Aus dem AO Forschungsinstitut, Davos
Direktor: Prof. Dr. sc. tech. E. Schneider

und der

Klinik für Wiederherstellende Chirurgie
Abteilung für Kiefer- und Gesichtschirurgie
Kantonsspital Basel – Universitätskliniken

Vorsteher: Prof. Dr. Dr. H.-F. Zeilhofer

Arbeit unter der Leitung von

Prof. Dr. Dr. B. A. Rahn
und
Dr. Dr. C. Leiggener

Early dural reaction to polylactide in cranial defects of rabbits

Inauguraldissertation
zur Erlangung der Doktorwürde der Zahnheilkunde
vorgelegt der Medizinischen Fakultät der Universität Basel

von

Dr. med. Andreas Albert Müller, Basel/BS, Oftringen/AG, Schweiz

Von der Medizinischen Fakultät der Universität Basel genehmigt auf Antrag von Prof. Dr. Dr. H.-F. Zeilhofer

Korreferent: Prof. Dr. G. Jundt

Tag der Promotion: 26. August 2004

From the AO Research Institute, Davos
Director: Prof. Dr. sc. tech. E. Schneider

and the

Clinic for Reconstructive Surgery
Division of Cranio-Maxillofacial Surgery
University Hospital Basel, Switzerland

Head: Prof. Dr. Dr. H.-F. Zeilhofer

Work under the direction of

Prof. Dr. Dr. B. A. Rahn
and
Dr. Dr. C. Leiggener

Early dural reaction to polylactide in cranial defects of rabbits

Inaugural thesis
in order to achieve the doctor degree of dental surgery
submitted to the Medical Faculty, the University of Basel

from

Dr. med. Andreas Albert Müller, Basel/BS, Oftringen/AG, Switzerland

Approved by the Medical Faculty of the University of Basel on the request of Prof. Dr. Dr. H.-F. Zeilhofer

Co-referee: Prof. Dr. G. Jundt

Day of promotion: 26. August 2004

Dedicated to my parents

Table of contents

1 Introduction

1.1 Calvarial bone defects

Bony defects of the craniofacial complex may be found as a result of congenital anomalies, posttraumatic, neoplastic, or infectious conditions. Such conditions are often associated with severe functional and aesthetic problems. Corrective treatment is often complicated by limitations in tissue adaptation (Bosch et al. 1995). Restoring bone integrity in these patients remains a challenging problem to cranio-maxillofacial surgery.

Menei et al. (1993) defined the bony vault of the cranium (calvaria) as that portion of the skull extending from the supraorbital ridge posteriorly to the external occipital protuberance. It comprises the paired parietal bones, the squamous portion of the occipital and temporal bones, the squama frontalis, and a small section of the greater wing of the sphenoid.

The calvaria and the facial bones are pure membranous bones, with the mandible and the greater wing of the sphenoid being exceptions. Anatomically, the calvaria consists of two cortical plates with regions of intervening cancellous bone similar to the mandible. Subtle differences exist between the microscopic structures and functions of the calvaria in different species; however, embryonic development is very similar (Menei et al. 1993).

The main cranial blood supply comes from branches of the middle meningeal artery to the inner table and to a lesser extent from small arteries of the outer surface. In contrast

to many long bones there is no primary nutrient artery in the human calvaria. Additionally, arterial blood enters the calvaria through the insertion of the temporalis muscle. However, because of the large area of the human skull that is devoid of muscle insertions, the blood supply to the human calvaria is poorer than in other mammals. As a result, even small defects in the adult human skull do not repair spontaneously. In this regard, the regenerative capacity of the calvaria of experimental animals can be considered better than that of humans (Schmitz et al. 1986).

As a result, cranial through-and-through defects, exceeding a certain size do not regenerate spontaneously by the complete ingrowth of bone tissue (Lundgreen et al. 1992). Without further treatment the defects regenerate incompletely with only a serrated edge of newly formed bone, originating from the perimeter of the defects, reducing the defect to some extent and sealing the defect margin with lamellar bone. The centre of the defect is repaired with dense, fibrous connective tissue only (Takagi et al. 1982).

This healing pattern is referred to as “bone repair”, defined (Leonhardt 1990) as the reestablishment of the continuity of disrupted tissue by another tissue that is not able to fulfil the structural and functional properties of the lost tissue. In contrast, “bone regeneration” has been defined as the biologic process that completely renews the architecture and function of lost bone (Leonhardt 1990).

1.2 Treatment of cranial defects

1.2.1 Trephination: one of the first surgical procedures

Historically, man made skull defects (Fig. 1) made by trephination is probably the oldest known surgical procedure, and archaeological studies have demonstrated that it was practiced in Europe in the Neolithic Period (7000 B.C. to 3000 B.C.), in Peru during the Columbian Period, and in Central America, Mexico, North America, and the south Pacific before the arrival of the European (Reid 1981).

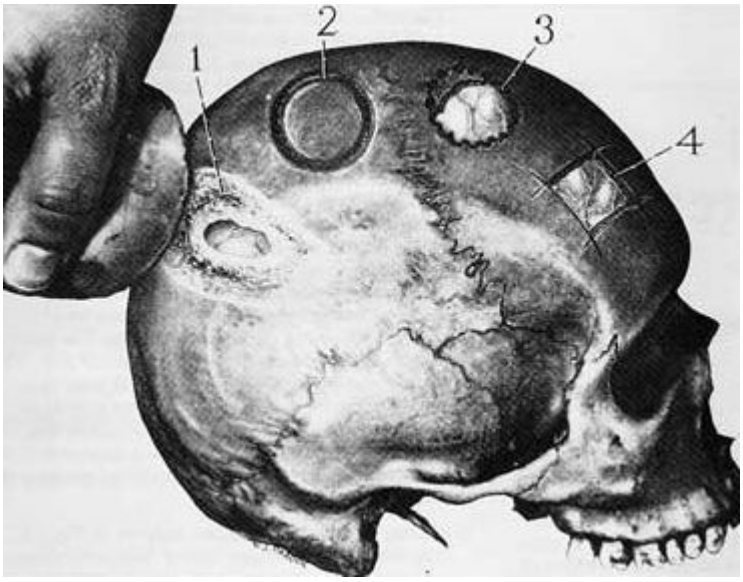

Fig. 1: Historical methods of trephination: 1. scraping, 2. grooving, 3. boring and cutting, 4. using intersecting incisions to remove a rectangular piece of skull (doi:10.4997/JRCPE.2010.215).

Hence cranioplasty to repair skull defects dates back centuries as well. In the 16th century the anatomist Gabriello Fallopio recommended the use of gold foil sheets to restore the cranial continuity. In the following century, Van Meekren implanted bone from a dog into the skull defect of a soldier. He later had to remove it on account of ecclesiastic pressure. It was reported that polished coconut shell was used to reconstruct

defects secondary to trephination. South sea islanders performed this technique in the early 19th century (Robinson et al. 1989).

1.2.2 Problems of fixation devices

Present-day cranioplasty is usually performed with autogenous bone. Donor sites for bone grafts include skull, iliac crest and ribs. However, there are certain disadvantages to autogenous bone grafting. The most important is the necessity of an additional surgical intervention at the donor site, with the possibility of increased morbidity. Other repeatedly reported problems (Gosain et al. 1999, Suuronen 1993) associated with bone grafting are: limited availability, especially in children, and the unpredictability in terms of incorporation and volumetric persistence.

There has always been concern about the use of rigid fixation with metallic plates and screws in craniofacial surgery. Some of the risks include extrusion of the implanted device, infection, visibility, cold sensitivity and palpability when placed under thin skin areas. Should any of these occur, removal of the fixation device as a secondary surgical procedure is necessary.

However, far more serious complications are growth alterations resulting from growth restriction, and passive translocation of the plates and screws inside the scull during skeletal growth (Goldstein et al. 1997). Thus, at least in children, metallic devices should be removed in a subsequent reoperation.

An ideal stabilization device needs to be adequately rigid and strong and should not cause any systemic or local reactions; i.e. it should not be allergenic, immunogenic, mutagenic, or teratogenic, it should not affect bone healing, and it should disintegrate totally after the bone had healed.

1.2.3 Guided bone regeneration

Removal or damage of the periosteum over a bone defect inhibits bone regeneration while its preservation enhances the healing capacity, acting as a natural physical barrier, which excludes the surrounding soft tissues and protects the haematoma in the bone defect. The periosteum provides both blood supplies to the cortical bone and osteoprogenitor cells for bone regeneration.

It has been shown that by mechanically excluding undesirable tissues from a bone defect space, by means of a foil, penetration of unwanted surrounding tissue and cells is reduced and desirable cells derived from the bone can repopulate the blood clot within that space. This is the biologic rationale of guided tissue regeneration (Bosch et al. 1995).

Guided bone regeneration can be defined as controlled stimulation of new bone formation in areas where a bony defect exists, either by osteogenesis or osteoinduction, re-establishing both, structural and functional characteristics of bone. This technique involves placing a mechanical barrier to protect the area of the defect from the ingrowth of non-osteogenic cells. It demands a space–maintaining capacity during the time period necessary for healing and an effective peripheral sealing to preclude soft tissue ingrowth (Lundgren et al. 1992). The procedure even enables the formation of new bone at sites where it is normally not present (Linde et al. 1993). This foil technique to enhance the defect healing and the generation of new bone was initially developed by Nyman et al. (1982) for periodontal surgery. In the meantime it has been successfully applied to other indications such as cranial defects (Dahlin et al. 1991, Levy et al. 1994, Lundgren et al. 1992).

Several investigations have been made to assess and to compare different techniques of foil applications for guided bone regeneration in cranial defects (Bosch et al. 1995, Levy et al. 1994). The results from previous studies clearly showed the advantage of a double foil technique with a spaceholder in between (sandwich technique) (Levy et al. 1994). It showed superior bone regeneration and more predictable results than techniques where only an outer foil or no foil was applied (Bosch et al. 1995).

Using non-degradable mechanical barriers to promote healing of skull bone defects bears the risk of possible late complications associated with permanent foil presence. On the other hand a second surgical procedure for foil removal may also be hazardous or even impossible to perform at least when the barrier has been placed on the inner surface of the skull. Hutmacher et al. marked 1996 a trend in guided tissue and bone regeneration research from non-resorbable towards biodegradable and bioresorbable foils.

In osseous defects, a stable, mouldable, slowly degrading fixation plate could serve as an osteopromotive underlay or overlay for guided tissue regeneration, bone graft or as framework on which bone fragments or grafts could be fixed by resorbable screws.

1.2.4 Types and definitions of polymer breakdown

Biodegradable, bioresorbable, and bioabsorbable are often used misleadingly in the literature of guided tissue and bone regeneration (Bauer et al. 2000, Hutmacher et al. 1996).

Biodegradable refers to solid polymeric materials that break down as a result of macromolecular degradation with dispersion in vivo. This definition excludes bacterial, fungal, and environmental degradation. There is no proof of elimination from the body.

Bioresorbable refers to solid polymeric materials that can degrade and further be resorbed in vivo. They are eliminated through natural pathways because of filtration or degradation of the by-products (low-molecular weight compounds) or because of their metabolization. Bioresorbable assumes that elimination is shown conclusively.

Bioabsorbable refers to solid polymeric materials that can dissolve in body fluids without any polymer chain cleavage or molecular mass decrease, e.g.: slow dissolution of water-soluble implants in body fluids. A bioabsorbable polymer can be bioresorbable if the dispersed macromolecules are excreted.

1.2.5 Polymers for bone repair

Biodegradable materials have been of interest for more than 20 years, both in maxillofacial and orthopaedic surgery disciplines, with initial studies focusing on suture materials. Inconveniences of metallic osteofixation devices like stress shielding, allergies, corrosion, infection, cold sensitivity, palpability, and scatter in computed tomography and magnetic resonance imaging, can be reduced by the use of biodegradable polymers.

The first experimental studies using resorbable materials for fracture fixation were conducted by Cutright and Hunsuck (1972) for the repair of traumatic orbital floor defects in monkeys using poly-L-lactic acid (PLA) materials. Since that time, various studies have been performed using PLA alone or in combination with other (co-) polymers like polyglycolic acid for reconstruction of traumatic orbital defects, treatment of unstable, displaced zygomatic fractures, and fixation of calvarial bone grafts.

Most of the absorbable bone fixation devices in clinical use so far in craniofacial surgery have been made of polylactide (PLA); its D- and L-isomeric forms, polyglycolic acid (PGA); or PLA-PGA copolymers.

1.3 Evolution and chemistry of polymers for implantation

A comprehensive overview of the history of polymers used in medicine was given by Hutmacher et al. (1996). The earliest studies of the use of biomaterials came from the Edgar Smith papyrus, which reports the use of sutures and other wound closure devices around 4000 BC and the use of metals in bone repair around 2000 BC. Ancient Indians are reported to have used silk and hair as suture materials.

Biomaterials used in surgery may be synthetic materials, such as poly-(amino acids), polyanhydrides, polyester, polyorthoester, and polyphophazenes; or they may occur naturally, such as albumin, chitin and chitosan, collagen and gelatine, and polysaccharides. Aliphatic polyesters constitute the most attractive family among which poly(a-hydroxy acids) constitute a class of polymers represented by the general formula –[O–CHR–CO]–n.

Poly(glycolic acid) (PGA) is the simplest alpha polyester. PGA was first synthesized in the 1930s by William Carothers, the father of nylon. At that time, it was noted that the major limitation of this polymer was its hydrolytic instability. It was this hydrolytic instability that led to the patents of Schmitt and Palistina (1967). They recognized that the alpha polyester analogs of the alpha polyamides might indeed be bioresorbable and, hence, a useful material for surgical applications. A search for a simplified synthetic alternative to collagen began in the early 1960s. Prior to this time, collagen was the only commercially available biodegradable material.

Poly(lactic acid) is the next homolog in the series of alpha polyester. Poly(lactic acid) with its asymmetric carbon atom is optically active. Poly(lactic acid) is more hydrophobic than poly(glycolic acid) due to the presence of an extra methyl group. This limits the water uptake and reduces the rate of backbone hydrolysis as compared to PGA.

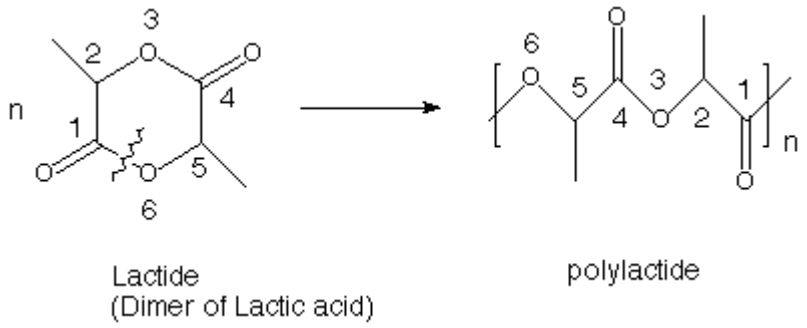

Fig. 2: Schematic synthesis of polylactide from the dimer of Lactic acid.

Since lactic acid is a chiral molecule, it exists in two stereoisomeric forms: poly(D-lactide) and poly(L-lactide). Poly(D,L-lactide) is the racemic polymer obtained from a mixture of D- and L-lactide. The polymer (Fig. 2) derived either from the optically active D- or L- monomers are semicrystalline materials, while the optically inactive copolymer of poly(D,L-lactide) is always amorphous. Therefore the amorphous poly(D,L-lactide) is usually considered for application such as drug delivery, when it is essential to have a homogeneous dispersion of the drug within a monophasic matrix. On the other hand, the semicrystalline poly(L-lactide) is preferred in applications when higher mechanical properties are required for items such as sutures, foils for guided tissue regeneration or orthopaedic devices for fracture stabilization.

The first experimental studies on the use of biodegradable devices for fracture-fixation were mainly carried out in maxillofacial surgery. The devices were used for fixation of mandibular fractures and orbital blowout fractures (Suuronen 1993).

1.4 Definitions and types of polymer breakdown

Erosion designates the loss of material due to monomers and oligomers leaving the polymer. Polymer degradation is the key process of erosion and describes the chain scission process during which polymer chains are cleaved to form oligomers and finally to form monomers. Polymer bonds can be cleaved either passively by hydrolysis or by active enzymatic reactions. For polylactides passive hydrolysis is the most important mode of degradation (Göpferich 1996).

The monomer degradation product of polylactide is lactic acid, which enters the Krebs' cycle and is consequently excreted by the lung as carbon dioxide and water. Vasenius et al. (1992) studied possible lactic acidosis in rabbits while intramedullary poly(DL-lactide) and poly(L-lactide) rods were implanted in both femurs for 60 weeks. The blood acid base balance remained normal throughout the study, and no rises above physiological levels of blood L- and D-lactated were observed during the follow-up.

Polymer degradation (Fig. 3) can be macroscopically described either as a surface (heterogeneous) or bulk (homogeneous) eroding process. Surface eroding polymers lose

material from the surface only, while in bulk eroding polymers the degradation is not confined to the surface only. Most polymers cannot be unequivocally assigned to one of the two classes. Polylactide shows mainly bulk erosion (Göpferich 1996).

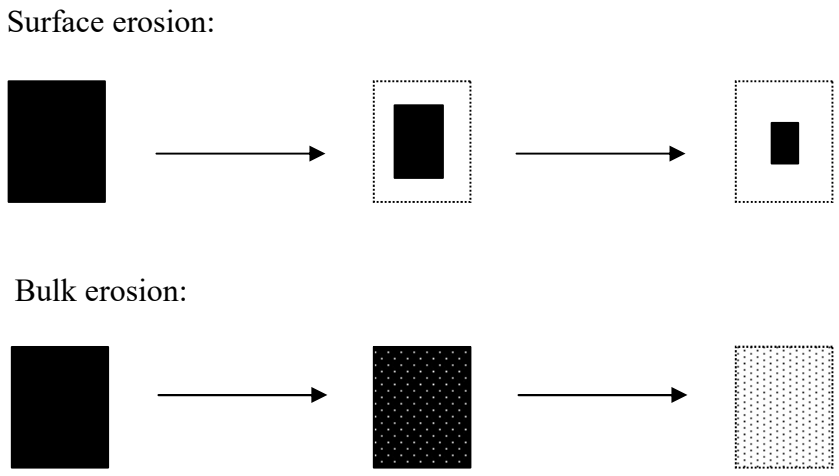

Fig. 3: Schematic illustration of surface and bulk erosion (adapted from Göpferich 1996).

The processes involved in the erosion of a degradable polymer are complex. Water enters the polymer bulk, which might be accompanied by swelling. The water triggers the chemical polymer degradation, which leads to the formation of pores, whereby oligomers and monomers are released. There are different factors that influence the velocity of this reaction e.g.: the type of chemical bond, pH, and the amount of water uptake. Further more these factors are changing because chemical and physical changes go along with the degradation of the polylactide, like the crystallization of oligomers and monomers or pH changes.

1.5 Biocompatibility of polymers

1.5.1 Definition of biocompatibility

“Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application.” (Williams 1988).

In the early stage of polymer degradation biological reaction depends on the material itself and on possible chemical residues from the processing, while in the later stage the biological reaction depends mainly on the degradation products and their molecular weight.

The complexity of the host responses results from the series of processes involving many closely interdependent mechanisms of material-tissue interactions. In biostable materials the primary goal is minimizing and adjusting material-tissue interactions. For biodegradable and bioresorbable materials, the situation is more complex because of the degradation by-products, which are able to strongly interact with living systems. For this reason biodegradable and bioresorbable polymers must be regarded as much closer to pharmacology than to material science (Hutmacher et al. 1996).

1.5.2 Interactions of polymers with adjacent tissues

The biocompatibility of resorbable materials has led to clinical applications, especially in paediatric patients. Polymer plates and dowels, placed on the outer or inner surface of the calvarial bone may undergo active or passive translocation due to skull growth, displacement during breakdown or external forces. Hence it is important to evaluate the polymer biocompatibility in respect to the adjacent tissues.

Regarding intraosseous placement of polymers, Peltoniemi et al. (1998) mention that degradation and resorption proceeds more slowly in an intraosseous environment than in the surrounding tissues, even if the bone is actively growing and remodelling. Moreover, they suspect that intraosseously placed polylactide devices may present a lower incidence of clinically manifest foreign body reactions than extraosseously implanted polylactide devices. Hence transient inflammatory tissue reactions to the absorbable implant have been considered to be milder in an intraosseous environment then in subcutaneous tissue.

1.5.3 Biocompatibility of different polymer types

Some clinical long-term follow up studies (Peltoniemi et al. 2001), are indicating that poly-L-lactide may be a more suitable material for biodegradable bone fixation devices than polyglycolide (PGA). Poly(L-lactide) miniscrews resulted in a considerably milder foreign body reaction than PGA implants.

Nevertheless intraosseously placed PLA devices appear sometimes to be associated with clinically manifest non-specific foreign body reactions (Suuronen 1993). Late inflammatory reactions to poly(L-lactide) have been reported (Bergsma et al. 1993, Bergsma et al. 1995) when bulky non-reinforced polymer plates, manufactured from highly crystalline material, have been used in the areas with minimal subcutaneous tissue. In that study polymeric remnants remained in the soft tissues until 3-6 years. Thus it has been concluded that the implant has to be small and the molecular weight of the polymeric material acceptable for biodegradation and total absorption. Further more, the plate has to be thin and screw heads flat or flattened at time of surgery (Böstman et al. 1995).

Before an application of such polymers in the neurocranium can be considered any hazards to neural tissues must be excluded. Up to now very little have been reported about the interaction between polylactides and the underlying dura mater (Peltoniemi et al. 2001).

2 Goals

Resorbable fixation plates have several applications in craniofacial surgery. As the skull consists mainly of non-weight bearing bones having often a thin soft tissue cover, the application of bioresorbable devices is here very suitable. The main interest consists in the use for internal fracture fixation and for guided bone regeneration. Implementation of the principles of guided bone regeneration in cranial defects has to result in the exclusion of both adjoining tissues: the brain with its surrounding dural tissue and the overlying scalp.

Any material implanted is suspected to induce a foreign body reaction in the surrounding tissue. Before an application of guided bone regeneration by biodegradable foils can be considered, the reaction of the underlying dural tissue which is in direct contact to the inner polylactide foil must be investigated. Any hazardous reaction must be excluded.

Especially a possible cellular reaction at the polymer-dura interface and the histological changes of the dura along the contact zone to the poly(L/DL-lactide) is of clinical interest. Histological specimens available form a study on “enhanced healing of cranial defects by bioresorbable poly(L/DL-lactide) foils in rabbits” (Leiggener et al. 2002) were investigated in respect to different poly(L/DL-lactide) foils and dowels at the inner surface of the neurocranium during the early stage of implantation.

Currently the only approved poly(L/DL-lactide) for clinical use is the amorphous poly(L/DL-lactide) 70/30. From the processing point of view it would be preferable to deal with a semicrystalline poly(L/DL-lactide) (chap. 5.4). Therefore the semicrystalline

poly(L/DL-lactide) 80/20 was included in the study and compared to the approved poly(L/DL-lactide) 70/30.

Information in this issue is also important as biodegradable plates and screws may passively undergo intracranial translocation, e.g. during skull growth of the paediatric patient, where resorbable polymers are preferably used.

3 Materials and Methods

3.1 Implants and rabbits

The implant systems consisted of an outer (epicranial) foil, four fixation dowels, a perforated strip serving as a spacer and an inner (endocranial) foil (Fig. 4). One series used in the investigation was manufactured from poly(L/DL-lactide) 70/30 the other was manufactured from poly(L/DL-lactide) 80/20.

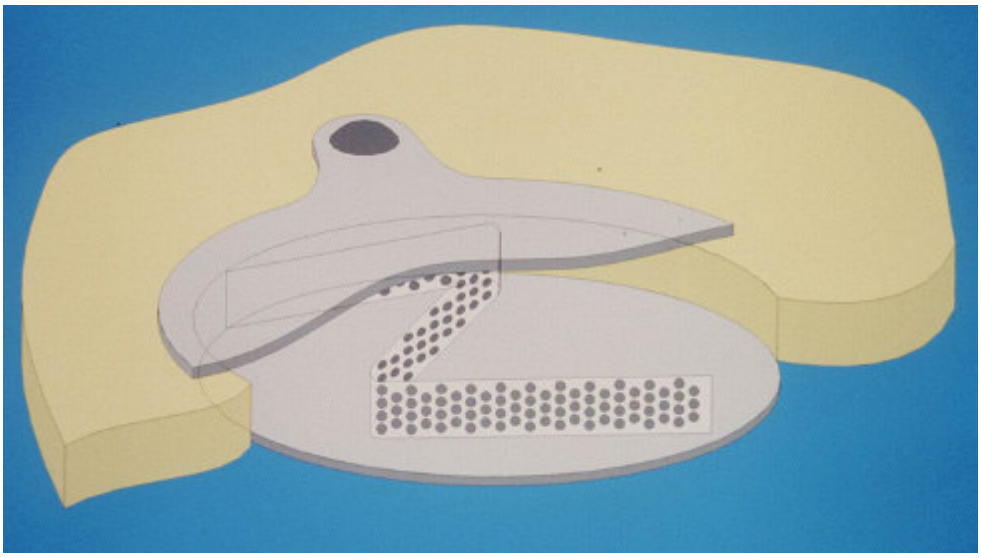

Fig. 4: Implant system for guided bone regeneration: inner foil, spacer and outer foil. (AO Research Institute Davos)

The raw material was purchased from Boehringer Ingelheim Ltd. The mean molecular weight of the raw material was approximately 660’000 Daltons and the intrinsic viscosity 6.69 g/dl and an inherent viscosity of 6.03 g/dl.

The outer foil had a diameter of 10.5 mm with four fixation straps, while the inner foil had a diameter of 8.2 mm, hence equal to the trephination diameter. They were both 0.3 mm thick and had no perforations.

To produce an open space, a “z”-shaped spacer separated both foils. The spacer was thermoformed from a rectangular perforated foil (15 mm × 3 mm × 0.3 mm, perforation diameter 0.5 mm) using sterile Ringers solution, heated in a water bath (Grant Instruments, Cambridge, England) at a temperature of 66° C. This strip was submerged for 15 second, then removed and put into a special tool to facilitate shaping. The covering foils and the spacer were manufactured by the melt extrusion process, followed by laser cutting.

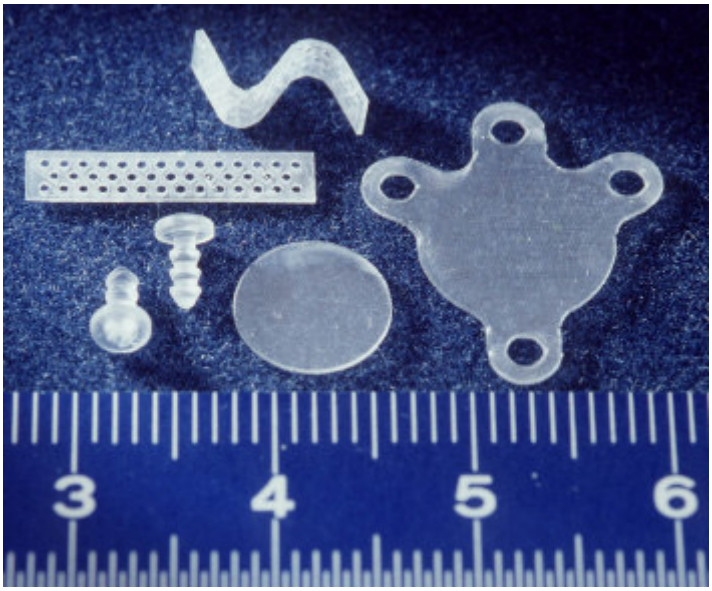

Fig. 5: Implant system for guided bone regeneration consisting of outer foil with fixation straps, inner foil, spacer before and after bending and fixation dowels. (AO Research Institute Davos)

The fixation dowels were also made of poly(L/DL-lactide) 70/30 or 80/20 produced by the injection moulding process. The dowel design was as follows: head diameter 3.0 mm, length 5.0 mm and shaft diameter of 1.6 mm.

All implants, as shown in Fig. 5, were sterilized by gamma irradiation (Co-60, dosage 30 kGy, B. Braun SSC AG, CH-8212 Neuhausen, Switzerland).

After processing and ?-sterilisation the specifications of the implant were measured again. The dowels showed a molecular weight of about 72'500 Daltons, an intrinsic viscosity of 1.22 g/dl and an inherent viscosity of 1.20 g/dl. The foils showed a molecular weight of about 84'100 Daltons, an intrinsic viscosity of 1.37 g/dl and an inherent viscosity of 1.37 g/dl.

Seventeen clinically healthy adult (12 month) female New Zealand White rabbits, ranging in weight from 3650 to 5350 g (mean 4103 g) and supplied by a single breeder (Charles River, Germany) were used in this study. Prior to experimentation, the animals were quarantined for four weeks. Surgery and animal maintenance were carried out according to Swiss Federal legislation (permit: GR 6/1996). The rabbits were housed in individual cages before and after surgery in a room with controlled light, humidity and temperature and were nourished with rations of food and fresh water ad libitum.

3.2 Surgery

Prior to surgery, the animals were sedated with subcutaneous injection of Ketamine (Ketasol 50, Graeub AG Bern, Switzerland: 32.5 mg/kg body weight) and Xylazine (Rompun 2 %, Bayer AG: 4 mg/kg body weight). General anaesthesia was induced by a combination of Ringer lactate-fentanyl (50 ml/h kg body weight) and maintained by a mixture of oxygen and halothane. The skulls of the rabbit were shaved and washed with iodine soap (Betadine, Mundipharma, Hamilton, Bermuda). The surgical area of the skull was disinfected twice with sterile iodine spray (Iodobac, Mundipharma) before

sterile drapes were applied. The animals were placed on a heating blanket maintained at 38°C.

The surgical procedure was performed according to Alberius et al. (1989). A mid-sagittal scalp incision was made from the frontal to the occipital region and full thickness flaps were reflected to expose the parietal bones. Two 8.3 mm diameter full thickness skull defects were made (Fig. 6), one in each parietal bone approximately 2 mm posterior to the coronal and lateral to the midline suture, not touching any suture. A trephine drill (Surgical Diamond Instruments, Munich, Germany) was used under constant irrigation with sterile physiological solution. The calvarial discs were gently removed and special care was taken not to damage the dura or to puncture the superior sagittal sinus. A drill guide was then used to position four holes around the defect.

3.3 Application of the implants

Implants were applied to the burr holes in a sandwich manner (Fig. 7). The endocranial foil was placed at the inner surface of the neurocranium in direct contact to the dural tissue without mechanical fixation. The perforated polylactide strip was then formed in a “z”-shape and placed perpendicular to the inner foil to maintain the chamber depth of 3 mm. The burr hole cover was then fixed onto the outer surface of the skull with four fixation dowels.

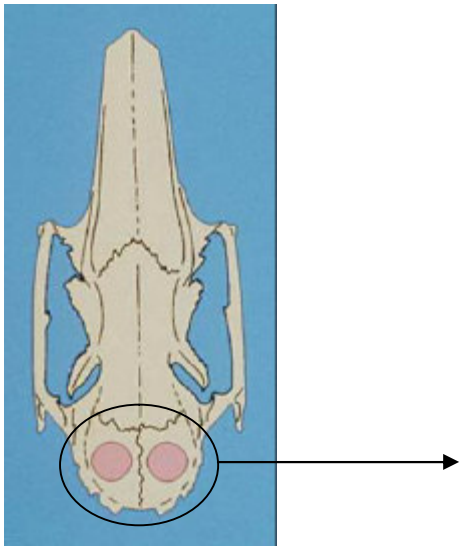

Fig. 6: Trephinations (Ø 8.3 mm), distal to the coronal, and lateral to the sagittal suture of the rabbit. (ARI)

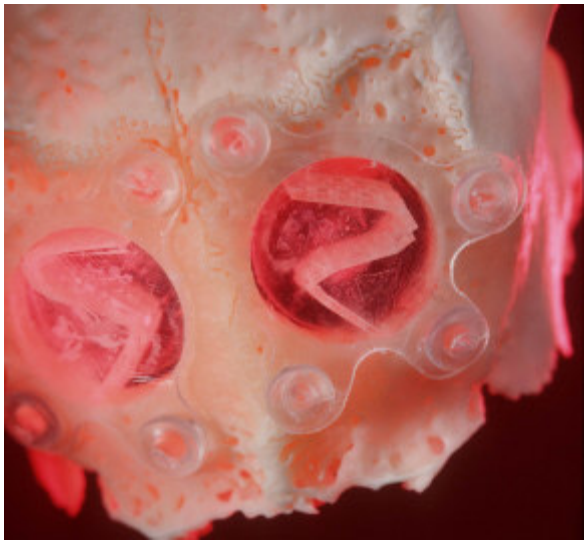

Fig. 7: One defect side covered with implants made of poly(L/DL-lactide) 70/30, the other side with 80/20. (AO Research Institute Davos)

On one side the defect was covered with inner and outer poly(L/DL-lactide) 70/30 foils and pins, and on the other side with poly(L/DL-lactide) 80/20. Each rabbit received both implants, one of each type. The side distribution of the two poly(L/DL-lactide) types was randomly assigned in each rabbit.

The periosteum was repositioned without sutures and the scalp was resutured with interrupted non-resorbable 5-0 Dafilon (Braun-SSC AG, Neuhausen, Switzerland) sutures. The sutures were left in place and were not removed. Temgesic (0.5 mg/kg body weight, Reckitt & Colman Products Ltd., Hull, England) was given subcutaneously for two days postoperatively for pain relief. No antibiotics were administered to the animals at any time during the experiment.

3.4 Postoperative fluorochrome labelling

Postoperative fluorochrome labelling of bone formation was performed by subcutaneous administration of Calcein green (Suzuki et al. 1966) 1 ml/kg body weight, after the second and fourth postoperative weeks and Xylenol orange (Rahn and Perren 1971), 1 ml/kg body weight, after the sixth week and 24 hours before sacrifice at eight weeks.

3.5 Controls

Four clinically healthy adult (12 month) female New Zealand White rabbits, ranging in weight from 3900 to 6090 g and supplied by a single breeder (Charles River, Germany) were used as controls. Compared to the trephined animals the life conditions were the same, but no fluorochrome labelling was performed.

For further comparisons the material of a previous study (Leiggener et al. 2002) was available. The study design was the same apart from the application of an outer foil only (no inner foil, no spacer) and the application of poly(L/DL-lactide) 70/30 only (no 80/20).

3.6 Tissue processing

Eight weeks postoperatively the animals were sacrificed by an overdose of barbiturate (Swiss Serum and Vaccine Institute, Bern, Eutha 77: 1ml/kg body weight) after a premedication. Immediately following euthanasia, the defect areas (block biopsies) were harvested with an oscillating orthopaedic saw.

For histological processing the calvariae were dehydrated in a series of ethanol immersions of increasing concentration (70 % to 99 %) and embedded without decalcification in LR White (London Resin Company, Reading, England).

The embedded specimens were sectioned at 300 µm (Leica SP1600-Saw Microtome, Nussloch, Germany) in a coronal direction.

Between 23 and 27 sections were made, running through the entire former defect area. Contact-radiographs of the operated rabbits were taken at 30 kV, 5 min of exposure time and 40 cm focus to film distance with a Faxitron 804 cabinet x-ray system (Field Emission Corp., Mc Minnville, OR) on AGFA Structurix D4 DW films (filter: Al, 5 mm). The contact-radiographs of the controls were taken under analogous conditions in Faxitron (mod 43855A).

In each defect two samples coming from the middle area were chosen for further evaluation. By measuring the defect diameters on the contact-radiographs (Nikon profile projector, model 6CT2, 10-fold magnification) two sections which showed nearly the burr hole diameter of 8.3 mm were identified. The dividing line between the old calvarial bone and the newly formed bone in the defect area was clear. The selected sections were ground to 70µm on a Micro Grinding System (Exakt 400CS, Exakt GmbH, Norderstedt, Germany) and one was stained with Giemsa-Eosin and the other was left unstained for fluorescence and polarizing microscopy.

For microradiographs the 300µm sections were ground to (70 ± 20) µm (Läppmaschine, Stähli AG, Biel, Switzerland). The microradiographs were taken at 25 kV on a High Resolution UF Plate (Microchrome Technology, San Jose, CA) in the same Faxitron unit.

3.7 Histology

3.7.1 Microscopic evaluation

The qualitative analysis of the Giemsa-Eosin specimens was made on a light microscope (Zeiss Universal and Zeiss Axioplan 2 imaging, Oberkochen, Germany) using magnification up to 400-fold.

Suspicious adverse histological signs were noted for all samples. The following histological changes were specially addressed:

- Dural tissue: general histological structure, variation of dural thickness, formation of fibrous- and granulation tissue, calcification or bone formation, artificial sinuses in the tissue, dural margins towards brain and polylactide, dystrophic signs, necrosis, inflammatory cells like foreign body giant cells, round cells, polymorphonuclear leucocytes.
- Interface between dura and polylactide: number of foreign body giant cells, adhesions.
- Polylactide foil and fixation dowels: position, form, material morphology, cellular and structural changes in the adjacent tissue.

The occurrence of inflammatory cells within the fields of view was assessed quantitatively using magnification 400-fold for cell identification. Statistical analysis was not performed because of the small number of inflammatory cells found.

Fluorescence microscopy was used to assess the site and time of bone regeneration and to reveal zones of active remodelling. As the fluorescence marker is acting on a

complex binding basis with calcium it will also show other calcifications than bone formation.

Polarisation microscopy was used to reveal zones of high crystallinity within the polylactides or their degradation products in the surrounding tissues.

The contact-radiographs were used on a Nikon profile projector (model 6CT2) with a 10-fold magnification to perform measurements of bony or calcified parts in the samples.

3.7.2 Program settings for image acquisition

Histological image acquisition was made on the Axioplan 2 imaging microscope using the Axiocam and the Axiovision program (Zeiss, Germany). Adjustments on the microscope were made as follows: light intensity was fixed on 3200K, apertures open, Köhler illumination. The settings in the Axiovision program were made as follows: no enhanced colour, exposure rate in a way that the logarithmic Histogram was completely included in the margins of 0 to 255. For Giemsa-Eosin microradiographs and polarization imaging the resolution was (1300 × 1030) scanned and the conversion was square root. For fluorescence imaging the resolution was (1300 × 1030) interpolated and the conversion was linear.

4 Results

4.1 Inspection of the rabbits

Sixteen New Zealand white rabbits recovered with no neurological complications from the surgical procedure. During the observation period of 8 weeks, all animals gained weight and showed no signs of systemic or local infection. No signs of neurological disturbances such as epilepsy or paresis were visible.

On inspection no swelling or thickening of the tissues as a sign of a foreign body reaction was present. At euthanasia, no instability of the implanted materials could be detected. Macroscopically there were no signs of inflammation at the implantation site.

After incision no liquid or serous fluid could be seen in the defect area. The implant was firm on palpation and covered by a thin soft layer of connective tissue (Fig. 9). All implants maintained their position and their original shape. The polymer foils turned to a white opaque aspect, but otherwise there were no signs of macroscopic degradation. No implant was rejected.

Some of the dowel heads turned also to a white colour while other heads remained completely clear (Fig. 10, Fig. 11). The distribution among the two different poly(L/DL-lactides) was scant. 53 from 64 dowel heads (83 %) made of poly(L/DL-lactide) 70/30 became white while 18 from 64 dowel heads (28 %) made of poly(L/DL-lactide) 80/20 became white. No structural changes of the shape of the dowel head were seen. 6 from 64 poly(L/DL-lactide) 70/30 dowels and 5 from 64 poly(L/DL-lactide)

80/20 dowels could be rotated in their implantation site. All other dowels were still fixed.

One rabbit died the day after surgery and was excluded from the study. On inspection the suture was intact and the wound showed no abnormal findings. On autopsy a thick haematoma extending between the dura and the endocranial foil was detected. The death was caused by a subdural haematoma most likely due to an intraoperative iatrogenic laceration of a vessel.

4.2 Inspection of the harvested implants

When the defect areas were harvested there were no adhesions between the dura and the brain (Fig. 8, Fig. 9). The calvarial bone including the dura was bluntly separated from the brain and the pia-arachnoid structures. Neither signs of an adverse tissue reaction nor subdural haematomas were visible

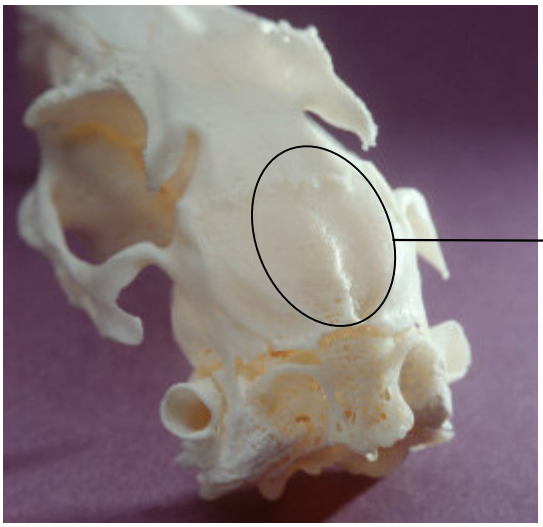

Fig. 8: Implantation site in a rabbit skull model. (AO Research Institute Davos)

Fig. 9: View on the outer polylactide foils and dowels after 8 weeks. (AO Research Institute Davos)

The view on the inner surface of the calvarial defect (Fig. 11) showed that the inner foil remained fixed at the implantation site, with no displacement. Thin newly formed bone islands were scattered over the inner foil with variable density and location.

Some dowel tips penetrated the inner surface of the calvaria and were covered by dural tissue, showing no signs of adverse reaction.

Fig. 10: Outer surface after 8 weeks: the foils and some of the dowels became opaque. (AO Research Institute Davos)

Fig. 11: Inner surface after 8 weeks: the inner foils are covered by newly formed bone islets. (AO Research Institute Davos)

4.3 Microscopy

4.3.1 Dowels

The polylactide dowels had become well incorporated and were surrounded by a very thin (~ 0.1 mm), dense connective tissue layer. The dowels were never in direct contact to the bone. The bone followed the windings of the dowel. The bone structure was well defined in the microradiographs up to the margin of the polylactide indicating no signs of bone lysis adjacent to the dowels. The fluorescence imaging showed slightly

increased bone remodelling activity in the vicinity to the dowel. At the interface between the bone and the dowels no specific cellular reaction was detected except some solitary giant cells.

As the dowels had a length of 5 mm and the cranial bone was about 3 mm thick, the dowel tips had often broken through the inner cortical bone and stayed in contact with the dura. At the top of the dowel tip the dura was very thin and in one third of the specimens ruptured but without a lack of dural tissue, indicating a preparation artefact.

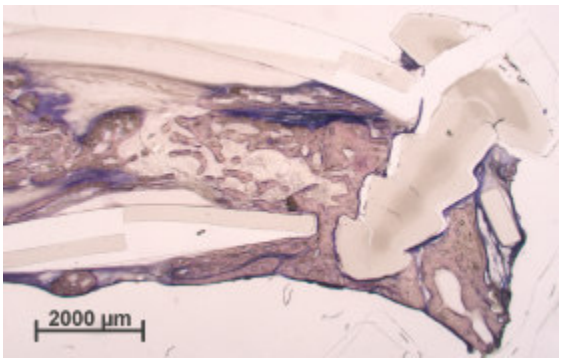

Fig. 12: Giemsa-Eosin microscopy: thin connective tissue layer surrounding the fixation dowel and bone following the dowel margin.

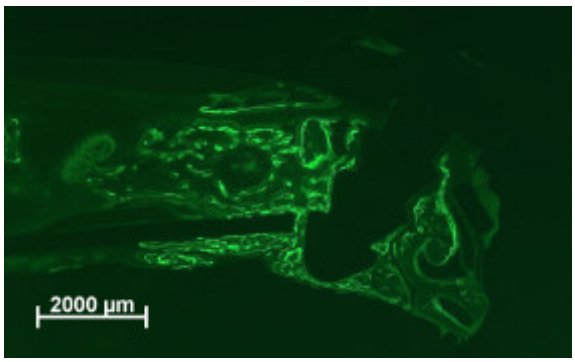

Fig. 13: Calcein-green fluorescence microscopy, revealing bone remodelling activity close to the implant (labelling week 2 and 4).

Not all dowels were represented on the histological slides, as the sections were made coronally through the centre of the defects. Totally 15 dowels made of poly(L/DL-lactide) 70/30 and 12 dowels of 80/20 were sectioned.

There were mainly two different structural changes in the polylactide dowels. Most dowels showed a homogeneous opaque cross section of the polymer bulk. They maintained the original shape with sharp outlines and only a few crackles.

A few dowels showed absence of polymer material inside the polymer bulk (Fig. 14) forming an optically empty space in the dowel core (3 out of 15 poly(L/DL-lactide) 80/20 and 1 out of 12 from the 70/30 type). There remained an inhomogeneous, granular polymer layer next to the bone, so the original dowel shape was still recognizable. Both patterns could be found in both materials, sometimes simultaneously.

Strongest birefringence was seen in those dowels where the polylactide bulk was still in the original form (Fig. 15). The polarizing areas inside the bulk where accentuated under the curved outlines of the dowel. Dowels with advanced structural changes exhibited practically no birefringence.

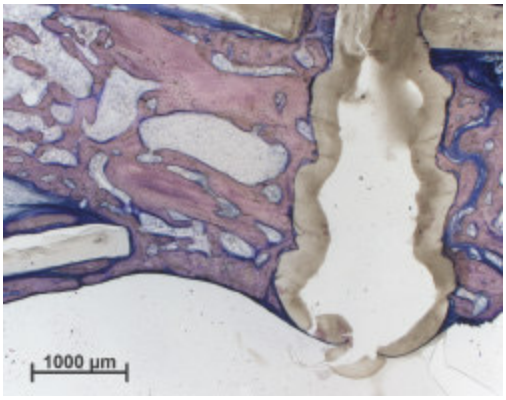

Fig. 14: Giemsa-Eosin microscopy: marked structural changes of a poly(L/DL-lactide) 80/20 dowel after 8 weeks.

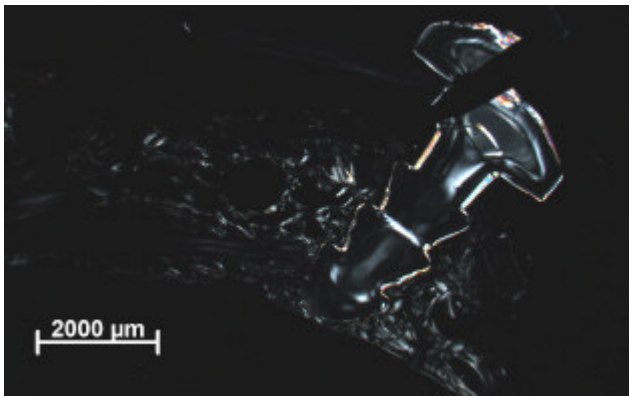

Fig. 15: Polarization microscopy (sample as shown in Fig. 12 and Fig. 13) revealing slight birefringence of the poly(L/DL-lactide) 70/30 dowels but not of the foils.

All described aspects were similar with no evident differences or preferences regarding the two types of the poly(L/DL-lactide) 70/30 and 80/20.

4.3.2 Foils

A thin, dense connective tissue capsule surrounded the polylactide foils. The foil was broken in 35 %, whereof 21 % were from the poly(L/DL-lactide) 70/30 and 14 % were from the poly(L/DL-lactide) 80/20. The edges along the fracture line were sharp and clearly delimited. The other 65 % of all specimens remained unbroken.

All foils prevented the brain from herniation into the bony defect. Half of the inner foils were even slightly bent towards the dura and brain. Three of the 32 inner foils had an oblique position protruding into the osseous defect, which did not affect the bone regeneration within the defect area.

There were four different grades of structural changes of the polylactide foils (Fig. 16, Fig. 17).

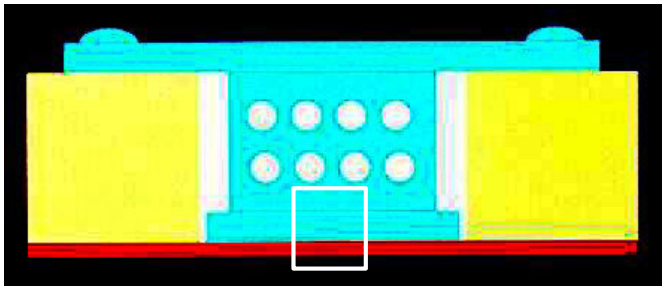

Fig. 16: Schematic cross-section: outer foil with dowels, perforated spacer, inner foil and dura. Different structural changes of the inner foil (framed area) are depicted in Fig. 17.

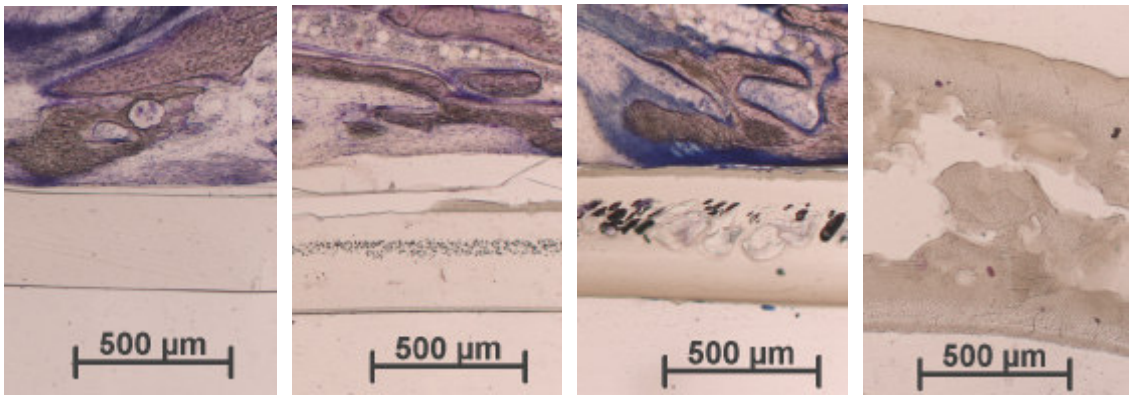

Fig. 17: Different polylactide foils after 8 weeks. From left to right with increasing structural changes: no visible signs, small vesicles along the core, bigger vesicles, breakdown along the core of the foil.

The first group showed no visible structural changes of the foils, neither inside nor along the outline of the foil. The form of this foil was mainly unchanged or very slightly bent. The second group showed innumerable small vesicles located along the core of the foil. The outline of the foil was still sharp and the form of the foils remained unchanged. The third group showed less and bigger oval and round vesicles, forming cavities along the core of the foil. The outline became now irregularly crinkled. The fourth group showed complete degradation of the core causing a lacuna in the centre of the foil with blurred margins. The outline of the foil became also blurred at some points. The form changed towards a blown or burst-out aspect.

Foils with some visible structural changes were more common among the outer foils than in the inner foils and were more common among the poly(L/DL-lactide) 70/30 than in 80/20. This trend is illustrated with numbers in Fig. 18.

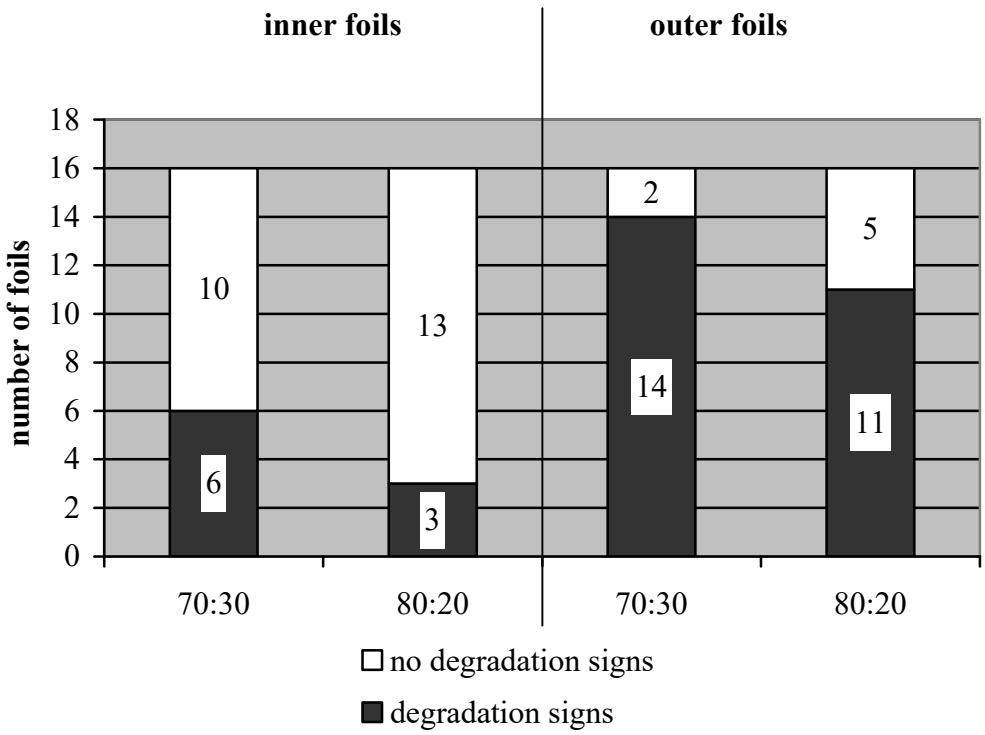

Fig. 18: Numbers of foils (grey) showing structural changes of the inner and outer foils. Structural changes were more common in the outer foils and in the poly(L/DL-lactide) 70/30.

In the group without any degradation the polarization microscopy exhibited no polarizing of the light, neither inside the foil nor in the neighbourhood. The strongest birefringence (Fig. 19 right) was seen in the intermediate stage of structural changes with cavities inside the foil (Fig. 19 left). The birefringence was along the core of the foil mainly in the bent areas of the foil. In the most degraded foils, birefringence was seen slightly along the blurred margins mainly inside the burst foils. Birefringence was always restricted to the foils and was never seen in their environment.

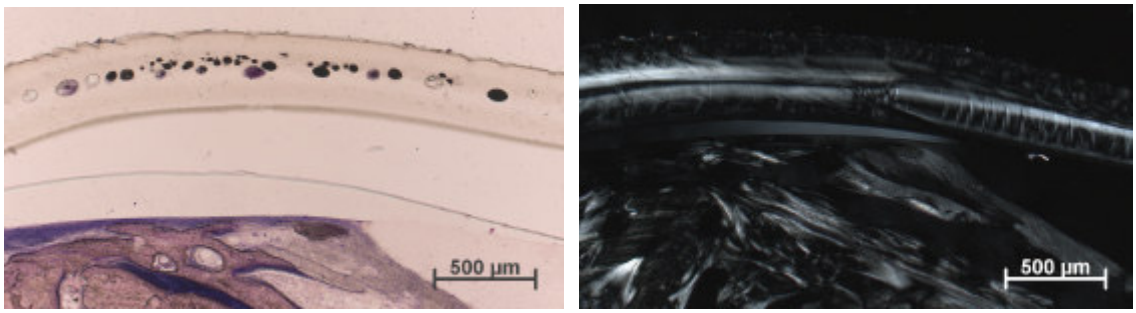

Fig. 19: Left: Giemsa-Eosin stained foil showing an intermediate stage of structural changes. Right: Polarization microscopy of the same sample exhibits strong birefringence along the core of the foil.

In the sample where the inner foil had an oblique position the structural changes showed an irregular distribution. The part of the foil intruding into the osseous defect showed small vesicles inside the foil, while the part of the foil protruding toward the dura showed no structural changes.

The rabbit which died the day after surgery, showed structural changes in the outer poly(L/DL-lactide) 70/30 foil, consisting of innumerable small vesicles along the core of the foil (Fig. 17, 2nd from left). This foil was markedly bent in order to be fixed on the little round skull of the rabbit, leading to fissures on the outer outline of foil. All other foils showed no structural changes.

4.3.3 Dura-polylactide interfaces

At the host-implant interfaces, a few foreign body giant cells (Fig. 21, Fig. 23) were detected. The giant cells were slightly more numerous at the foil-dura interface (framed area in Fig. 20) than on the foil-bone interface. The number of giant cells was variable. There was a mean of three giant cells at the foil-dura interfaces. In 5 % of the foil-dura interfaces there were more than 10 giant cells with a maximum of 20 giant cells. On the

opposite foil-bone interface all specimens showed less than 10 giant cells, with a mean of 2 giant cells.

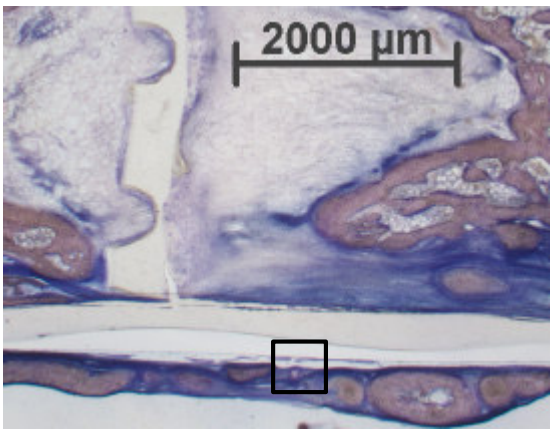

Fig. 20: Foreign body giant cells, Giemsa-Eosin. More numerous at the foil-dura interface (framed area) than at the foil-calvarial interface.

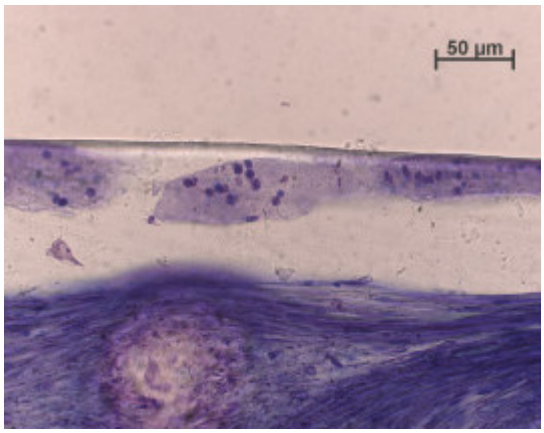

Fig. 21: Foreign body giant cells between the dura and the polylactide foil (framed area in Fig. 20).

The occurrence of the foreign body giant cells was almost equal in both tested isomeric poly(L/DL-lactide) mixtures. 52 % of all giant cells were in contact to a poly(L/DL-lactide) 70/30 foil where as 47 % of all giant cells were in contact with a poly(L/DL-lactide) 80/20 foil. Neither lymphocytes nor round cells, nor polymorphonuclear granulocytes were present. Neither signs of osteolytic reactions nor dystrophic calcifications were seen.

4.3.4 Dura

The dura had a smooth surface towards the brain as well as towards the poly(L/DL-lactide) foil. The fibrous part of the dura was consisting of a dense connective tissue and a large amount of fibroblasts. Most fibres run longitudinally and regularly (Fig. 21, Fig. 22).

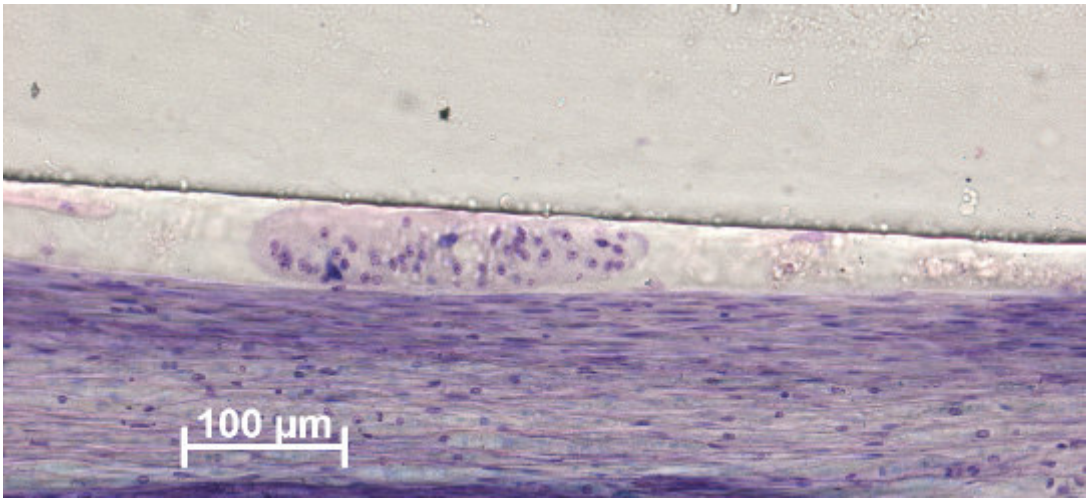

Fig. 22: Dural tissue with regular order of the cells and fibres. Giant cell is lying between the polylactide foil and the dura.

The dura was ruptured in some slides, but no lack of dural substance was noticed and the tissue edges were sharp. It was ruptured in one third of the poly(L/DL-lactide) 70/30 specimens and in half of the poly(L/DL-lactide) 80/20. The site of rupture was most commonly on the medial or lateral edge of the foil.

No specimen showed acute reactions as e.g. necrosis, abscess, lysis of the dura and no infectious or immunological signs were detected. No accumulation of round cells or polymorphonuclear cells was detected. No granulation tissue, no remnants of haematomas, no cartilage formation and no dystrophic calcifications were seen. There were no signs of swelling in the vicinity of the polylactide; neither in the dura nor in the regenerating bone.

In 90 % of the specimens some conglomerates of acellular material were detected in the subdural space (Fig. 23). They were mainly localised beneath the polylactide foil at the edge of the burr hole but also along the polylactide foil in the defect area. The size was variable reaching diameters of about 0.3 mm. In the Giemsa-Eosin slides they were oval

with a centre of reddish inhomogeneous stained granular mass surrounded by thin fibres of dural connective tissue. In fluorescence microscopy (Fig. 24) these conglomerates showed the same intensity as the newly formed bone. The fluorescence emitted by the Calcein green was clearly more intense than that of the Xylenol orange. The microradiographs showed the conglomerates being radiodense.

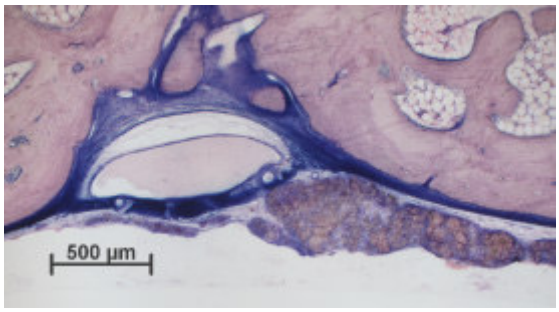

Fig. 23: Conglomerate of acellular material in the subdural space beneath the sinus sagittalis.

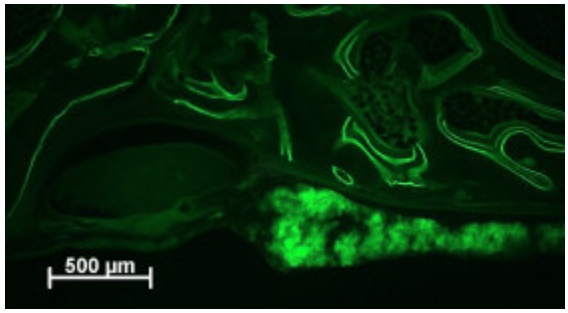

Fig. 24: Calcein green fluorescence microscopy exhibits fluorescence of the conglomerates seen in Fig. 23.

4.3.5 Dural bone formation

In about 93 % (63 of 66 slides and 15 of 16 rabbits) we found bone islets within the dura. The formation of bone islets was limited to the dura section along the implant (Fig. 25). As the slides were consisting of central cuts of the round poly(L/DL-lactide) foil, this dura section had a length of about 8.2 mm.

The bone islets raised at several places in the dura, with no visible preference. The central part of the dura was regularly the origin of bone formation. In average there raised about four to five osseous islets (maximum 10) in the dura. The osseous islets never stayed in direct contact to the poly(L/DL-lactide) foil nor to the underlying neural tissue. They were always delimited by a thin (<0.1 mm) dural tissue layer. The

thickness of this delimitation was variable on both sides of the osseous islets. No side was regularly thicker or thinner.

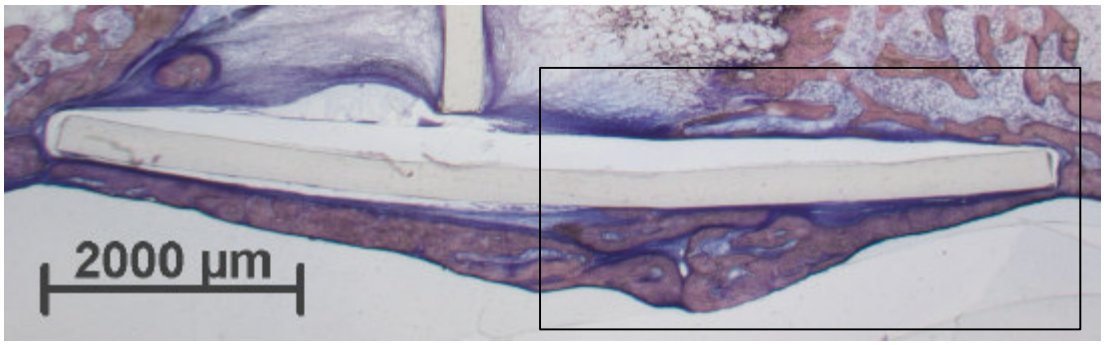

Fig. 25: Dural bone islets: pronounced bone formation within the dural part in the former defect area, next to the inner polylactide foil. Bone islets are completely surrounded by dural tissue. Some islets are fused to a more compact structure. Framed area is depicted in Fig. 27.

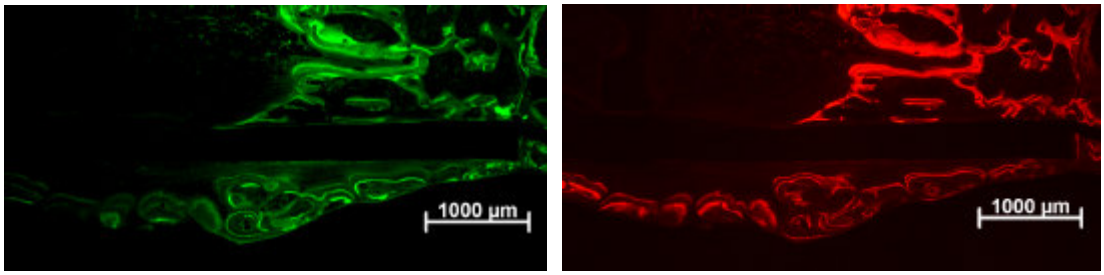

Fig. 26: Dural bone formation in the fluorescence microscope, framed area of Fig. 25. Bone remodelling processes are labelled at week 2 and 4 by Calcein green (left) and at week 6 and 8 by Xylenol orange (right).

The thickness of the osseous islets was variable (mean 0.65 mm) reaching maximally 1 mm while the calvarial bone had a diameter of about 3 mm in this area. Osseous fusion of adjoining islets was frequent giving to the fused bone regions an aspect of lamellar bone with little spaces. No clear differences regarding the frequency, amount or characteristics of the dural bone formation could be detected between the two types of poly(L/DL-lactide).

Fluorescence microscopy revealed the first signs of ossification, as visualized by Calcein green (Fig. 26, left), after two weeks. In a few samples, thin bridging across the centre of the defect might have already been present up to week 4, as visualized by Calcein green. Complete bridging in most samples is obvious at week 6 to 8, as visualized by Xylenol orange (Fig. 26, right).

In each case bone formation started on the border of the defect and simultaneously within the dura. The dural bone formation started at various sites within the dura.

4.4 Controls

4.4.1 Dura of non-operated controls

The 4 non-operated specimens showed a normal dura consisting only of dense connective tissue (Fig. 28). No bone formation or inflammation signs were seen in the dura. The cranial bone thickness showed an interindividual variation from 1.75 mm to 3.5 mm and an intraindividual variation of about 1 mm along the bone part which was removed in the operated rabbits (Fig. 27).

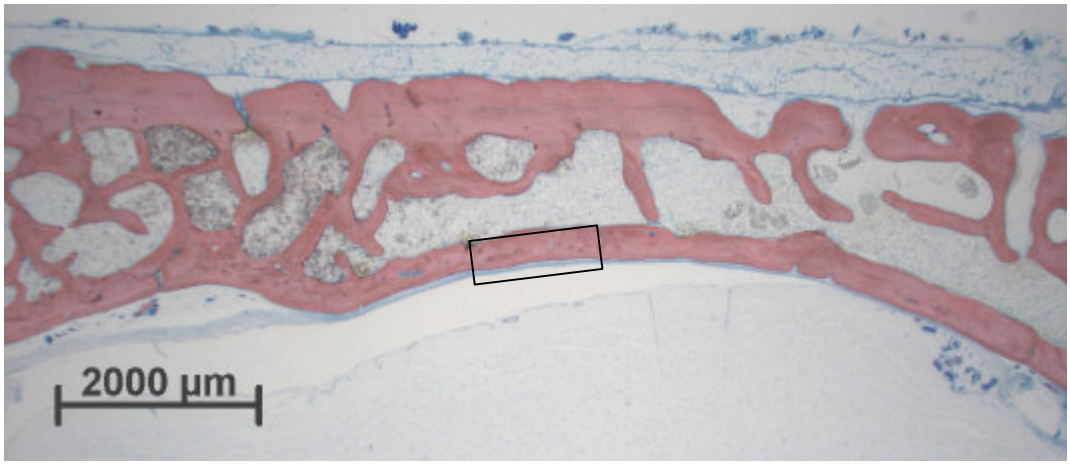

Fig. 27: Controls: calvaria, dura and brain of a mature rabbit (Giemsa-Eosin). Variable thickness of the cranial bone and the dural tissue.

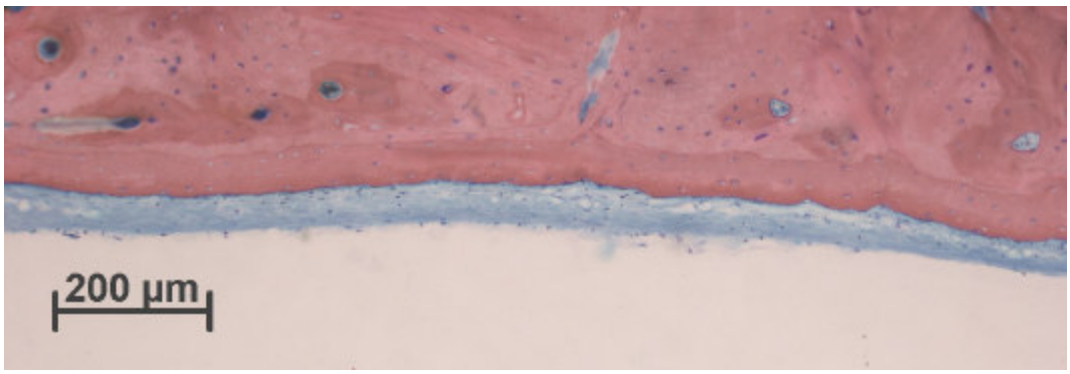

Fig. 28: Dural tissue (framed area of Fig. 27), showing dense connective tissue, no giant cells, no dural bone formation.

4.4.2 Dura without direct contact to polymers

For comparison specimens from a former investigation were used. In this group, all 16 rabbits received a poly(L/DL-lactide) 70/30 foil only on the outer surface of the skull. The amount of bone regeneration in the defect area was considerably lower than in the group with the sandwich technique (inner foil, spacer, outer foil). The bone regenerated with a pronounced internal bony table on the dural side and a more cancellous structure

in the diploe region. Dural bone regeneration was more effective than the centripetal bone regeneration from the defect edge to the centre of the defect (Fig. 29).

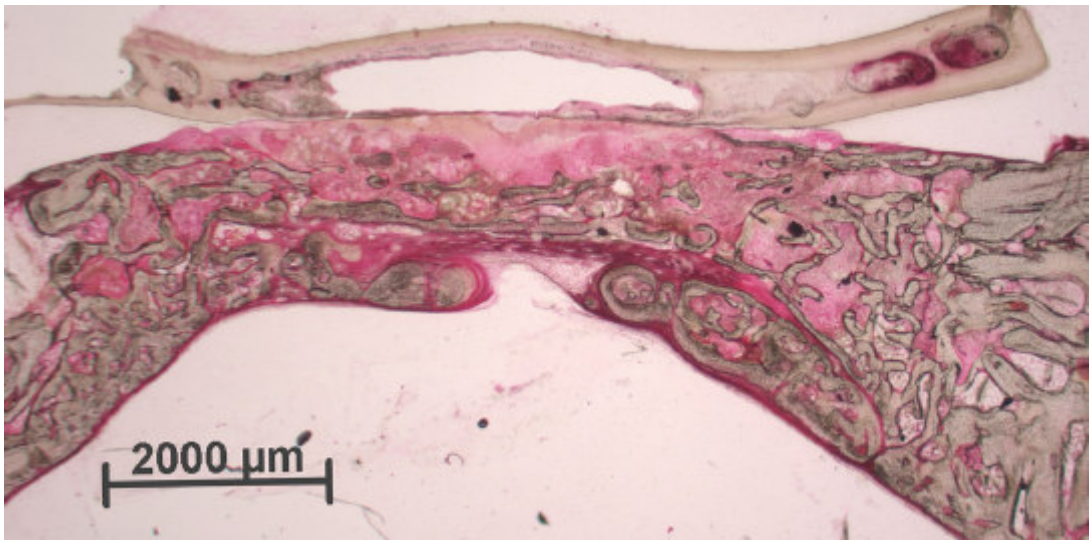

Fig. 29: Specimen with a polylactide foil only at the outer surface: compact dural bone regeneration at the dural side, more cancellous regeneration under the outer poly(L/DL-lactide) 70/30 foil. Bony structures are difficult to refer to their dural or diploic origin. The polylactide foil is showing marked structural changes.

In contrast to the series where an inner foil was applied, the dural bone formation was not separated from the bone regeneration in the defect area. Therefore dural bone formation merged continuously with the bone regeneration in the diploe region. Hence it was difficult to refer bone structures to their dural or diploic origin. 3 of 16 (19 %) rabbits showed neither dural bone formation nor any bone regeneration in the defect area. Hence it can be said, that maximally in 13 of 16 (81 %) rabbits the dural bone formation contributed to the bone regeneration. In contrast we found dural bone formation in the double foil group in 15 of 16 rabbits.

Fluorescence microscopy revealed the first signs of ossification and the earliest signs of bridging across the centre of the defect after two weeks, as visualized by Calcein green.

In each case bone formation started on the border of the defect and simultaneously on the dural side at various nucleation sites that merged later to compact structure (Fig. 29).

In contrast to the series with the double foil technique no foil remained unchanged. The outer foil always showed marked structural changes (Fig. 29). The core of the foil has often been completely absent, leaving a lacuna inside the foil. As a result the outline of the foil was broken up in several portions.

5 Discussion

5.1 Calvarial critical size defect in rabbits

In newborn rabbits and infants, the cranium can spontaneously regenerate following ostectomy of the calvaria. Because of their enormous capacity to repair bone defects, weanling rabbits are unsatisfactory subjects for experiments on bone regeneration in the calvaria. Hence we used skeletally mature rabbits, all aged 12 month. It is known that in infant rabbits, regeneration may occur not only from the defect edges, but also from bone deposits in the underlying dura (Alberius et al. 1990). The maintenance of the dura's integrity is essential for complete regeneration.

The skull trephine defect has become a standard experimental test system of autologous bone substitutes for almost a century. In 1889, Senn substituted xenogenic (bovine) bone matrix demineralized in hydrochloric acid for a blood coagulum and investigated effects on the healing of skull trephine defects in 14 normal dogs (Senn 1889). In order to obtain a standard model of calvarial nonunions, Schmitz et al. (1986) assessed and reviewed different sizes of calvarial defects in various animals. In rabbits calvarial defects exceeding 15 mm of diameter will always fail to regenerate spontaneously (critical size defect) and are therefore recommended for investigation on enhanced bone regeneration. Kramer et al. (1968) showed that 8 mm defects in rabbits do not always regenerate spontaneously up to 16 weeks as well. As our study was aimed to investigate the early reactions against two different polylactides, we decided to test without the interference by sutural growth with 8 mm defect diameter, allowing comparing two defects at once in one rabbit.

Alberius et al. (1990) investigated the pattern of bone regeneration in growth areas of the sutural region to that of the calvarial bone plate. Only minor divergences in healing capacity between the two defects were found. Nevertheless we paid attention not touching any suture when placing the defect areas.

5.2 Biomaterials in maxillofacial surgery

Gosain et al. (1999) refers to the exhaustive review of the complications and toxicities of implantable biomaterials used in facial surgery made by Rubin and Yaremchuck (1997). They reviewed nearly 200 clinical studies reporting series of patients with implantable biomaterials in the face. Metal fixation devices had an overall infection rate of 7.4 % and an exposure/ extrusion rate of 3.3 %. 9.3 % of implants were removed due to implant-related complications. Polymer and ceramic materials in the face had an overall infection rate of 3 % and an exposure/ extrusion rate of 1.2 %. 4.6 % of implants were removed due to implant-related complications.

They concluded that it is difficult to attribute many of the complications solely to the implant material itself, and that there is much overlap between surgical technique, host response, and potential toxicity of the implant. They also noted that the biocompatibility of a material varies depending on the conditions under which the implant is placed. Although current implant materials have favourable complication rates in most craniofacial applications, a biomaterial which behaves fairly well in one clinical circumstance may not be ideal for all applications in facial reconstructive surgery.

We investigated therefore not only the amount of bone regeneration in our series but directed our investigations as well towards the biocompatibility and the characteristics of the biodegradable material in this special setting adjacent to dural tissue.

5.3 Rationales of double foil technique with spacer (sandwich)

Lundgren et al. (1992) compared the results of bone healing in 8 mm full-thickness trephine skull defects in 2 rabbits by using either double foil technique (inner and outer biodegradable foil) or only an outer foil to cover the defects. He achieved better bone healing after 6 weeks with the double foil technique. He mentioned islands of bone seemingly surrounded by dural tissue only in the outer foil samples. He used infant (5 month) rabbits. The inner biodegradable foil on the dura was made of plasticized, non-porous polylactic acid (10 mm × 0.2 mm).

Bosch et al. (1995) investigated the amount of bone formation in an experimentally created parietal bone defect in rats, protected with one or two polytetrafluorethylene foils (Gore-Tex) compared to a contralateral control defect. He concluded that the use of two foils in order to preclude the defect area from the overlying tissues and underlying brain enhances bone regeneration.

Levy et al. (1994) investigated bone regeneration in 15 mm full-thickness cranial defects in 50 skeletally mature rabbits. He compared 4 different follow-up periods (4, 8, 16, and 24 weeks) and 3 different cover methods (no implant, outer foil only, inner and outer foil). Their amorphous poly(DL-lactide) had a molecular weight (MW) of 435’000 Daltons before gamma sterilization. They found only after 24 weeks a significantly better bone healing in the group being treated with an inner and outer foil compared to all other groups. This result might be due to an insufficient space maintaining capacity of their implantation model. First they used no spacer between the inner and the outer foil and additionally they used amorphous poly(DL-lactide) which is known to loose the mechanical stability faster than for instance the semicrystalline

poly(L-lactide). No bone formation within the dura was mentioned, but generally they found more new bone at the dural surface than at the pericranial surface. This finding was not further discussed.

The results of various previous studies prompted us to assess bone regeneration by a double foil technique with a spacer in between.

5.4 Rationale of semicrystalline poly(L/DL-lactide) 80/20

One series of late tissue response to poly(L-lactide) implants has been reported by Bergsma et al. (1995) in for of 10 patients with zygomatic fractures. Due to mild intermittent painless swelling of the implant area the material was explanted. Histology showed a foreign body reaction without signs of inflammation around the poly(L-lactide). The authors hypothesized that the foreign-body reaction was due to crystalline poly(L-lactide) fragments.

Since then it has often been speculated, that the lack of crystallinity of a polymeric material might be one of the most important determinants of complete, nonreactive resorption of an implanted degradable device. Hence this was the first rationale for the common use of amorphous poly(L/DL-lactide) 70/30 for implants. Secondly, amorphous polymers are supposed to have faster material resorption as compared to semicrystalline polylactides.

The result of a long-term in vivo study (Büchel 2002) indicated that both, amorphous poly(L/DL-lactide) 70/30 and the semicrystalline poly(L/DL-lactide) 80/20 degrade at comparable rates and evolve similar tissue reaction. Hence it can be concluded that the

reason for preferring poly(L/DL-lactide) 70/30 over poly(L/DL-lactide) 80/20 for the preparation of implants would be no longer evident.

Additional benefits from using semicrystalline polylactides instead of amorphous ones are production-related. Thus, implants from resorbable polymers produced from the melt are usually annealed above the glass transition temperature (T_g) to release the stress concentration. While implants from poly(L/DL-lactide) 80/20 can be safely annealed at temperatures exceeding the T_g, implants from amorphous poly(L/DL-lactide) 70/30 produced by injection-moulding and/or extrusion undergo plastic deformation when heated above T_g. It should also be realized that due to the development of crystallinity, stronger implants could be produced from semicrystalline polymers than from amorphous ones (Büchel 2002).

5.5 Reports about dural bone formation

5.5.1 The physiology of the dura

The periosteum is a vascular foil that cannot withstand pressure but rather responds to surface tension (e.g. muscles, ligaments) in osteogenesis. The dura likewise functions as periosteum on the endocranial side of the calvaria. Since both the endo- and ectocranial surfaces of the calvaria are areas of bony deposition, this phenomenon results in thickening of the flat bones of the cranial vault (Reid et al. 1981).

In literature, the dura mater is suspected being a critical regulator of successful calvarial reossification in immature animals (Greenwald et al. 2000). It is known for example that subtotal calvariectomy with dural preservation in children less than 2 years of age and immature animals is associated with complete reossification. It seems that calvaria

regenerates structurally and functionally complete in any respect. This effect gets lost in an age related fashion.

During early calvarial reossification, greater bone deposition is normally noted at the dural margin (endocranium) further implicating the dura mater being a tissue capable of osteoinduction.

5.5.2 Age related dural osteogenesis

It is known since a long time (Moss 1954, Sirola 1960) that the osteogenic potential of the periosteum diminishes with increasing age of the animal. It also diminishes with damage to either the dural or pericranial component.

Reid et al. (1981) confirmed as well statistically the clinical impression that bone regeneration of such defects occurs to a greater degree in young individuals as compared to adult. Until about eight years of age, spontaneous cranial repair from the dura takes place, but after this age the ability to do so declines (Robinson et al. 1989). This capacity generally starts to decrease in children older than 2 years of age and is lost in mature individuals. Hence the dura has been implicated as a regulator of calvarial reossification.

In a study of age related changes in periosteum and chondrogenesis O'Driscoll et al. (2001) assessed the decrease of chondrogenesis in rabbits. Chondrogenesis declined with age and was maximal in 1.5 to 2 month old rabbits. Explants from 6 month old rabbits formed 50 % less cartilage and by 12 month chondrogenesis reached a steady state minimal level. Analogous to the decrease of chondrogenesis there was a marked decrease (87 %) of the thickness and total cell number in the cambium layer of the periosteum between 2 and 12 months. These data confirm a decline in the chondrogenic

potential of periosteum with aging. The investigated rabbits were skeletally mature at 6 months and stopped gaining weight at 12 months.

5.5.3 Site-specific dural bone formation

Alberius et al. (1990) explored the pattern of bone regeneration in two different defect sites in young rabbits. He found only minor divergences in healing capacity between the osteotomies in the sutural region and the osteotomies in the bone plate.

Yu et al. (1997) investigated the differences of dural osteoinduction in different areas of the skull. In neonatal rats he compared osteoinduction of the dura underlying the flat portions of the calvaria and the dura underlying the sutural portion of the calvaria. He found that heterotopically transplanted neonatal dura from both regions can induce osteogenesis after 2 weeks. Bone formation was only seen when the dura was transplanted into an epithelio-mesenchymal pocket (thinned skin flap to expose hair follicles) on the back of the rat, but was never seen after transplantation into a pure mesenchymal pocket. This could indicate that the mesodermal dura needs an ectodermal stimulus, normally given by the central nervous system, for osteoinduction.

5.6 Dural bone formation in our series

5.6.1 Dural bone formation in adult rabbits

In our study we used skeletally mature rabbits of 12 months of age. New Zealand white rabbits reach skeletal maturity at 9 to 10 months when epiphyseal closure of the distal humerus and proximal tibia takes place. They gain weight till 10 to 12 months up to an average of 4270 g (Habermehl et al. 1980).

The fact of a dural bone formation in about two thirds of our rabbits was therefore unexpected. Further there was a tendency that the dura being in contact with poly(L/DL-lactide) 70/30 showed more often dural bone formation than the dura in contact with poly(L/DL-lactide) 80/20. However the number is not big enough to argue conclusively. Although the rabbits were skeletally mature it must be admitted that they stopped growing recently, so their dura might have preserved some of its infant potency of bone formation. In this case our results indicate that dural bone formation in rabbits is less strictly related to infancy as it has been reported previously.

The contribution of the dura to the regeneration of cranial defects was shown in a number of studies. Due to our concept of a double foil application with spacer, the dural bone islets can no longer merge with bone regeneration in the defect area. Nevertheless the better effect of space maintaining seems to overact considerably the contribution of dural bone formation, because dural bone formation in the double foil series was even more often present than in the outer foil series of Leiggener et al. (2002).

5.6.2 Dural bone formation adjacent to the polymer foil

The bony islets were found in very close contact to the polylactide foil, only separated by a very thin layer of dural tissue. The dural tissue layer surrounding the bony islet had the same thickness both towards the polylactide foil and towards the bony defect. We conclude therefore first, that the poly(L/DL-lactide) seems not to hinder the physiological dural bone formation in any respect. Second that the poly(L/DL-lactide) seems not to increase the connective tissue formation in the dura adjacent to the polylactide foil. It remains the open question whether these dural bone islets would

merge with the regenerated calvarial bone after the complete degradation of the polylactide foil.

5.7 Clinical consequences of dural calcifications

In our series, dural bone formation was separated from the defect area by the inner polylactide foil. Although we assume, that after degradation of the inner foil, these bone islets would merge with the regenerating calvarial bone, there could be a risk of a remaining artificial dural calcification.

In adults dural calcification may occur. The main factors contributing to dural calcification are considered to be aging, long-term irritation of lesions, head injuries and endocrine disorders.

Robinson et al. (1989) reported about an 18 year old man who underwent bloc resection of a squamous cell carcinoma of the fronto-ethmoid complex. Three months later he underwent a course of radiation therapy, receiving 6’000 cGy over six weeks. 14 months after primary surgery computed tomography showed complete dural calcification of the treated side. Neurological sequels due to the dural calcifications were no stated.

In a study of dural calcification Tanaka et al. (1974) radiologically detected 164 cases of dural calcification or ossification among 1162 neurological and neurosurgical patients. The most frequently affected sites were the falx cerebri 10 %, tentorium cerebelli 2 % and petroclinoid ligament 2 %. The 164 cases were referred to the hospital for the following reasons: head injury (103), headache (18), cerebrovascular disease (14), epilepsy (12) and miscellaneous diseases (17).

It seems that there is no specific clinical manifestation of dural calcification in humans and that the occurrence of symptoms is rare. None of our animals showed any neurological symptoms during the observed time period, especially no signs of abnormal behavior or paresis.

5.8 Sufficient source of osteoblasts in bone marrow

Osteoinduction reflects the recruitment of perivascular connective tissue cells (pericytes) from a fibrogenetic to an osteogenetic pathway.

Sato and Urist (1985) demonstrated a dual origin of new bone in regenerating calvaria. They reported bone development by proliferation of pre-existing osteoprogenitor cells lining the diploe, and by perivascular cells of the endosteal and bone marrow stroma.

Perivascular loose connective tissue that accompanies the proliferating capillaries represents the source of osteoprogenitor cells, as demonstrated in endochondral ossification of the epiphyseal plate or in the early stage of fracture healing. The intimate correlation between newly formed blood vessels and de novo bone formation was also elucidated in a case report of guided bone regeneration in a rabbit from Schmid et al. (1997).

Bosch (1995) et al. concluded from his results, that the adult skull is an extremely limited source of osteoblasts and new bone. In the adult, osteoblast precursors must be recruited from the perivascular dural tissues to complete cranial bone regeneration.

We found superior bone formation in the double foil technique compared to the series of outer foils only. The hindered recruitment of pericytes from the dural tissue, due to the overlying foil, seems not to jeopardize the complete calvarial bone regeneration.

The source of osteoprogenitor cells in the bone marrow stroma seems to be sufficient, as long other conditions for the regenerating bone are favorable, especially the appropriate space maintenance due to a spacer.

5.9 Chemical aspects of polylactides and their breakdown

5.9.1 Factors influencing the polymer breakdown

The resorption of a polymeric device is influenced by many factors, including the size, and shape of the implant, its molecular weight and crystallinity, and site of implantation (Eppley et al. 1997). We will discuss the structural changes of the polylactides (Fig. 17 p.36) in our series in the light of some of these factors.

Factors inducing the in vivo and in vitro degradation and resorption of polymers (Hutmacher 1996):

1. Chemical structure
2. Chemical composition
3. Distribution of repeat units in polymers
4. Presence of ionic groups
5. Presence of unexpected units or chain defects
6. Structure of configuration
7. Molecular weight
8. Molecular weight distribution (polydispersity)
9. Presence of low-molecular weight compounds (monomers, oligomers, solvents, plasticizers, drugs, etc.)
10. Method and conditions of processing
11. Design
12. Method of sterilization
13. Morphology (amorphous / crystalline, microstructure, presence of residual stress)
14. Annealing
15. Storage history
16. Site of implantation

17. Adsorbed and absorbed compounds (water, lipids, ions, etc.)
18. Physicochemical factors (ion exchange, ionic strength)
19. Physical factors (mechanical stress, shape and size changes, variations of diffusion coefficients, cracking)
20. Mechanism of hydrolysis (hydrolysis / enzymes)

Chemical and physical changes go along with the degradation of biodegradable polymers, like the crystallization of oligomers and monomers or pH changes. Some of these factors can have a substantial feedback effect on the degradation velocity (Göpferich 1996). Usually the rate of polymer degradation and resorption is lower for polymers with high molecular weight, high crystallinity, and a strong orientation. It is mainly the type of bond within the polymer that determines the rate of hydrolysis. The relatively slow degradation of polylactide is partially due to the steric effects, because the voluminous alkyl group hinders the attack of water. Polylactide is a lipophilic polymer and shows therefore a small amount of water uptake.

Poly(L/DL-lactide) 70/30 has a bending strength of about 123.7 MPa. The decrease of the mechanical properties of poly(L/DL-lactide) 70/30 starts at 9 months (Hutmacher et al. 1996) while mass loss and resorption takes considerably longer, up to estimated 3.5 to 4 years.

A faster chain scission of polylactide at low pH explains the bulk (heterogeneous) erosion due to autocatalysis (Fig. 3, p. 15). The generated monomers, which are carboxylic acids, accelerate polymer degradation by lowering the pH. pH-values as low as 1.8 were measured inside the eroding polymer rods, due to the autocatalytic process and relatively high solubility and a low pK_a of polylactide. The loss of tensile strength is known to precede by far the complete degradation of poly(L-lactide).

5.9.2 Inverse bulk degradation in both types of polylactides

Inversely moving erosion fronts have been observed for the autocatalytic degradation of poly(DL-lactide) that moves from inside of the polymer outwards (Göpferich 1996). In our series the group with the most advanced degradation signs showed such structural changes (Fig. 17, p. 36). This inversely moving erosion fronts were seen in the foils (Fig. 29, p. 42) as well as in the dowels (Fig. 14, p. 34) and for both types of poly(L/DL-lactide). It can be concluded that the semicrystalline poly(L/DL-lactide) 80/20 shows light-microscopically similar degradation as the amorphous poly(DL-lactide) and the poly(L/DL-lactide) 70/30. This degradation process can be described as inverse bulk degradation.

5.9.3 Unexpected variability of structural changes

The variations of structural changes among the same type of polylactides are difficult to explain. First, all rabbits were sacrificed at the same time after 8 weeks. The aspects of the macroscopic changes are similar to the degradation process described in the normal inverse bulk degradation. It must be admitted that the time of seeing these changes and the wide range, from no signs to advanced stages, is unexpected as the complete degradation time for our polylactides is reported to be about 3.5 to 4 years.

We applied two 8 mm defects in each rabbit skull. By fixing the outer foil with dowels onto the concave skull surface the outer foil underwent a bending stress to some extent. It is possible, that this procedure resulted in small fissures on the foil surface. These fissures would enhance the penetration of water into the foil, resulting in an enhanced degradation process.

There was a tendency that the outer foils showed more often and more advanced signs of degradation (Fig. 18, p. 37). This would corroborate the explication that bent foils show more often an enhanced degradation. Further it is known that the implantation site has an impact on the degradation dynamics. Generally polymer degradation is faster in highly vascularized tissue and in an abundant soft tissue surrounding. These factors are enhancing the hydrolysis and the clearance of degradation products. This mechanism might also have had an effect on the observed tendency.

Bos et al. (1989) have published on the use of high molecular-weight poly(L-lactide) plates and screws in mandibular fractures in sheep and dogs. The results indicated that the plates subjected to loading had lost more of their tensile strength (about 90 %) in 11 weeks than those not under stress (about 80 %). This illustrates the known impact of mechanical stress on the degradation dynamics.

Further it can be argued that the material quality might not have been constant for both polymers throughout the processing. There are numerous factors influencing the degradation dynamics of the polymer (chap. 5.9.1). Every production process varying one of these factors is therefore suspicious of having contributed to this variability.

In the rabbit which died the day after surgery histology revealed as well structural changes in the foil. The outer foil made from poly(L/DL-lactide) 70/30 showed innumerable small vesicles located along the core of the foil (Fig. 17, p.36), which corroborates that the first stage of structural changes had already existed at the time of application or they are due to the application itself.

5.9.4 Opaque dowels

Pins made of poly(L/DL-lactide) 70/30 turned a lot more often to a white opaque colour; while only a few pins of poly(L/DL-lactide) 80/20 did so (Fig. 10, Fig. 11). Poly(L/DL-lactide) 70/30 is highly amorphous while poly(L/DL-lactide) 80/20 is semicrystalline, hence the pins made of poly(L/DL-lactide) 70/30 showed more frequently macroscopic signs of starting hydrolysis. The water enters the polymer and scissors the polymer bonds by hydrolysis, the increasing liquid part changes the refraction of the polymer, resulting in macroscopical opacity.

5.9.5 Crystallinity in the polarization microscopy

Due to the faster erosion of amorphous compared to crystalline polymer regions, the overall crystallinity of samples increases, and has been measured for poly(L-lactide) (Göpferich 1996). Crystallinity also increases during the erosion of intrinsically amorphous polymer like quenched samples of poly(L-lactide). When introducing these samples to erosion media, their glass transition temperature is lowered due to the uptake of water, which leads to the recrystallization of the polymer.

Initially, the devices had a transparent optical quality at the time of placement (Fig. 5, p. 22). At the time of implantation the polarization microscopy showed slight birefringence of both poly(L/DL-lactides) 70/30 and 80/20, as seen in the died rabbit. This indicates that at the beginning in both poly(L/DL-lactides) there are some crystalline structures. This can most probably be attributed to the manufacturing process. The mixture of L/DL is not 100 % accurate; there is variability up to 10 %. Therefore the composition differences of poly(L/DL-lactide) 70/30 and 80/20 might be

less than those theoretically expected and therefore not visible in the polarizing microscope.

After 8 weeks birefringence microscopy revealed no remaining birefringence in those foils without any degradation signs. An explication could be that the water uptake permitted the hydrolytic process to start and to break up the parallel orientation of the polymer molecules, thus nearly eliminating any birefringence properties. This process is microscopically invisible and results in no structural changes.

Strongest birefringence was seen in intermediate stages with cavities inside the foil (Fig. 12, p. 33). The birefringence was along the core of the foil mainly in the bent areas of the foil. In the most degraded foils, only slight birefringence was seen along the blurred margins mainly inside the burst foil. Birefringence was always restricted to the foils and was never seen in their environment. No highly crystalline parts could be detected during the advanced stages of degradation, not even in the semicrystalline poly(L/DL-lactide) 80/20. The polarizing microscopy revealed mainly stress zones, where the molecules are pressed into a crystalline like order.

All findings were analogously seen in the dowels. It must be stated again that no differences could be detected between the poly(L/DL-lactide) 70/30 and 80/20 in respect to their birefringence. This indicates that there are no major differences of the crystalline character in these two polylactides.

5.9.6 Molecular weight changes during processing

Due to the susceptibility of polylactides to hydrolysis, conventional methods of sterilization cannot be used. Currently available methods for sterilization of

biodegradable implants include gamma irradiation and ethylene oxide (EtO) sterilization.

Gamma irradiation is known to induce structural changes such as main chain scission and or cross-linking in the irradiated polymer. These changes may affect the polymer and implant properties such as mechanical strength, degradation kinetics, molecular weight, and implant design.

Ethylene oxide (EtO) is used in medical supplies and devices that are heat sensitive. EtO is known to be a very reactive agent and residuals of EtO are considered to be a risk for patients. It has been shown (Hutmacher 1996) that it takes approximately 6 to 8 weeks of degassing to reduce an initial EtO residue level of 40 ppm to the tolerable level of 3 ppm for a bioresorbable pin made of poly(L/DL-lactide) 70/30. Therefore we used in our series sterilization by gamma irradiation.

The molecular weight decreased by gamma irradiation from initially 660’000 Daltons of the raw material to 72’500 Daltons in the dowels and to 84’100 Daltons in the foil. These differences are due to the different production processes.

The dowels were made by injection moulding what implicates, that out of a reservoir of melted polymer the dowels were injected into a form step by step. The longer the polymer is exposed to heat the more bonds will be cleaved due to the thermal energy resulting in a gradual decrease of the molecular weight.

The foils are made by melt extrusion. In this process the polymer is transported by a kind of Archimedes screw and melted at the same time. Then the polymer is immediately squeezed onto a conveyor band. Hence the polymer is less long exposed to the degrading heat.

5.10 Biocompatibility

5.10.1 Review of polymer biocompatibility

Bioresorbable and biodegradable fixation devices for maxillofacial surgery have been reviewed (Anthanasiou et al. 1996, Gosain et al. 1999, Hutmacher et al. 1996, Suuronen 1993). Several investigations showed an excellent biocompatibility of poly(L/DL-lactide) devices with no signs of clinically relevant inflammatory reactions; however mild unspecific inflammatory reactions have frequently been seen around implants.

It appears that biocompatibility of bioresorbable polyesters depends primarily on factors other than the polymers themselves. The leaching of low-molecular mass compounds, either because of degradation or because of the presence of leached out impurities, seems to be the major source of triggering inflammation. Further more the size, shape and surface structure of the implants seems also to influence tissue reactions (Suuronen 1993).

One other important factor is the location of the implantation. If the capacity of the surroundings to eliminate the by-products is small because of poor vascularization or low metabolic activity, chemical composition of the by-products may lead to temporary local disturbances. One example is the increase of osmotic pressure or pH manifested by local fluid accumulation or transient sinus formation. Hence, biocompatibility of bioresorbable polylactide is thought to depend mainly on biodegradation and bioresorption.

5.10.2 Biocompatibility in our series

In our series we have seen only a few foreign body giant cells in the interface (Fig. 21, p. 39), indicating a mild sterile inflammation. There were no signs of adverse tissue reactions in the adjacent tissue, neither in the regenerating bone nor in the dural tissue. No signs of acute or chronically inflammation were seen. In this respect no difference between poly(L/DL-lactide) 70/30 and 80/20 was seen.

The results must be limited to the observed time period, representing the early stage of interaction between host and implant. During the early stage any impurities or residues from processing are expected to induce a biological reaction if they are present. As no such reaction was present the material can be considered as being properly processed in this respect. However we cannot exclude that some processing variability might have contributed to the different degradation behaviours, varying from no visible signs to advanced stages of degradation.

We found no correlation between the crystallinity of the material and its induced biological reaction throughout the different degradation stages. Further more, polarization microscopy showed no differences between the birefringence property of poly(L/DL-lactide) 70/30 and 80/20. Hence we conclude that the amount of crystallinity is very similar in both polylactides and that moreover slight differences in crystallinity have no major effect on the biological reaction during the early stage of implantation.

5.11 Conclusions and perspectives

To enhance the healing of cranial bone defects the method of guided bone regeneration, using a polylactide foil at the inner and outer calvarial surface with a spacer in between,

is very effective. Before application of this method in humans can be considered more has to be known about the reaction of its underlying brain and dural tissue. This investigation was addressed to the dural reaction during the early stage of polylactide foil implantation.

The dural tissue lying next to the inner foil showed no acute adverse reactions. Biocompatibility of the mechanically preferable poly(L/DL-lactide) 80/20 was good and equal to the approved poly(L/DL-lactide) 70/30.

The physiological dural bone formation was not hindered by the adjacent polylactide foils. However, the open question remains whether the dural bone islets would merge with the calvaria after complete foil degradation. The observed dural bone formation led to no adverse neurological findings during the observed time period.

There were slightly less structural changes in the foils made of poly(L/DL-lactide) 80/20 foils than in the 70/30, and in the inner foil than in the outer foils. These findings are consistent with the current knowledge about chemical and physical factors influencing the degradation dynamics.

For information about late tissue reactions and degradation dynamics a long-term study would be necessary. Complete degradation can be expected earliest at 3 to 4 years. Since degradable polylactides became especially useful in paediatric cranial surgery long-term investigations should also consider the interaction with the skull growth and with the described dural bone formation.

Nowadays many efforts are made in medicine to reduce the permanent application of any foreign materials. This vision is partially realized by the biodegradable materials which remain only transiently in patients. A potential next step might exploit the known

calvarial regeneration by extensive dural bone formation during early infancy. Any therapeutic intervention that could re-establish this potency in mature individuals could lead one day to a successful calvarial regeneration without any implants.

6 Abstract

Cranial bone defects, exceeding a certain size do not heal spontaneously and therefore frequently require surgical treatment. One approach uses the principle of guided tissue regeneration. The application of biodegradable foils leads to various advantages, e.g. no growth restrictions and spontaneous breakdown in case of translocation during skull growth. Hence, polymers became especially useful in paediatric cranial surgery. Any hazards must be excluded before application in the neurocranium and therefore in vicinity to neural tissue can be considered.

We investigated the dural reaction during the early stage after implantation of polylactide implants into cranial defects in rabbits. Implants were applied to 8 mm calvarial defect in a sandwich manner: an endocranial foil at the inner surface in direct contact to the dura, a spaceholder bridging the defect area and a foil on the outer surface of the skull. One series was manufactured from amorphous poly(L/DL-lactide) 70/30 the other was manufactured from semicrystalline poly(L/DL-lactide) 80/20.

All 16 rabbits tolerated the implants with no adverse signs during the observed time period of 8 weeks. The cellular histological findings were about the same in both types of poly(L/DL-lactide). There were only few giant cells along the membrane-dura interface. No lymphocytes or round cells were detected. The dura itself showed no adverse reactions. In 15 of 16 specimens the physiologically known dural bone formation was found, and seemed not to be hindered by the adjacent inner polymer foil.

It can be concluded that both polylactides showed a good and equal biocompatibility with the dura during the early stage of implantation.

7 Literature

Alberius P, Isaksson S, Klinge B, Sjögren S, Jönsson J. 1990. Regeneration of cranial suture and bone plate lesions in rabbits. Implications for positioning of osteotomies. J Cranio Max Fac Surg. 18:271-9

Alberius P, Klinge B, Isaksson S. 1989. Management of craniotomy in young rabbits. Lab Anim. 23:70-2

Athanasiou KA, Niederauer GG, Agrawal CM. 1996. Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. Biomaterials. 17:93-102

Bauer TW, Muschler GF. 2000. Bone graft materials. An overview of the basic science. Clin Orthop. 371:10-27

Bergsma JE, Bruijn WC, Rozema FR, Bos PRM, Boering G. 1995. Late degradation tissue response to poly(L-lactide) bone plates and screws. Biomaterials. 16:25-31

Bergsma JE, Rozema FR, Bos RRM, de Bruijn WC. 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. 51:666-70

Bos RRM, Rozema FR, Boering G, Nijenhuis AJ, Pennings AJ, Verwey AB. 1989. Bio-absorbable plates and screws for internal fixation of mandibular fractures. A study in six dogs. Int J Oral Maxillofac Surg. 18:365-9

Bosch C, Melsen B, Vargervik K. 1995. Guided bone regeneration in calvarial bone defects using polytetrafluoroethylene membranes. Cleft Palate Craniofac J. 32:311-7

Böstman OM, Pihlajamäki HK, Partio EK, Rokkanen PU. 1995. Clinical biocompatibility and degradation of polylevolactide screws in the ankle. Clin Orthop. 320:101-9

Büchel P. 2002. Long-term in vivo degradation and bone reaction to resorbable implants from selected polylactides. POLY(L/DL-LACTIDE) 80/20 % and POLY(L/DL-LACTIDE) 70/30 %). Thesis. The University of Basel.

Cutright DE, Hunsuck EE. 1972. The repair of fractures of the orbital floor using biodegradable polylactide acid. Oral Surg Med Oral Pathol. 33:28-34

Dahlin C, Alberius P, Linde A. 1991. Osteopromotion for cranioplasty. An experimental study in rats using a membrane technique. J Neurosurg. 74:487

Eppley BL, Reilly M. 1997. Degradation characteristics of PLLA-PGA bone fixation devices. J Craniofac Surg. 8:116-20

Goldstein JA, Quereshy FA, Cohen AR. 1997. Early experience with biodegradable fixation for paediatric craniofacial surgery. J Craniofac Surg. 8:110-15

Göpferich A. 1996. Mechanisms of polymer degradation and erosion. Biomaterials. 17:103-14

Gosain AK, Persing JA. 1999. Biomaterials in the face: benefits and risks. J Craniofac Surg. 10(5):404-14

Greenwald JA, Mehrara BJ, Spector JA, Chin GS, Steinbrech DS, Saadeh PB. 2000. Biomolecular mechanisms of calvarial bone induction: immature versus mature dura mater. Plast Reconst Surg. 105:1382-92

Groeneveld EHJ, Burger EH. 2000. Bone morphogenic proteins in human bone regeneration. Europ J Endocrinol. 142:9-21

Habermehl KH. 1980. Die Altersbestimmung bei Versuchstieren. Berlin Hamburg: Parey; 40-6

Hutmacher D, Hürzeler MB, Schliephake H. 1996. A review of material properties of biodegradable and bioresorbable polymers and devices for GTR and GBR applications. Int J Oral Maxillofac Implants. 11:667-78

Kramer IR, Killey HC, Wright HC. 1968. A histological and radiological comparison of the healing of defects in the rabbit calvarium with and without implanted heterogeneous anorganic bone. Archs Oral Biol. 13:1095

Leiggener CS, Curtis R, Pfluger D, Rahn BA. 2002. Effect of perforations in burr hole covers on cranial bone regeneration in rabbits. J Biomed Mater Res. 61:131-137

Leonhardt H. 1990. Histologie, Zytologie und Mikroanatomie des Menschen. 8th ed Stuttgart: Thieme; 88-9

Levy FE, Hollinger JO, Szachowicz EH. 1994. Effects of a bioresorbable film on regeneration of cranial bone. Plast Reconst Surg. 93:307-11

Linde A, Thorén C, Dahlin C, Sandberg E. 1993. Creation of new bone by an osteopromotive membrane technique. An experimental study in rats. J Oral Maxillofac Surg. 51:892-7

Lundgren D, Nyman S, Mathisen T, Isaksson S, Klinge B. 1992. Guided bone regeneration of cranial defects, using biodegradable barriers: an experimental pilot study in rabbit. J Cranio Max Fac Surg. 20:257-60

Menei P, Daniel V, Montero-Menei C, Brouillard M, Pouplard-Barthelaix A, Benoit JP. 1993. Biodegradation and brain tissue reaction to poly(D, L-lactide-co-glycolide) microspheres. Biomaterials. 14:470-8

Moss ML. 1954. Growth of the calvaria in the rat. The determination of osseous morphology. Am J Anat. 94:333

Nyman S, Gottlow J, Karring T, Lindhe J. 1982. The regenerative potential of the periodontal ligament: an experimental study in the monkey. J Clin Periodontol. 9:257-65

O'Driscoll SW, Saris DB, Ito Y, Fitzimmons JS. 2001. The chondrogenic potential of periosteum decreases with age. J Orthop Res. 19(1):95-103

Peltoniemi HH, Hallikainen D, Toivonen T, Helevirta P, Waris T. 1999. SR-PLLA and SR-PGA miniscrews: biodegradation and tissue reactions in the calvarium and dura mater. J Cranio Max Fac Surg. 27:42-50

Peltoniemi HH, Tulamo RM, Toivonen T, Pihlajamäki HK, Pohjonen T, Törmälä P. 1998. Intraosseous plating: a new method for biodegradable osteofixation in caniofacial surgery. J Craniofac Surg. 9:171-6

Rahn BA, Perren SM. 1971. Xylenol orange, a fluorochrome useful in polychrome sequential labeling of calcifying tissues. Stain Techn. 46:125-9

Reid CA, McCarthy JG, Kolber AB. 1981. A study of regeneration in parietal bone defects in rabbits. Plast Reconst Surg. 67:591-6

Robinson AC, O'Dwyer TP, Gullane PJ, Dolan EJ. 1989. Anterior skull defect reconstruction with methyl methacrylate. J Otolaryngol. 18(5):241-4

Rubin JP, Yaremchuck MJ. 1997. Complications and toxicities of implantable biomaterials used in facial reconstructive and aesthetic surgery: a comprehensive review of the literature. Plast Reconst Surg. 100:1336-53

Sato K, Urist MR. 1985. Induced regeneration of calvaria by bone morphogenic protein (BMP) in dogs. Clin Orthop. 197:301

Schmid J, Wallkamm B, Hämmerle CHF, Gogolewski S, Lang NP. 1997. The significance of angiogenesis in guided bone regeneration. A case report of a rabbit experiment. Clin Oral Impl Res. 8:244-8

Schmitt EF, Palistina RA. US Patent No 3.371.069(1967), No 3.463.158(1969), No 3739.773(1973).Cited by Hutmacher et al (1996)

Schmitz JP, Hollinger JO. 1986. The critical size defect as an experimental model for craniomandibulofacial nonunions. Clin Orthop. 205:299-308

Senn N. 1889. On the healing of aseptic bone cavities by implantation of antiseptic decalcified bone. Am J Med Sci (New series). 98:219-43. Cited by Takagi et al (1982)

Sirola K. 1960. Regeneration of defects in the calvaria. An experimental study. Ann Med Exp Biol Fenn. 38(Suppl 2):1

Suuronen R. 1993. Biodegradable fracture-fixation devices in maxillofacial surgery. J Oral Maxillofac Surg. 22:50-7

Suzuki HK, Mathews A. 1966. Two color fluorescent labeling of mineralizing tissues with tetracycline and 2,4-bi[N, N'-di-(carbomethyl)aminomethyl]fluorescein. Stain Techn. 41:57-60

Swart JGN, Allard RHB. 1985. Subperiostal onlay augmentation of the mandible: a clinical and radiographic survey. J Oral Maxillofac Surg. 43:183-7

Takagi K, Urist MR. 1982. The reaction of the dura to bone morphogenic protein (BMP) in repair of skull defects. Ann Surg. 196(1):100-9

Tanaka Y, Takeuchi K. 1974. Dural calcification from the neurosurgical point of view. Neurol Med Chir. 14(1):5-10

Vasenius J, Majola A, Miettinen E-L, Törmälä P, Rokkanen P. 1992. Do intramedullary rods of self-reinforced poly(L-lactide) or poly(DL/L-lactide) cause lactic acid acidosis in rabbits? Clin Mater. 10:213-18

Williams DF. 1988. Consensus and definitions in biomaterials, in: C. de Putter, K. de Lange, K. de Groot and A:J. C. Lee, eds., Advances in biomaterials. Amsterdam: Elsevier Science Publishers B. V. 8:11-16

Yu JC, McClinctock JS, Gannon F, Gao XX, Mobasser JP, Sharawy M. 1997. Regional differences of dura osteoinduction: squamous dura induces osteogenesis, sutural dura induces chondrogenesis and osteogenesis. Plast Reconst Surg. 100(1):23-31

Acknowledgement

At the first place I want to express many thanks to my supervisor and mentor Prof. Dr. Dr. B. A. Rahn. With his enormous experience and sense for research he gave me precious feedbacks and guidance, always helping me to find back on the right way. I learned a lot from you, thank you very much.

Secondly I'd like to give a very special thank to Dr. Dr. C. Leiggener, who supported me cordially and generously with his knowledge and his previous work done in the similar field. Additionally I thank a lot to Prof. Dr. S. Gogolewski for giving me an understanding of the chemical basics of polylactides with all his passion.

Especially I thank Prof. Dr. Dr. H.-F. Zeilhofer for his kind attendance submitting my dissertation to the Medical Faculty of the University of Basel and helping to set the final improvements.

Moreover I thank a lot to the team from the histology laboratory and from the library at the AO-Institute; they gave me very fundamental support during this work.

Finally but all the more I want to express my gratitude to Prof. Dr. E. W. Morscher, representing the Margarete and Walter Lichtenstein Foundation, who gave me the needful financial support during my dental studies, permitting me to invest my forces into this project.

Curriculum vitae

Personal data

Name	Dr. med. Andreas Albert Müller
Parents	Dr. med. dent. Rudolf Müller and Doris Müller-Tschan
Date of birth	2nd of May 1975
Place of origin	Basel BS, Oftringen AG, Switzerland
Military service	Medical doctor

Academic course

2004	**Internship:** University Hospital, Basel, Neurosurgery, Surgical intensive care
2000–2003	**Study of dentistry:** University of Zurich Dental student association University of Zurich: President 2001/2
25/01/2001	**Thesis in human medicine:** University of Basel **"**Additional malformations in cleft lip and palate patients"
1994–2000	**Study of medicine:** University of Basel, Switzerland **Practical Trainings:** Hospital Italiano, Buenos Aires: Head and neck surgery University Hospital, Basel: Paediatric surgery, Pathology Academic Hospital, Pretoria: General surgery, ENT, Obstetrics Pitié-Salpêtrière, Paris: Maxillofacial surgery, Anaesthesia Triemli Hospital, Zurich: Internal medicine
10/2000	**United States Medical Licensing Examination:** Step 2

Research

15–19/09/2002	**2nd world cleft congress, Munich, speech:** Multiple malformations among cleft lip and palate patients
22/05/2002	**Swiss Society for Biomaterials, annual meeting, speech:** Early dural reaction to polylactide in cranial defects of rabbits
2001/2002	**AO-Institute, cooperation in the maxillo-facial research group:** Dural reaction to polylactide implants (Prof. Dr. Dr. B. Rahn)

School

1991–1994	**Senior high school, Basel:** Matura in maths and natural science
1987–1991	**Junior high school, Allschwil**