBN 978-951-42-8575-2 (Paperback)
ISBN 978-951-42-8576-9 (PDF)
ISSN 0355-3221 (Print)
ISSN 1796-2234 (Online)