*ORIGINAL ARTICLE*

# Reconstruction of Craniofacial Defects with Bone-Marrow-Coated Polycaprolactone Scaffolds — An Animal Study in the Yorkshire Pig*

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**ABSTRACT**

**Background**. Alloplastic materials offer a number of advantages over bone autografts in the reconstruction of craniofacial defects, including lack of donor site morbidity, unlimited quantities of available material and the possibility to conform exactly to the defect. While an ideal bioresorbable material would degrade sufficiently slowly, and have osteoconductive properties to allow replacement and remodelling by osseous tissue, this is seldom observed, with the materials instead being replaced by fibrous tissue. Polycaprolactone (PCL), an FDA-approved bioresorbable polymer, has several properties that might make it suitable for reconstruction of craniofacial defects. The technique of fused deposition modelling (FDM) allows for the fabrication of highly reproducible bioresorbable 3D scaffolds, and the nature of the fully interconnected pore network might enhance vascular ingrowth and osteoconductive properties. It was also hypothesised that coating the scaffolds in bone marrow might enhance bone formation due to the osteoinductive nature of the bone marrow mesenchymal cells. This study aimed to test these hypotheses in the pig model.

**Methods**. Defects measuring 1.5×2.0 centimetres were surgically created in each orbit of ten adult Yorkshire pigs. Silicone casts were made of the defect. These were used to produce a PCL scaffold that fit exactly to the defect. Three days following the first surgery, reconstruction of the defects was performed. The orbits were divided into four groups: Group 1, no reconstruction (control), Group 2, reconstruction with PLLA/PDLA foils, Group 3, reconstruction with PCL scaffolds, and Group 4, reconstruction with PCL scaffolds coated with bone marrow. The results were evaluated after three months by radiologic and histologic analyses.

**Results**. Defects in Group 1 were bridged by fibrous tissue. In Group 2, the defects were bridged by the PLLA/PDLA foil, covered with fibrous tissue, with some new bone formation at the edges. In Groups 3 and 4, the pore network of the scaffold was filled with cellular tissue, with new bone formation at the edges. Defects in Group 4 showed a greater amount of bone formation than Group 3, with bone observed at the middle as well as border zones.

**Conclusion**. Defects reconstructed with PCL scaffolds appear to demonstrate superior bone formation compared to those reconstructed with PLLA/PDLA. Coating of the PCL scaffolds with bone marrow appears to enhance bone formation.

*Keywords:* bone marrow, craniofacial defects, polycaprolactone scaffolds, tissue engineering

## INTRODUCTION

Enophthalmos is a common post-traumatic finding following complex orbital and midface fractures. The primary and secondary reconstruction of the orbits has been widely discussed in the literature.[1-5] The correct reconstruction of the orbital walls is the most important aim of treatment towards achieving a normal anterior projection of the eye with normal function. Bone autograft is currently

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the standard reconstructive material for orbital osteoplasties.

Evidence-based studies have shown that successful craniofacial surgical reconstruction with patient's own bone is the graft material against which all others are measured.[6] Unfortunately, autografted bone is limited in amount, shape and desired morphology. In addition, the use of patient's own bone is associated with donor-site morbidity and graft resorption. When autogenous tissue is not available, or its use is limited because of defect size or shape, a variety of biomaterials are used for orbital reconstruction. Metallic, ceramic, natural and synthetic polymer materials are readily available. All metals, most ceramics, and many polymers are not designed to degrade and resorb, and the potential for replacement by host tissue does not exist. Clinically, foreign body reactions are observed and long-term problems include minimal potential for new bone growth and poor remodelling adjacent to the reconstructed area. A logical solution would be the ready availability of transplants, which require a minimal donor site, which are a perfect match for the defect, are highly biocompatible, and which would be remodelled by the host bone. Although tissue engineers are used to dealing with these requirements, such grafting materials do not yet exist.

However, craniofacial reconstruction via tissue engineering techniques is one of the most promising treatment concepts that is presently being developed.[7] Dean *et al*[8] and Zellin *et al*[9] studied the osteogenic potential of different bone marrow coated scaffold materials in critical size defects. Dean *et al*[8] reported that polymethylmethacrylate (PMMA) discs coated with bone marrow impregnated PLGA foam demonstrated increased bone formation after three and six weeks as compared with non-coated PMMA discs in a rabbit model. However, the authors conclude that the ideal scaffold would be completely degraded and resorbed so that the entire defect can be regenerated by bone.

The shortcomings of the current scaffold fabrication techniques had encouraged Hutmacher *et al*[10,11] to apply a solid-free form fabrication technology, known as Fused Deposition Modeling (FDM) in combination with polycaprolactone (PCL), an FDA-approved bioresorbable polymer. The computer-controlled FDM process allowed the design and fabrication of highly reproducible bioresorbable scaffolds.

The cell culture studies showed that fibroblasts and osteoblast-like cells as well as chondrocytes did proliferate, differentiate, and produce cellular tissue in an entire interconnected three-dimensional PCL matrix. The results from several nude mice experiments revealed that culturing of human osteoblast cells in combination with a bioresorbable PCL scaffold could be used in vivo for intrinsic bone formation.

Studying tissue engineering concepts, which are based on a new scaffold technology, in an immunocompetent model, is essential for the development of engineered human bone autografts. In an attempt to build on the work of Dean *et al*[8] the objective of our study was to evaluate the potential of bone marrow coated polycaprolactone scaffold in regenerating a critical size defect in the orbit of the pig.

## METHODS

### *Scaffold and Bone Defect Preparation*

Scaffold specimens were fabricated using filaments made of custom-made polycaprolactone (Sigma-Alderich, NJ) on a FDM 3D Modeler rapid prototyping system from Stratasy Inc. (Eden Prairie, MN). PCL scaffolds measuring 20×30×2 millimetres with a 65 percent porosity and a lay-down pattern of 0/60/120° were fabricated for the experiment.

A poly (lactide-co-D,L-lactide) 70/30 sheet (Figs. 2a–c) measuring 600×600×0.5 millimetres was compression molded using a heat press (Dr Collin GmbH, Ebersberg Germany) at a temperature of 200°C and a pressure of 5 bars. Intra-operatively, the bioresorbable foils were cut to the exact shape to fit the individual bone defects.

Ten adult Yorkshire pigs were chosen for the animal model because of the similar anatomy of the orbits in comparison to the human orbit.[12] The animals were housed in the animal holding facility at the Department of Experimental Surgery, Singapore General Hospital, for the entire duration of the experiment. Housing and feeding were according to standard animal care protocols. The study was approved by the Animal Welfare Committee of the Singapore General Hospital and was licensed by the National Institute of Health's Guide for Care and Use of Laboratory Animals.

The pigs were premedicated with ketamine, orally intubated after induction with pentobarbitane and

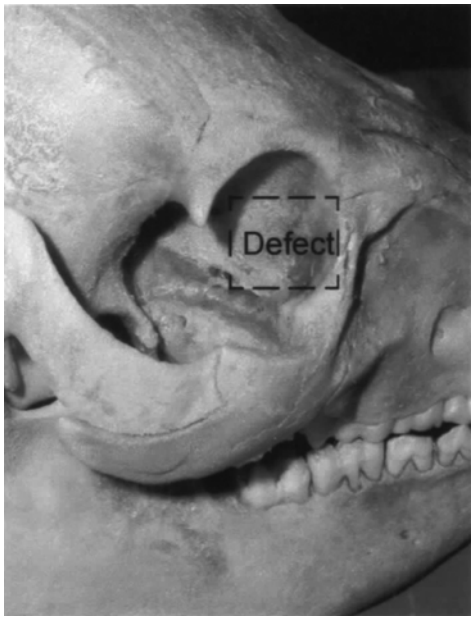


Fig. 1. Skull demonstration with prospected size and shape of the defect.

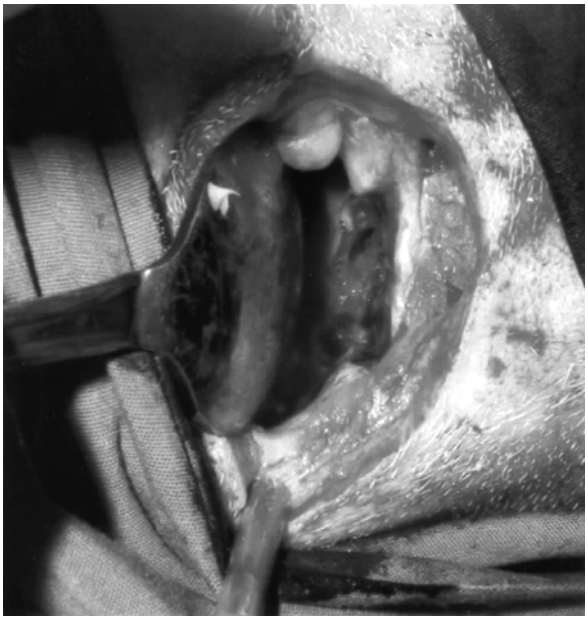

Fig. 2. Defect created at the medial orbital wall with direct visualisation of the paranasal sinus.

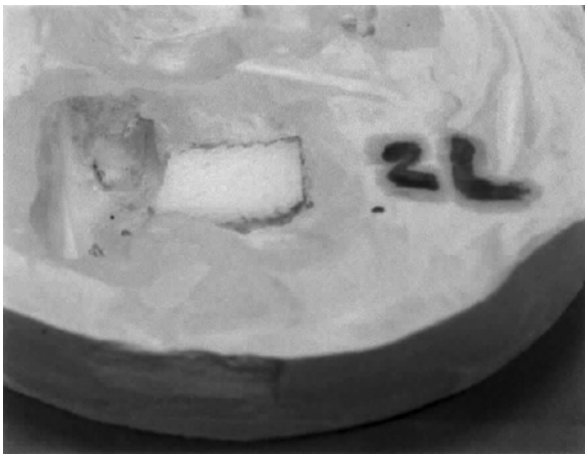


Fig. 3. A mould from dental cement is used to form exactly the shape of the scaffold.

anaesthesia was maintained with 1% halothane. The periorbital region was prepared with 1% centrimide solution and 0.05% chlorhexidine acetate aqueous antiseptic solution. A transconjunctival approach with lateral canthotomy was provided as access to the orbit. A defect of 1.5×2.5 centimetres at the medial wall of both orbits was created (Figs. 1 and 2). The defect was cast with a silicone material (Zerosil Supersoft®, Drewe AG, Germany). The wound was rinsed with 0.9% saline solution and the incision closed with absorbable suture material (Vicryl®, Ethicon).

Post-operatively the silicone cast was used to produce a custom-made mould from dental cement. The PCL scaffold was three-dimensionally formed in heated saline solution (37°C) on the cement mould to fit the geometrically complex bone defect exactly (Fig. 3).

***Reconstruction of Orbital Bone Defect***

Three days after a critical size defect was created, the second phase of surgery was carried out, for the reconstruction of the orbital defect. Four different reconstruction techniques were studied in this project:

Group 1: no reconstruction (control group)

Group 2: reconstruction with PLLA/PDLA foil (0.5 millimetres)

Group 3: reconstruction with PCL scaffold

Group 4: reconstruction with bone marrow-coated PCL scaffold

The technique for the reconstruction was randomised for each orbit and none of the animals had the same technique in both orbits. With the same measures and precautions as in phase 1, the animals were premedicated and anesthetised during the second phase surgery. Group 1 was used as a control group and therefore the defect was not reconstructed. In group 2, the reconstruction with PLLA/PDLA-sheets was made as an onlay technique with fixation of the sheets with a resorbable pin (Resor Pin, Geistlich, Wolhusen,

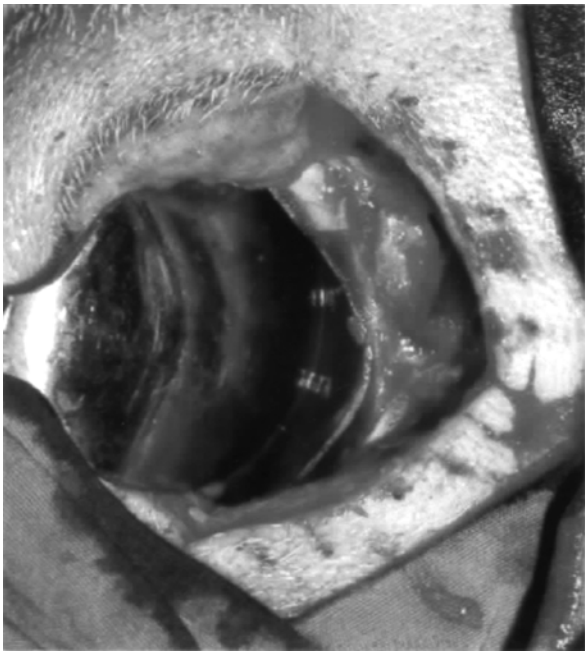

Fig. 4. Orbital wall defect bridged with a PLLA/PDLA sheet, which is fixed to the orbital rim with a resorbable pin.

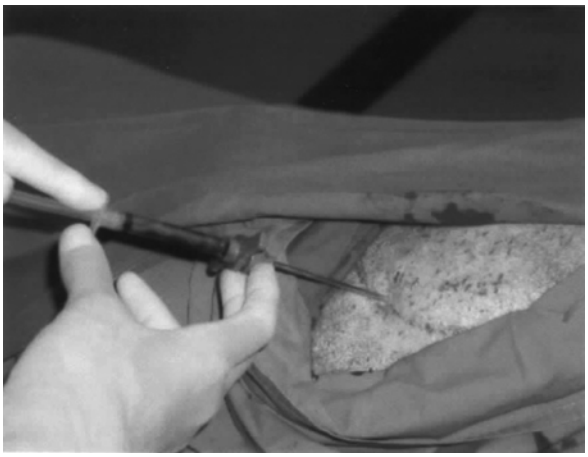

Fig. 6. Harvesting of bone marrow from iliac crest using a trephine needle.

Switzerland). It was the surgical aim that the rather stiff PLLA/PDLA-membrane overlap the edges of the host bone at least for one millimetres at all sites (Fig. 4). In group 3, non-coated PCL scaffolds were placed into the defect without additional fixation (Fig. 5). The PCL scaffolds of group 4 were submerged 30 minutes in heparinised bone marrow (20 millilitres) which was harvested from the iliac crest using a trephine needle under aseptic conditions (Fig. 6).

The wounds were carefully rinsed with 0.9% saline solution and closed with absorbable suture material. All pigs received amoxicillin (Ampicillin®) for five

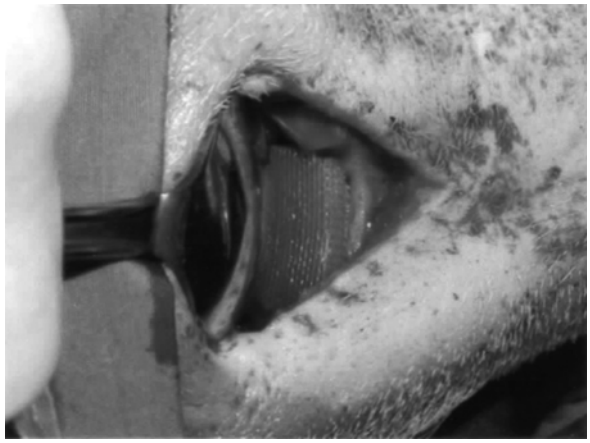

Fig. 5. Orbital defect reconstructed with a PCL scaffold.

consecutive post-operative days. The pigs were sacrificed by an intravenous injection of euthanasia solution after three months. The heads were disarticulated and CT scans in coronal projection were done at the Center for Diagnostic Radiology, National University Hospital of Singapore. Bone sections including the defects were harvested for histological analysis.

*Histology*

The amount of tissue formation and the tissue/tissue interaction between the implant and the surrounding bone were analysed by standard histology. The samples were fixed and stored in 10% neutral buffered formalin until ready for embedding. Standard dehydration in sequentially increasing ethanol solutions to 100% ethanol was performed followed by immersion in Hemo-De paraffin.

Tissue blocks were sectioned and stained for visualisation of cells and extracellular matrix with HE-staining and with Von Kossa Staining and Trichrom staining after Mason-Goldner. The section blocks were prepared and mounted. Using a Leitz 1600 microtome (Leitz, Germany) 10µm thick sections were obtained. The sections were stained with Goldner solution and analysed. Goldner solution stained osteoid and new bone formation red but colours mineralised bone and collagen green. This enabled us to determine whether new bone was laid down at the defect site.

## RESULTS

### *Clinical Findings*

The healing period was uneventful in all the pigs. The defects in group 1 were covered with fibrous

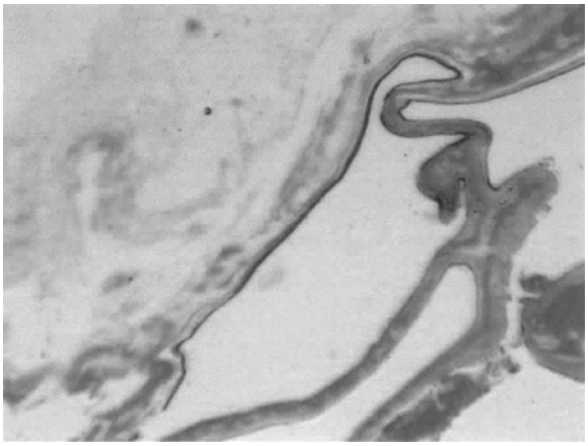

Fig. 7. Histology of a defect that has not been reconstructed. The defect is covered with a thin epithelial soft tissue.

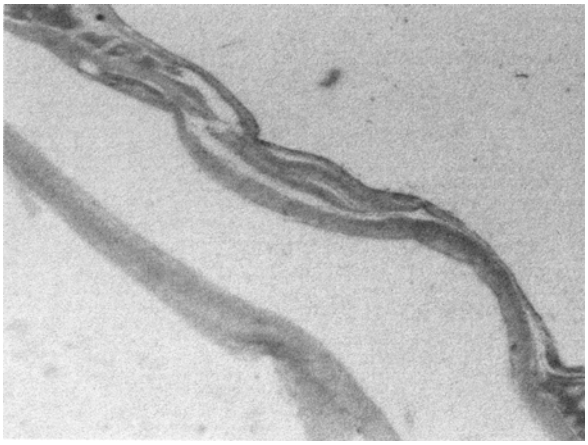

Fig. 8. Defect, which has been reconstructed with a PLLA/PDLA sheet. The sheet has been dissolved during embedding. Around the sheet a thick layer of soft tissue can be seen. There is only little new bone formation at the edges of the bony defect.

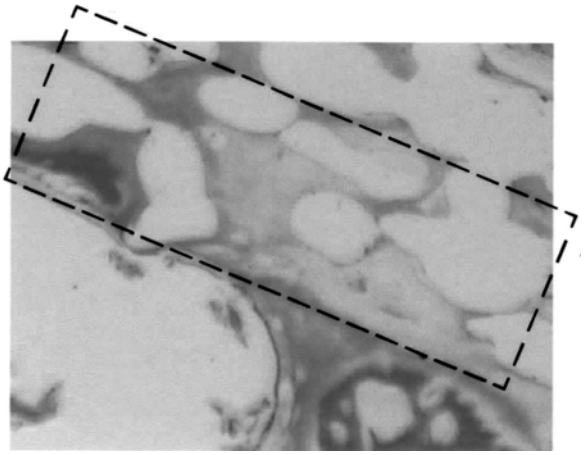

Fig. 9. PCL scaffold has been dissolved during embedding. The round form of the filaments can be seen as white spots. Around these spots the spaces are filled by cellular tissue. From the left side new bone formation starts to invade the scaffold.

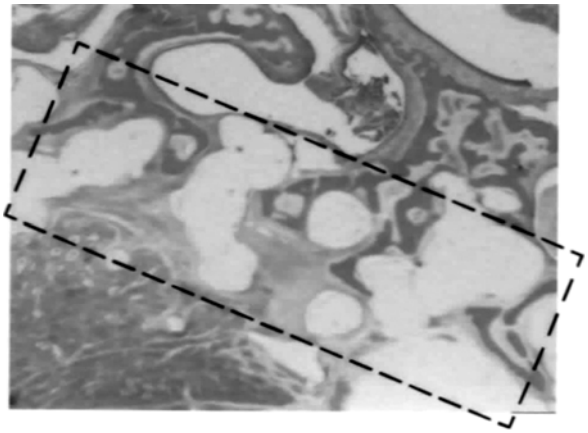

Fig. 10. The bone marrow coated PCL scaffold is half filled with new bone. There is a significant higher amount of remodeling in comparison to the non-coated PCL scaffold.

tissue, which was depressed in the defect. Therefore the orbital volume was increased and the animals showed an enophthalmos. In group 2, the defects were bridged with the PLLA/PDLA sheets, which were covered with fibrous tissue. In groups 3 and 4, the defects were reconstructed with 3D scaffolds. A thin fibrous soft tissue covered the defects. No signs of infection or inflammatory reaction could be detected in all four groups.

### *Histological Findings*

The typical cross-sections of the orbits were done in a coronal plane and included on one side the orbital cavity and on the other side the nasal and antral cavity.

*Group 1 = No Reconstruction (Fig. 7)*

The defect was bridged with fibrous soft tissue. The surface towards the nasal cavity was lined with epithelium. The border zone of the defect showed little signs of new bone formation. There was no inflammatory reaction.

*Group 2 = PLLA/PDLA-sheet (Fig. 8)*

The defect was completely bridged with the PLLA/PDLA sheet, which had completely dissolved during the process of decalcification. The nasal surface was lined with epithelium. The area around the sheet showed a thick fibrous capsule of soft tissue. Within this fibrous tissue some giant cells could be detected surrounding small particles of foreign body material. No significant inflammatory

reaction was present. There was some new bone formation starting from the border zone of the defect.

*Group 3 = Polycaprolactone Scaffold (Fig. 9)*

The entire defect was completely reconstructed with the scaffold. The honeycomb structure of the PCL scaffold was filled by cellular tissue. New bone formation growing into the scaffold could be detected starting from the nasal and paranasal area. At the interface between fibrous tissue and scaffold, some giant cells could be seen. This giant cell reaction was clinically not detected.

*Group 4 = Polycaprolactone Scaffold Coated with Bone Marrow (Fig. 10)*

All the defects were completely reconstructed with the scaffolds. These bone marrow coated scaffolds showed a higher amount of bone remodelling in comparison to group 3. On the one hand, the new formation of bone started from the nasal and paranasal area. On the other hand, there was bone remodelling starting in the middle of the scaffold, which might be based on mesenchymal stem cells from bone marrow. There were some giant cells within the scaffold as well, but without clinically detectable inflammatory signs.

## DISCUSSION

Aesthetic and functional considerations often dictate the use of malleable implant materials for the reconstruction of craniofacial defects.[13-15] In most cases, these three-dimensional shaped implants must also provide immediate structural integrity. A great number of materials are available. These include autogenous bone, cartilage or fascia; non-absorbable implants such as silicone, polyamide, hydroxyapatite and polyethylene; titanium implants; bioresorbable implants such as Vicryl mesh, gelatin films, polylactide and polydioxanone membranes. It is obvious that with this many choices the perfect material for craniofacial and especially orbital reconstruction has yet to be found.

While autogenous bone remains the 'gold standard' in reconstruction of craniofacial defects' disadvantages include limited quantity, a morphology that may not conform perfectly to the defect, and donor site morbidity. Other materials available include metals, ceramics and synthetic polymers. With the non-resorbable materials, there is no potential for replacement by host tissue. With many of the resorbable materials evaluated, limitations include significant foreign body reactions and minimal potential for new bone growth. Merten and Luhr[16] evaluated polydiaxonone (PDS), which is a commonly used resorbable material, for repair of experimentally created orbital wall defects in pigs. They found that these implants were completely absorbed after 29 weeks, replaced by fibrous tissue and covered by mucosa on the sinus side. They concluded that PDS allowed sufficient bridging of defects, but noted that the hydrolytic breakdown products of the implant material produced irritation of the surrounding tissues causing a secondary foreign-body reaction.

Then, what material would best be used to cover an orbital wall defect? As mentioned before, the ideal material should be easy to obtain, available in sufficient quantities, conform to the regional anatomy, easily shaped and rigid enough to withstand compressive forces, osteoinductive and bioresorbable with minimal foreign body reaction.[17] Bioresorbable implants should on the one hand minimise the foreign body reaction and on the other hand allow for new bone remodelling. The degradation and resorption process has to be very slow with a minimal time of six months.[18] Rozema *et al*[19] showed in an animal study with Poly-(L)-Lactide (PLLA) sheets for the reconstruction of orbital floor defects new bone formation at 19 weeks. At 78 weeks of observation, new bone had fully covered the membrane and no inflammation or rejection of the PLLA implant was seen. In another animal study de Roche *et al*[20,21] showed bony new formation in orbital defects reconstructed with Poly-(L/DL)-Lactide (PLLA/PDLA) membranes at four months. The PDS implants in the control group showed significantly less bone formation. The PLLA/PDLA membrane showed an osteoconductive characteristic with bone remodelling along the surface. We could notice the same results in our study. The PLLA/PDLA implants initiated new bone formation at the surface. In contrast, the PCL implants with its fully interconnected architecture were completely filled by cellular tissue and showed new bone formation within the scaffold. The amount of new bone formation was obviously higher in both PCL groups compared to the group with PLLA/PDLA sheets. The bone marrow-coated PCL implants started the bone remodelling within the scaffold. This produced evidence for the osteoinductive characteristic of the mesenchymal cells that have been imported from bone marrow.

Foreign body giant cells could be detected around PCL and PLLA/PDLA implants. However, even with the histological findings of some giant cell formation, there were clinically no signs of inflammatory reaction. Long-term studies might clarify and specify the amount of foreign body reaction, bone formation and degradation of bone marrow coated PCL scaffolds in comparison to non-coated scaffolds and to PLLA/PDLA implants.

Referring to these findings, one could say that the amount of bone remodelling might be increased using isolated pure mesenchymal stem cells instead of full bone marrow. Therefore, the present research group has already started an animal study with PCL implants that are coated with isolated pure mesenchymal stem cells to increase and accelerate new bone formation. These studies might give us the necessary information to start prospective clinical trials for the reconstruction of craniofacial defects.

## CONCLUSION

This study indicated that the use of polycaprolactone scaffolds for the reconstruction of craniofacial defects was superior regarding new formation of bone in comparison to the well-tested PLLA/PDLA material. Furthermore, the coating of the PCL scaffolds with bone marrow seemed to be a promising technique. A combination of this technique with isolated pure mesenchymal stem cells has been initiated in another animal study. However, only long-term observation of degradation and foreign body reaction will give us a conclusive judgment of this material and technique. In any case, the clinically uneventful course showed that in the future polycaprolactone might be an important allogenic material in the field of reconstructive craniofacial surgery.

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