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APPLICATIONS OF CORALLINE HYDROXYAPATITE WITH BIOABSORBABLE CONTAINMENT AND REINFORCEMENT AS BONE GRAFT SUBSTITUTE

An experimental study

by

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Academic dissertation

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ABSTRACT

Hydroxyapatite (HA) is chemically identical to the inorganic matrix of living bones. It can be processed synthetically or by a hydrothermal exchange process from marine coral skeletons. As derived from marine corals, HA preserves the porotic structure which is dependent on the coral species used. As pure material HA is bioactive and non-resorbable, but when it is porous, it is also osteoconductive. In clinical practise HA has been used as bone graft substitute in alveolar ridge augmentation, in filling of bone defects and cysts, and in vertebral arthrodeses. The aim of the present experimental study was to find out if the clinical applications of HA can be improved by using bioabsorbable containment for a particulate form of coralline HA in mandibular contour augmentation or by using bioabsorbable fibre-reinforced coralline blocks in filling bone defects and in anterior lumbar interbody fusion. The studies were conducted with a special reference to the interface of HA and host bone as well as to the connective tissue and the bone ingrowth properties.

A separate curved containment device made of polyglycolide (PGA) was used in the created subperiosteal pocket of the sheep mandibular contour augmentation aiming to keep the particulate HA layer in shape until it was ingrown by connective and bone tissue, compared to the conventional HA augmentation when only the particulate HA was administered into the subperiosteal pocket. In addition, a fast resorbing binding substance for particulate HA, polyglycolide/polylactide (PGA/PLA) copolymer, was used with the curved containment. The studies revealed a higher risk for wound dehiscense and infection when curved containments were used. Radiologically, the use of containment reduced HA migration, but the augmenting effects did not differ significantly. Histologically, the connective tissue ingrowth was more abundant in augmentations with the PGA containment, but, instead, the bone ingrowth was best in conventional augmentation. It is noteworthy, however, that no bone ingrowth was shown in the cases where the polymer composite bound the HA particles together and, related to that, foreign-body type cells were seen in the interface between the HA and alveolar bone.

The coralline HA blocks were reinforced with PGA and poly-dl/l-lactide (PDLLA) fibres to improve their mechanical properties. When implanted in the created metaphyseal or diaphyseal defects of the rabbit tibiae these implants showed rapid bone ingrowth exceeding its final stage already at six weeks. Both PGA and PDLLA fibres induced an inflammatory fibrous reaction around themselves, which did not, however, hinder the bone ingrowth. A significant finding was that the spatial bone ingrowth pattern was directed according to the loading conditions so that the load-carrying cortical ends of the implants as well as the implants sited in the diaphyseal defects were the most ossified, i.e. the bone ingrowth followed Wolff's law.

The reinforced HA implants were further used as stand-alone grafts in lumbar anterior interbody implantation in pigs. The aim was to find out if the HA blocks could maintain the disc space, serve as bone growth conductor, and, finally, accomplish

the fusion. In minipigs synthetic sintered porous HA and reinforced coralline HA blocks were compared. The lowering of the implanted disc spaces and the fracturation and migration of the three different kinds of implants were similar. Histologically, synthetic sintered porous HA implants showed no ossification, and in the reinforced coralline implants, one in the PDLLA- and the other in the PGA-reinforced implant, small islets of new bone formation were shown. Radiologically the grade of ossification was better than histologically, and, when related to the histologic findings, CT was more dependable than the plain films to show ossification of the implanted disc space.

Additionally, a lumbar interbody implantation study was performed in growing pigs using PDLLA-reinforced coralline blocks with up to 16 weeks of follow-up. Implants showed minor displacements but no dislocations and from six weeks onwards they started to fragmentize. While fragmented, the inner structure of the implants was lost, the bone ingrowth was minimal, and the disc was replaced by fibrous connective tissue. Local kyphosis was a frequent finding along with anterior bone bridging and ligament ossification, as a consequence of instability of the implanted segment. The height of the disc spaces was gradually lost similarly to that of the discectomized disc spaces. Conclusively, lumbar interbody implantation after removing the disc and roughening the end-plates did not allow sufficiently stable condition for the fibre-reinforced porous HA implant to be stabilized by tissue and bone ingrowth.

LIST OF ORIGINAL PUBLICATIONS

The present study is based on the following articles that are referred to in the text by their Roman numerals (I to V).

I Ylinen P, Raekallio M, Toivonen T, Vihtonen K, Vainionpää S. Preliminary study of porous hydroxylapatite particle containment with a curved biodegradable implant in sheep mandible. J Oral Maxillofac Surg 49: 1191–1197, 1991

II Ylinen P, Suuronen R, Taurio R, Törmälä P, Rokkanen P. Use of hydroxylapatite/polymer-composite in facial bone augmentation. An experimental study. Int J Oral Maxillofac Surg 31: 405–409, 2002

III Ylinen P. Filling of bone defects with porous hydroxyapatite reinforced with polylactide or polyglycolide fibers. J Mater Sci Mater Med 5: 522–528, 1994

IV Ylinen P, Kinnunen J, Laasonen EM, Lamminen A, Vainionpää S, Raekallio M, Rokkanen P, Törmälä P. Lumbar spine interbody fusion with reinforced hydroxyapatite implants. Arch Orthop Trauma Surg 110: 250–256, 1991

V Ylinen P, Raekallio M, Taurio R, Vihtonen K, Vainionpää S, Partio EK, Törmälä P, Rokkanen P. Coralline hydroxyapatite reinforced with polylactide fibres in lumbar interbody implantation. J Mater Sci Mater Med 16: 325–331, 2005

ABBREVIATIONS

ALIF	anterior lumbar interbody fusion
BCP	biphasic calcium phosphate; mixture of tricalciumphosphate and hydroxyapatite
BMP	bone morphogenetic protein
Ca:P	atomic ratio of calcium to phosphorus
CT	computed tomography
DBM	demineralized bone matrix
FG	fibrin glue
HA	hydroxyapatite or hydroxylapatite
i.m.	intramuscular
IU	international unit
i.v.	intravenous
MRI	magnetic resonance imaging
OTC	oxytetracycline
OP-1	osteogenic protein-1
PDGF	platelet derived growth factor
PDLA	poly-dl-lactide acid or poly-dl-lactide
PDLLA	poly-dl/l-lactide acid or poly-dl/l-lactide
PFC	purified fibrillar collagen
PGA	polyglycolic acid or polyglycolide
PLA	polylactide acid or polylactide
PLIF	posterior lumbar interbody fusion
PLLA	poly-l-lactide acid or poly-l-lactide
PMMA	polymethyl methacrylate
s.c.	subcutaneous, underneath the cutis
SEM	scanning electron microscopy
TCP	tricalciumphosphate
TGF-β	transforming growth factor-beta

DEFINITIONS IN BIOMATERIALS

Most of the terms used in the present study have been accepted by the European Society of Biomaterials to be used within science and applications of biomaterials (Consensus Conference 1986, Williams 1987). Definitions of terms relevant for this thesis are presented as follows.

Graft: a piece of viable tissue or a collection of viable cells transferred from a donor site to a recipient site for the purpose of reconstruction of the recipient site

Allograft: a graft taken from another individual of the same species as the recipient

Autogenous graft (autograft): a graft taken from a source in the individual who receives it

Xenograft: a graft taken from an individual of a different species to the recipient

Bioabsorbtion: the process of removal by cellular activity and/or dissolution of a material in a biological environment, synonym to bioresorption

Bioactive material: a material that has been designed to induce specific biological activity. Capable of bonding with the surrounding bone

Biocompatibility: the ability of a material to perform with an appropriate host response in a specific application

Biodegradation: the gradual breakdown of a material mediated by a specific biological activity

Bioinert: material having no action or reactions with biologic system or elements

Bioresorption: the process of removal by cellular activity and/or dissolution of a material in a biological environment

Biotolerant: material being able to endure without effect or action of any biologic system or element

Bone ingrowth: the term has several synonyms such as bone ongrowth, osseointegration, and biological ingrowth. In the present study the term describes bone tissue found in direct contact with implant material inside the outer boundaries of the implant

Host response: a reaction of a living system to the presence of a material

Implant: a medical device made from one or more biomaterials that is intentionally placed in the body, either totally or partially buried beneath an epithelial surface

Osteoconductive: the ability of an implant to guide or direct bone growth

Osteoinductive: differentiation of uncommitted connective tissue cells into bone-forming cells in the presence of an inductive stimulus

Particulate material: a material having granular structure

Coralline hydroxyapatite: a porous hydroxyapatite material prepared by hydrothermal conversion of the specific marine coral carbonate matrix to the pure hydroxyapatite preserving the unique skeletal structure of the coral, also called replamineform hydroxyapatite

Replamineform: a term for materials, including metals, ceramics, and polymers which have replicated form of the specific invertebrate marine coral skeletal microstructure with specific pore size and complete interconnection of pores

TECHNICAL DEFINITIONS

The technical terms used in the present study are defined as follows.

Load: the external force (F) applied to a material

Shear: the motion of two parallel surfaces relative to each other

Stress: the force per unit area that develops within a body in response to externally applied forces (MPa)

Ultimate strength, Ultimate compressive load: the maximum stress which a material can withstand before failure (MPa)

Compressive stiffness: the relation between applied load and deformation obtained during compressive test, represented by the slope of the load-deformation curve (MPa/m^2)

Energy absorption capacity: energy absorption at the interface before failure determined from the area under the load-deformation curve (integral) until peak load

1. INTRODUCTION

Normal bone consists of organic and inorganic fractions being in continuous interchange with each other for bone-forming and resorbing, i.e. for the remodelling process. The main part of the hard, inorganic structure of the bone is chemically hydroxyapatite $\mathbf{Ca_{10}(PO_4)_6\ (OH)_2}$. In a living organism it forms a crystalline microstructure called whitlockite and allows circulations of body fluids. Synthetically hydroxyapatite (HA) can be processed by using different methods and it has been shown to be a bioinert and bioactive bone substitute. Problems in processing arise with the whitlockite microstructure combined with the mainly porous macrostructure found in the living bone.

The widespread use of HA for applications in bone prosthetics is based on the structural analogies and the biological inertness with the living bone. HA belongs to the apatite minerals which are chemically calcium phosphates. Depending on their atomic Ca:P ratio, different kinds of calcium phosphate ceramics can be synthesized being then also biologically different. Generally, calcium phosphate biomaterials with a capacity to resorb are based on tricalciumphosphates (TCP) characterized by a Ca:P atomic ratio of 1,50. The Ca:P ratio of pure hydroxyapatite, 1,67, clearly exceeds that ratio. Pure HA has been shown to be non-resorbable, or at least the resorption is so slow that it can not be measured reliably.

The structure of HA can be fabricated as dense or porous. The porotic structure allows new bone ingrowth inside the structure, a property that is called osteoconductivity. This was utilized in clinical practice first in the field of facial surgery, when porous HA was used as onlay graft for bone reconstruction. Oral surgeons have long used the particulate form of porous HA to augment an atrophic alveolar ridge and to improve retention of a prosthetic denture. Many other clinical applications of porotic blocks of HA have been considered, but the brittleness of HA is the limiting factor. Studies on the strengthening of porous HA with different kinds of composite materials have been performed in several institutions.

The present study aims at making a better use of the applications of HA by using absorbable covering material for particulate HA containment. In alveolar ridge augmentation, the migration of granules is the main problem. To eliminate migration, a study on curved absorbable implants for HA particle containment in mandibular ridge augmentation was carried out. Likewise, due to their brittleness the use of porous, block form of HA implants is limited to non- or low-weight bearing situations. A special method for reinforcing porous HA blocks with absorbable fibres was developed. Two types of reinforcement fibres were studied using reinforced HA blocks in filling cavities in non-weight-bearing applications in bone defects of the rabbit tibiae. Furthermore, reinforced blocks were tested in demanding, cyclic loading applications in the experimental ventral stand-alone interbody fusions in minipigs and growing pigs.

2. REVIEW OF THE LITERATURE

2.1. HISTORY OF BONE TRANSPLANTATION

Some suggestions exist, that transplants of animal tissues to man have been tried by ancient Egyptians and at the time of Hippocrates. According to the first reliable document, Job van Meekren in Holland was the first to transplant bone from animal to man in 1668, as cited by de Boer (de Boer 1988). He filled a cranial defect in a male with a piece of the dog skull. This xenograft was to be removed later because of the Church instructions. From the late 17th century on there are reports of gunshot fractures successfully splinted with animal bone (de Boer 1988). Attempts in bone grafting from one species to another, i.e. xenografts, were, however, mainly disappointing, mostly due to suppuration and extrusion or sepsis. Originally the word "graft" was defined as "anything inserted into something else so as to become an integral part of the latter" (Stedman's Medical Dictionary 1957). Thus, the first bone transplantations did not fulfil the definition of grafting, because they failed to become an integral part of the organism.

Citing by LeVay (1990), Senn filled bone defects in the late 19th century with chemically decalcified bone. The results were encouraging and raised a question whether it was the revascularized graft or the host bed that contributed to the successful grafting. The use of spongy bone for grafting became popular after the works of Phemister (1914), Gallie (1931), and Matti (1932). Taken from the patient himself, i.e. autogenously, spongy bone was successfully used in filling cavities of benign bone lesions or in artrodeses. Phemister (1931) treated delayed unions or non-unions by simply laying a massive graft as a splint alongside the disturbed shaft and then closing the soft tissues tightly. This is still the method of choice in the treatment of delayed unions in tubular bone fractures. Autogenous cancellous grafting became popular in the treatment of non-unions and malunions during World War II and it was especially used by plastic surgeons in filling facial bone defects.

Obviously autogenous bone was and still is ideal in bone grafting for immunologic reasons. However, harvesting of autogenous bone has proved to have a number of disadvantages: 1) inadequacy of its supply for large defects or needs, 2) risks of postoperative morbidity at the donor site (pain, haemorrhage, infection, nerve damage, cosmetic disability), and 3) disability to have optimally functional shapes of grafted tissues (Lane and Sandhu 1987). For the reasons above, substitutes for autogenous bone were needed and they were and continue to be under development. Allogenous bone grafts with freezing and banking were the solution (Inclan 1942, Wilson 1947, 1951) which is still today mostly used if the need of bone is demanding, like in revision arthroplasties or reconstructions after bone tumor resection (Alho et al. 1989, Aho et al. 1994, 1998). Another approach in bone grafting was the use of "prepared" bone. Among others, Senn's decalcified bone, citing by LeVay (1990), was the first alternative and its successful use seemed to depend on the chemical agent employed. Orell (1934) in Sweden developed "os purum" by removing

chemically all soft tissues from bone in an attempt to obviate its antigenicity. This type of transplant provided a scaffold or a latticework into which new bone could grow having originated from the host bed. To control immune mechanisms preserved calf-bone had its advocates (Basset and Creighton 1962), but both frozen and freeze-dried calf bones showed unsuccessful. "Kiel bone" was deproteinized calf bone and it was found to be rather useful in small cavities (Maatz and Bauermeister 1957). Kiel bone was prepared from the calf bone by washing it in water, then treating with hydrogen peroxide, fat solvents and acetone and sterilizing with gamma radiation. It was very weakly antigenic but possessed no bone inducing properties. The Kiel graft could be fabricated in any desired shape providing also scaffolding. Furthermore, bone ingrowth properties and vascularization were shown to improve by mixing it with red marrow aspirated from the patient to be grafted (Salama and Weissman 1978). The preparations of an inorganic matrix as in "Kiel bone" or "Oswestry bone" (Williams and Irvine 1954) were ambitious attempts to develop a totally purified inorganic bone matrix which is chemically of HA. An inorganic matrix forms a crystalline lattice, which is biologically inert, not at all or very slowly resorbing, and has no inductive properties. Theoretically it is ideal for expanding an inadequate supply of the patients own, autogenic grafts. In the recipient site this requires, however, a well-vascularized bony bed and a number of osteoblasts but in larger cavities its use appeared questionable (Wilppula and Bakalim 1972).

2.2. BIOLOGY OF BONE GRAFTING

Bone grafts, whether autogenous or allogenous, undergo a well-described sequence of histologic events that lead to incorporation. According to De Boer (1988), Barth in Germany in the late 19th century was the first to use the phrase "schleichenden Ersatz" which was then introduced by Phemister into the English literature as the term "creeping substitution" (Phemister 1914). This concept describes bone resorption and a new bone formation process. The authors showed that the transplanted bone was invaded by vascular granulation tissue, causing the transplanted old bone to be resorbed while the host with new bone replaced it. The new bone formation occurred at the site of the transplanted autogenous bone originating from the surrounding mesenchymal cells. The mesenchymal cells differentiated into the osteoblasts to make new bone. The new bone was deposited on the necrotic trabeculae of the graft. The stimulus that the autogenous graft gives to the mesenchymal cells is still not completely understood. It is involved to the concept of osteoinduction, introduced by Urist and McLean (Urist and McLean 1952). Contemporary views of the osteoinduction process are based on the studies of Urist and his co-workers with the chemical mediator that is released from the necrotic bone graft. This mediator is called "bone morphogenetic protein" (BMP). The protein, or a group of proteins, has probably its source in the transforming growth factors-beta (TGF-β). Supposedly, BMP found so far is not the only mediator in the osteoinduction process; intensive research continues on the subject.

In addition to osteoinduction, the bone-forming process preconceives scaffolding in which the viable bone formed by the mesenchymal cells can be replaced in the creeping substitution process. Osteoconduction is the term for the passive scaffolding process of the necrotic graft that makes the creeping substitution possible. Osteoconduction means the ability of a graft or an implant to guide or direct bone growth. It is well established that bone will ingrow into the inert porous system, provided the implant is stabilized or only minimal movement occurs between the implant and host bone (Cameron et al. 1973). In the studies of the bone substitutes it has been shown that the pore size of the scaffold for osteoconduction to occur should be minimally 100 μm (Klawitter and Hulbert 1971), but a pore size greater than 150 μm seems to be required for osteon formation. The osteoconducive pore size is also dependent on the porous material. Klawitter et al. (1976) found bone ingrowth into high-density polyethylene with a pore size of only 40 μm and Bobyn et al. (1980) noticed bone ingrowth into porous surfaced cobalt implants with a pore size range of 50–400 μm. Accordingly, the biology of the bone graft includes both osteoconductive and osteoinductive mechanisms when new bone is produced. Naturally, the bone grafts may also have some bone forming capasity, i.e. osteoprogenitor cells which contribute to bone production or osteogenesis. In the creeping substitution the necrotic bone graft is gradually resorbed and replaced by viable new bone. A review of the current understanding of osteoconduction suggests that this process follows the same principles observed during fracture repair and bone remodelling.

Autogenous bone is still the most advantageous graft material available in most clinical situations. When needed in massive osseous defects like in hip and knee revision surgery or in large-scale posterolateral spinal fusions fresh frozen allogenous bank bone is used more and more alone or in combination with autologous bone. Fresh frozen bone acts also as a scaffold in which the new bone grows in the manner of creeping substitution. But fresh frozen allografts encounter increased risks for transmitting infections and immunologic complications. Through the immunogenicity a slow rejection reaction always prevails leading to some resorption of the graft which may occasionally even totally ruin the result.

In summary, the incorporation of bone grafts, whether autogenic, allogenic, xenogenic, deproteinized or synthetic, occurs by three mechanisms, osteoinduction, osteoconduction, and osteogenesis:
Osteoinduction brings about differentiation of uncommitted connective tissue cells into bone forming cells in the presence of an inductive stimulus.
Osteoconduction means that the graft acts as a scaffold in which the new bone grows by creeping substitution.
Osteogenesis is the formation of new bone by osteogenetic progenitor cells.

2.3. ARTIFICIAL BONE GRAFTS

Biomaterials can be classified according to their chemical composition or biologic interaction. According to their chemical composition the materials are

– **ceramics**
– **metals**
– **polymers or**
– **composites**

According to their biologic interaction, the synthetic materials are classified as

– **biotolerant materials,** being able to endure without effect or harmful action of their biologic environment. Typically these agents evoke a tissue response which encapsulates these materials with a connective tissue layer, e.g. stainless steel and cobolt-crome
– **bioinert materials,** which build up direct contact with surrounding bone. These materials have an oxide layer at their surface, e.g. aluminum, zirconium, titanium, and carbon materials
– **bioactive materials** which build up a direct chemical bond with the surrounding bone, e.g. calcium phosphate ceramics and glass ceramics.

Bioactive materials such as calcium phosphate ceramics fulfil the linguistic criterion for grafting materials: "to become an integral part of the organism". Biotolerant or bioinert materials such as metals, carbon, and most polymers are implants, because, once inserted, they never become an integral part of the organism.

2.3.1. Bioceramics

In material science, ceramics are non-metallic and inorganic materials. Ceramics that are used for biologic implants are of three types: 1) oxide ceramics such as aluminiumoxide Al_2O_3 and zirconiumoxide ZrO_2, 2) calciumphosphate containing glass ceramics and glasses such as silica-based glasses or glass ceramics and pyrolytic carbons, and 3) calcium phosphate ceramics such as tricalciumphosphate TCP, hydroxyapatite HA, and biphasic calcium phosphates (BCP), which are HA/TCP composites (Heimke and Griss 1980, Jarcho 1981, Heimke 1986, Damien and Parsons 1991, Bohner 2001). Bioceramics fulfil the requirements for biologic applications. Their most important property is biocompatibility, which means that they evoke no tissue response and do not show any kind of foreign-body reaction against themselves. Aluminium and zirconium are used as articulating surfaces in the hip and knee joints. Glass ceramics have been studied as bone substitute and surface material for orthopaedic devices. Pyrolytic carbon has been used in artificial heart valves, mostly because of its ability to resist blood clotting, and also in arthroplasties of the small joints (e.g. fingers).

Calcium phosphates are generally considered the materials of choice to be used as artificial bone substitutes. Several calcium phosphate ceramics are biocompatible and most of them are resorbable, dissolving in physiological environment. They have long been used in bone repair, since, structured as porous, they mimic both the bone structure and chemistry. The porous calcium phosphates have been adopted as scaffolds for bone restoration, because they can induce bioactive osteogenesis though they are not osteoinductive. The porous structure invites soft tissues and bone ingrowth into the implant, which is called the osteoconductive property. Cameron et al. (1977) demonstrated that TCP ceramic implants placed on intact living bone cortex did not show any bone ingrowth but were resorbed over time. But when placed subperiosteally and in tight contact with the host bone, bone ingrowth took place. Studies of McDavid et

al. (1979) with the millipore chamber techniques showed that TCP implants formed bone only in the presence of bone marrow, i.e. the implants were not osteoinductive.

Traditional calciumphosphates used in medicine are HA, TCP, and HA/TCP composites (=biphasic calcium phosphates, BCP) (Bohner 2001). Bioceramic interactions in air, water, biologic fluids and their solutions as function of pH and temperature should be known before their clinical use (Lemons 1990). Generally, HA is classified as bioactive and non-resorbable, whereas TCP is classified as bioactive and bioresorbable. The solid and porous forms of TCP and HA bioceramics have been used as bone substitutes, fillers for bone voids or cysts, and carriers for medications (e.g. antibiotics) or bioactive substances (BMP, growth factors). HA coating has also been used in bone implants, especially in joint replacements (Osborn 1987, Geesink et al. 1987,1988). In relation to their biologic use, the most important considerations for usability include chemical analyses, elastic modulus, porosity, microstructure, and atomic structure (Lemons 1990). There have been no reports of the toxicity, hypersensitivity or carcinogenicity of these biomaterials. The main reason for the limited clinical use of ceramic implants is that they are brittle and have low impact and fracture resistance.

The physico-chemical and biological characteristics of calcium phosphate ceramics have been studied and described extensively in the literature (Young 1975, Jarcho et al. 1977 Rejda 1977, Harms and Mausle 1979, Denissen 1980, Denissen et al. 1980, Christoffersen 1981, de With et al. 1981, Winter et al. 1981, Jarcho 1981, Driessen et al. 1982, Flatley et al. 1983, Klein et al. 1983, 1984, Uchida 1984, de Groot 1980, 1983, Patka et al. 1985, Shimazaki and Mooney 1985, Holmes 1979, Holmes et al. 1984,1986, van Blitterswijk et al. 1985, 1986, Sartoriset al. 1986a, 1986b, 1986c, Bucholz et al. 1987, Christel et al. 1989, Damien and Parsons 1991, Bohner 2001). One of the main objects of interest has been the degree and rate of bioresorption of calcium phosphate ceramics, and some controversy and even ignorance still exist on this question. There are two different biologic resorption pathways, the *solution-mediated* process where the material dissolves in physiologic solutions and the *cell-mediated* process where the material resorbs in consequence of cellular activity through phagocytosis (Jarcho 1981). According to the definitions of the biomaterials, both processes can be defined as bioresorption, i.e. the process of removal by cellular activity and/or dissolution of a material in a biological environment. The surface area is one of the major factors in determining the dissolution rates. Generally, dense materials resorb less than chemically identical, but highly porous materials. Furthermore, the solubility of calcium phosphate ceramics in waters and in physiologic solutions is dependent on the atomic Ca:P ratio rather than on the material itself, and, in general, it increases as the Ca:P atomic ratio decreases. Accordingly, pure HA with a Ca:P ratio of 1,67 is not soluble, but different TCPs with higher Ca/P ratios are soluble. The essential characteristics of TCP and HA are described in the following chapters.

2.3.2. Tricalciumphosphate

Tricalciumphosphate (TCP), $\mathbf{Ca_3(PO_4)_2}$ (Fig 1.) is available in two crystallographically different forms, α-TCP and β-TCP.

The crystallographic form of the β-TCP is often called whitlockite. The β-TCP is less soluble in water than the α-TCP (Bohner 2001). Both α- and β-TCP are obtained by thermal treatment or sintering by heating TCP above ~ 700 °C. The TCP fabricated in low (room) temperature is also called calcium-deficient hydroxyapatite and its solubility in water is close to that of β-TCP (Bohner 2001). The low-temperature TCP has chemically and crystallographically a different structure than β-TCP, though the atomic Ca:P ratio is in all TCPs 1,50. The solubility and bioresorption of different TCPs are dependent also on the possible incorporation of foreign ions (i.e. fluoride and magnesium) and on the size of the particle crystals. The smaller the particle size, the greater is the solubility (Bohner 2001).

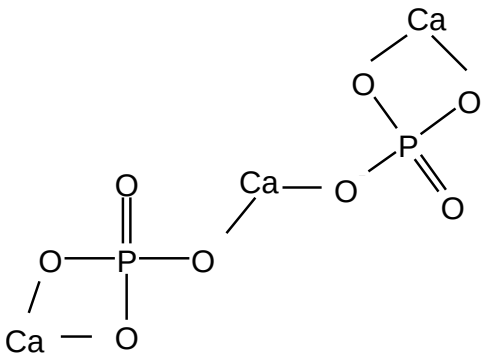

Fig 1. Chemical structure of tricalciumphosphate, $Ca_3(PO_4)_2$

According to a number of studies, ceramic TCP undergoes bioresorption (Ferraro 1979, Hoogendoorn et al. 1984, Jarcho et al. 1977, Jarcho 1981), and dense TCP implants exhibit decreased bioresorption compared to corresponding porous implants. All calcium phosphate ceramics such as TCPs that have a high bioresorption rate have also been shown to exhibit large surface areas. Additionally, it is assumed that large surface areas can adsorb endogenous proteins such as growth factors, from adjacent tissues which indicates that they can theoretically be indirectly osteoinductive and could therefore be considered to be used even as materials for tissue engineering (Bohner 2001).

The TCPs traditionally used in medicine are synthetic-sintered, high-temperature ceramics, mostly β-TCP or β-TCP/HA composites. Due to its fast soluability α-TCP has not been used as bone substitute. The bioresorption of β-TCP takes place typically in the range of 1–2 years (Bohner 2001). In the buffered acid and basic media TCP dissolved 12,3 and 22,3 times faster than HA, respectively. According to Eggli et al. (1988), cell-mediated bioresorption occurs via the osteoclastic activity, i.e. via phagocytosis.

2.3.3. Tricalciumphosphate and biphasic calcium phosphate in experimental use

TCP has been studied alone in its different forms or in combination with HA as biphasic calcium phosphate, BCP. Due to its bioresorption TCP intends to have a positive influence on the local tissue interactions for a short time period to provide an optimal interface and tissue ingrowth for a long-term function. In BCPs the bioresorption rate is directly proportional to the TCP content of that material (Jarcho 1981). At the very early stage, Albee and Morrison (1920) demonstrated even the osteogenicity of TCP, but, stimulation of osteogenicity was seen only on the bone fracture sites, and, in all likelihood, osteoinduction was not the issue.

Shima et al. published in 1979 the first report of using synthetic TCP implants for interbody fusion in the dog cervical spine.

The TCP implants were inserted into the third cervical disc place while the fifth disc place was grafted with an autologous bone graft. After the follow-up of three, six, 12, 18, and 22 weeks the TCP implants were displaced anteriorly or posteriorly in 70 % of the cases. Some compression of the spinal cord due to posterior implant displacement or fibrous connective tissue reaction was seen in 55 % of the cases. Microscopically, no complete interbody fusion was seen with TCP implantation in 22 weeks, whereas fusion was complete when autologous bone was used. The implants were infiltrated by the meshwork of vessels, and only some islands of newly formed bone were seen. Radiologically, half of the implants were crushed at three weeks and all at six weeks. The implants were still seen at 22 weeks surrounded by anterior osteophytes.

In the study of Nasca et al. (1989) TCP was used in augmenting and enhancing lumbar posterior spinal fusion in dogs. The TCP used was the porous particulate form and it was compared to the dense particulate HA and the particulate glass ceramic. The study demonstrated that TCP and HA incorporate within the fusion mass, but a fibrous tissue layer encapsulated the glass ceramic. Posterior fusion occurred only with the addition of autogenous iliac bone. TCP showed little evidence of resorption. The main conclusion was that TCP and HA can be used as filling device but they do not enhance new bone formation.

Steffen et al. (2001) made a vertebral body-filling test with TCP. They plugged the experimentally created bone defect in the lumbar vertebral body in a baboo model using a β-TCP plug and β-TCP plugs impregnated with TGF-β3. In their study all the β-TCP plugs were histologically completely osseointegrated at six months. The plugs loaded with the growth factor TGF-β3 exhibited no incremental benefit. Since then, the authors have used ceramic plugs to fill the defect after harvesting adjacent vertebral bodies for a local autogenous bone graft in anterior fusion surgery.

Steffen et al. (2001) performed a further study using stand-alone titanium cages in the anterior lumbar fusion in sheep. The cages were filled either with autologous bone or β-TCP granules and they were compared to empty cages. At 16 weeks the ossification of the β-TCP group was graded almost as high as that of the autologous bone group, but at 32 weeks autologous bone showed the best results. Histologically new bone was seen invading the β-TCP granules, and good osseointegration was often found inside the cage filled with β-TCP granules. The newly-formed bone was in direct contact with the implant surface inside the cage but, conversely, outside the cage there was a fibrous tissue layer encapsulating the implant as a sign of segmental instability due to stand-alone interbody fusion. In the experimental canine lumbar spine model by Ohyama et al. (2002) the fusion rates inside the cage were 42 % if the autograft was used and 50 % if β-TCP was used. Bone ingrowth areas within the cage were 55 % and 54 %, respectively.

The experimental use of TCP in combination with HA in anterior spinal fusion is reviewed in Chapter 2.6.1.

2.3.4. Tricalciumphosphate and biphasic calciumphosphate in clinical use

The surgical treatment of idiopathic scoliosis and multilevel posterolateral spondylod-

esis requires large quantities of bone graft. Autologous grafting is often insufficient and may additionally increase morbidity while harvesting bone from the pelvis. Due to these problems, osteoconductive ceramics have been used as bone graft extender or alone in clinical situations.

Passuti and co-workers (1989) performed posterolateral spinal fusion in 12 patients using macroporous BCP (60 % HA, 40 % β-TCP, pore size 400–600 μm). The patients had severe neurologic scoliosis or osteogenesis imperfecta. The fusion was performed by placing BCP blocks into the facet-joint articular cavities after removing the inferior articular process and locking it into that position with the Cotrel-Dubousset instrumentation rods. Additionally, in eight cases autologous cancellous bone mixed with BCP blocks (1:1), and, in four cases, only BCP blocks mixed with fibrin glue were used. After 15 months, no differences were observed between the two groups: a) no change in curvature, b) no loosening of hooks or rods, and c), radiologically, continuous bony fusion was observed. The histology revealed new bone formation inside the macropores, and the use of fibrin glue did not seem to modify the bone ingrowth.

The TCP mixed with autograft was compared to allograft mixed with autograft in the treatment of scoliosis in 28 patients (Muschik et al. 2001). The computed tomograms showed after a mean follow-up of 13 months the mineral bone density of mean 430 mg/cm^3 in the TCP group versus 337 mg/cm^3 in the allograft group. Radiologically the segments were fused at six months in both groups.

Successful results using TCP in scoliosis surgery have also been reported by Le Huec et al. (1997), and Ransford et al. (1998). Linovitz and Peppers (2002) performed an anterior lumbar interbody fusion (ALIF) or a posterior lumbar interbody fusion (PLIF) with an allograft bone ring and β-TCP. The fusion was accomplished with supplemental posterior instrumentation, and retrospective evaluation revealed radiologically solid interbody fusion in all patients.

2.4. HYDROXYAPATITE

Hydroxyapatite (HA) is the most used polycrystalline calcium phosphate ceramic mineral as an artificial bone graft substitute. Mature bone consists of 60–70 % calcium phosphates of its dry weight. HA is the main element in the mineral bony substance of the vertebrate's skeleton, where the organic matrix is embedded. The chemical composition of pure HA is $Ca_5(PO_4)_3(OH)$, pentacalsium-hydroxy-triphosphate, but it is usually written $\mathbf{Ca_{10}(PO_4)_6(OH)_2}$ to denote that the crystal unit cell comprises two molecules and its Ca:P atomic ratio is 10:6 = 1,67. Its crystalline structure is hexagonal.

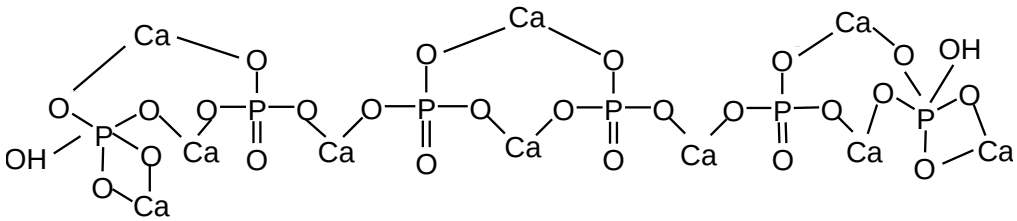

Fig 2. Chemical structure of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$

Apatite as a term refers to mineral compounds, which have a similar three-dimensional structure of constituent ions (e.g. hydroxyapatite, fluorapatite, and chlorapatite) (Young 1975, Posner 1985). The term "apatite" is derived from the Greek word "απαταω" (apatao) which means "to deceive". Probably, the structural similarity of different possible mineral compositions was deceiving or misleading to ancient Greek researches. HA can be processed synthetically or from marine corals with a so-called replamineform process. The synthetic forms of HA have been shown to be chemically and crystallographically similar (Alexander et al. 1987), though they are not identical with the natural HA (LeGeros et al.1988). Several studies have shown that in biologic implantations HA evokes no local or systemic toxicity and no inflammatory or foreign-body reactions (Jarcho 1981). New bone forms a direct contact with HA without any intervening fibrous or inflammatory layer (Tracy and Doremus 1984, Ricci et al. 1986). However, according to Kitsugi et al. (1987), the inflammatory phase after HA implantation causes an invasion of various cells including macrophages which phagocytize dead cells and debris also on the HA surfaces. As HA is identical with biological apatite, it produces a roughened chemical apatite layer enabling the intimate bonding of the host tissue.

Intimate bonding is the main advantage of using HA as bone graft substitute. Another advantage is that HA is not resorbing or the resorbing process is so slow that it is not measurable. Several investigators have reported that HA does not exhibit bioresorption and is inert (Jarcho et al. 1977, Jarcho 1981, Hoogendoorn et al. 1984). However, LeGeros et al. (1988) concluded that all calcium phosphate ceramics including HA bioresorb in varying degrees. Klein et al. (1983) showed that the bioresorption was a function of the microporosity of the ceramic. Micropores are found irregularly distributed within the gross crystalline structure and are frequently localized in the junctions of individual crystals (Rejda et al. 1977). The theory postulates that during the dissolving process some separate crystals, especially at the grain boundaries, become detached or broken-up through micropores and some of these crystals or their fragments are small enough to be phagocytosed by macrophages. Consistent with that theory, Ferraro (1979) found ceramic fragments inside these cells. Even dense HA has shown to degrade slightly, in spite that it does not contain pores.

In the order of solubility in water the most known ceramics are the following:
tetracalcium phosphate ($Ca_4P_2O_9$) >
amorphous calcium phosphate >
α-tricalciumphosphate ($Ca_3(PO_4)_2$) >
β-tricalciumphosphate ($Ca_3(PO_4)_2$) >>
hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$)

Hydroxyapatite is, so far, most unlikely to be dissolved under physiological conditions compared to the other calcium phosphate ceramics. Experimental and clinical applications have also shown that pure HA, in any of its forms, does not have any significant tendency to bioresorb in either dissolving or cell mediated-processes (Nery et al. 1980, Jarcho 1981). Due to its biochemical stability, HA has widely replaced other calcium phosphate ceramics including tricalciumphospate.

2.4.1. Preparation of hydroxyapatite

Studies on preparing calcium phosphates as dense and porous forms for bone substituting applications started in the early 1970's. HA has been the most widely investigated material. Both dense and porous forms can be manufactured as blocks of different sizes or in the granular form. The mean pore size and porous volume in blocks or granules are dependent on the techniques used in the production and, naturally, on the intended applications of the HA ceramic.

2.4.1.1. Synthetic hydroxyapatite

Initially the high-pressure powder compaction technique was used in producing dense HA. In that method HA powder is first pressured into a distinct shape by either the uniaxial or isostatic compression method (Jarcho 1981). In uniaxial compaction the pressure is applied on one axis and in isostatic compaction on all directions. The pressures used at this stage are up to 80 MPa. After the compression the powder exceeds the so-called "green state" which is then sintered in high temperatures, usually 1200–1300°C to produce dence HA material. The sintering process fuses individual HA crystals together in their grain boundaries. Sintering can also be accomplished in one step by pressing and heating the HA powder simultaneously (hot pressing). In another method the pure HA is first in an aqueous solution forming HA clay which is then dried and sintered (Jarcho 1981).

The synthetic method to prepare porous HA is based on the studies of Klawitter and Hulbert (1971). Appropriately-sized naphthalene particles are mixed within the HA powder and the mixture is compressed in the uniaxial or isostatic manner to exceed the "green state". In that state the naphthalene particles are removed by sublimation, and the porous green state is produced. The integrity of the individual crystals is produced by sintering the porous green state. Hydrogen peroxide solution (0,3 to 1%) can also be used instead of naphthalene (Rejda 1977) to produce a porous structure.

2.4.1.2. Coralline hydroxyapatite

Implant materials derived from the mineral content of marine corals are generally called coralline materials. A replicated life-form process to produce "replamineform" ceramics from the coral skeleton was developed by Roy and Linnehan 1974. The issue is a hydrothermal exchange process to produce calcium phosphate replicas of marine coral structures by heating and pressuring corals in the presence of an aqueous phosphate solution. A phosphate solution is needed to replace calcium carbonate by calcium phosphate in the specific skeletal coral structure. The mean pore size and porous volume are dependent on the coral species used. In the original technique, sections of coral together with weighed quantities of reactants and water were sealed in a gold tube, heated in a specified temperature and pressure for a period from 12 hours to one week (Roy and Linnehan 1974, White et al. 1975). The exchange reaction with the marine coral species of *Porites* is as follows:

$$10CaCO_3 + 6(NH_4)_2HPO_4 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6(NH_4)_2CO_3 + 4H_2CO_3$$

where *Porites* calciumcarbonite (aragonite) is replaced by hydroxyapatite, and, accord-

ing to the microscopical and X-ray diffraction studies by Roy &Linnehan's (1974) the skeletal structure is preserved and the Ca:P ratio tends to be 1,67 being identical with that of pure synthetic hydroxyapatite. The coral species with a magnesium-rich skeletal structure has been shown to build up a whitlockite crystalline structure (TCP) under the same hydrothermal exchange reaction. The rationale for using natural corals as original structure is that they mimic better the structure of the human cancellous or cortical bone and, in all likelihood, allow better circulation of body fluids and increase the potential for tissue and bone ingrowth. A limited number of stony corals show such an interconnected porous structure and minimally a 100 µm pore diameter to be useful for manufacturing bone substitutes. The most used species meeting the above requirements are *Porites* and *Goniopora*. Both materials contain longitudinal channels with transverse pores allowing interconnections between the channels, i.e. open porosity. Their structure resembles that of trabecular bone in contrast to some other coral species such as *Favites* or *Lobophyllia* the skeletal structure of which resembles compact diaphyseal bone, as a dense outer wall surrounds the inner septa, i.e. the porosity is closed (Guillemin et al. 1987). In *Porites*, the channel walls are approxymately 95 µm thick, the channel diameters are approximately 190 µm, and the transverse pores are about 190 µm in diameter. In *Goniopora*, the channel walls are about 130 µm thick, the channel diameters about 600 µm, and the transverse pores approxymately 130 µm in diameter (Tencer et al. 1988). Most products available in the market are based either on *Porites* with a mean pore diameter of 200 µm and porosity of 50 % or on *Goniopora* with a mean pore diameter of 500 µm and porosity of 65 % (e.g. Interpore 200™ and Interpore 500™, Interpore International, Irvine, CA, USA).

2.4.1.3. Natural coral

Natural coral is also used for bone substitute. Coral directly obtained from the sea is cleaned of all organic material and then sterilized and manufactured as blocks and in a granular form (Biocoral™) (Guillemin et al. 1987, Shors 1999). Calciumcarbonite in its skeletal structure is mainly preserved, making it highly resorbable. The resorption is brought about by osteoclasts observed histologically in contact with coral. Also the enzymatic process via carbonic anhydrate dissolves the carbonated coral skeleton. The term *natural coral* is to be distinguished from *coralline* materials in which calcium carbonate is converted to hydroxyapatite through a replamineform process.

2.4.2. Bone bonding of hydroxyapatite

HA, either as porous or dense implants or in a granular form, has shown to become directly bonded to the surrounding bone. Direct bonding includes a lack of local inflammatory, foreign-body or local toxicity reactions. As a consequence, no intervening fibrous tissue between the implant and bone is developed. This direct bonding is often called a "chemical bonding", "bone-bonding" or "natural bone cementing" mechanism (Jarcho 1981). In fact, it applies to all calcium phosphate implant materials. This bone cementing mechanism has been investigated in in vitro chemical, histochemical, and electron microscopic studies (Jarcho et al. 1977). The studies have given evidence

of the existence of a bonding zone that is a narrow 3–5 μm wide amorphous band with no structural details located between the surfaces of HA and the host cells. According to one explanation, a chemical exchange of ions takes place between the natural bone and HA ceramic, most of the ions being calcium and phosphate ions from both sites. Ion exchange is part of the solid-solution equilibrium which ultimately calcifies forming mineral crystals depositing on the surface of HA (Jarcho et al. 1977, Ogiso et al. 1980, Daculsi et al. 1990). This is also supported by the findings that Ca:P ratio measured with the electron probe microanalysis technique around the implants increases during six months from 1,50 to about 1,67, the last ratio corresponding that found in the normal bone mineral around the implant. The study by Jarcho (1981) of the calcium accumulation in the body with the use of radio-labeled (Ca^{45}) ceramic implants showed that implant-derived calcium accumulates locally in the body pool (Jarcho 1981). However, this was not verified by the study of Christel et al. (1989) who found no detectable radio-labeled Ca^{45} in the newly formed bone in the implanted areas. According to all findings detected in the zone of bone bonding, the bonding zone appears to be similar to the acellular bone matrix derived from differentiating osteoblasts. Another electron microscopic study revealed a direct chemical bonding between bone and HA without any unmineralized tissue layer at the interface (Tracy and Doremus 1984).

The conclusion of the interface studies of HA is that HA is not considered a foreign material but rather a normal bone component due to its chemical resemblance to bone, and, consequently, bone grows directly onto the surface of HA.

2.4.3. Mechanical properties of hydroxyapatite

The crucial question in the use of calcium phosphates for bone grafting is what kind of porosity of the implant is the most effective to promote ingrowth and yet strong enough to resist compressive stresses found in the place to be grafted. It is known that the ability for bone ingrowth increases and the compressive strength decreases when the porosity of the ceramic is increased. Porous ceramic has good ingrowth properties but may fracture. Dense implants remain intact but may be surrounded by fibrous tissue (Toth et al. 1995).

The mechanics of coralline materials is essential for their clinical applications. Due to their porous structure, the mechanical properties of coralline materials resemble more the properties of cancellous than cortical bone. In Table 1 the compressive and tensile strengths and the Young modulus for bone and calcium phosphate materials are presented according to Jarcho (1981), Holmes et al. (1984), and Shors (1999).

Table 1. The mechanical properties of bone and calcium phosphate implant materials. According to Jarcho 1981, Holmes et al. 1984, and Shors 1999.

Material	Compressive strength (MPa)	Compressive stiffness (MPa/m^2)	Young modulus (MPa)
Cortical bone	138*–170**	10	14
Cancellous bone	7,5**–41*	0,8	–
Porous calcium phosphates	7–69*	0,36	–
Dense calcium phosphates	207–896*	10–28	35–103
Coralline HA 200	10 (6–12)	0,8	–
– load parallel to channel axis			
Coralline HA 500	4 (2–6)	0,6	–
– load parallel to channel axis			

*Jarcho 1981
**Holmes et al. 1984

Both the total porosity and the pore size influence the mechanical strength. Compressive strength decreases with increased porosity of HA implants, and it has also shown that the resistance of implants is dependent on the dimensions of the pores (Le Huec et al. 1995). Furthermore, the proportions of macropores (> 100 μm) and micropores (< 10 μm) in the total porosity of implants have an influence on the compressive resistance; in each glass of pore, implants with the same porosity but a greater proportion of micropores are more resistant to compression. In spite of porosity, the ultimate strength and the energy-absorption capacity depend upon the orientation of HA implant channels in relation to the applied load, i.e. the channel axis-load axis angle (Tencer et al. 1985, 1988, Bucholz et al. 1987). The maximum compressive strength is achieved with a channel axis-load axis angle of 0°, and the minimum values are measured at the orientation of 90°. In coralline HA implants the maximum compressive strength is only approximately half of that of cancellous bone, but after bone ingrowth for six months the implants may exhibit values even three times greater than those of cancellous bone (Bucholz et al. 1987).

To conclude, it is desirable to minimize the porosity or size of pores to increase the strength of HA implants, but the porosity should be optimized with bone ingrowth properties. In the optimal implanting of HA the orientation of its porosity should be parallel with that of the host bone.

2.4.4. Hydroxyapatite composites

Combinations of porous HA with synthetic polymers or metallic agents are called HA composites. They have been developed and studied in purpose to improve the mechanical properties of porous HA, or vice versa, to develop partially biodegradable artificial bone grafts for tissue engineering. The most common composites are those with biode-

gradable polymers such as lactide or glycolide polymers. (Higashi et al. 1986, Cehreli et al. 2003, Ishii et al. 2003). Also combinations of HA and TCP with different types of collagen derived from living organisms have been studied (Iwano et al. 1991). Theoretically, biodegradable grafts replaced gradually by bone can be used even to guide or direct bone or tissue ingrowth, i.e. tissue engineering. HA composites can also be used as drug carriers, e.g. as bone growth stimulating factors.

The idea in synthesizing composites from biodegradable polymers and HA aims at producing biodegradable artificial bone which is gradually degraded and replaced by new bone. Higashi et al. (1986) studied polymer-hydroxyapatite composites as biodegradable bone fillers. They used polydl-lactide acid (PDLA) and incorporated it with HA by hot-pressing with HA powder, through polymerization or by melting and mixing. Experimental studies in the rat femur showed that after PDLA was degraded new bone was grown into direct contact with HA. HA had an important role in new bone formation, because in cases of implanting pure PDLA no bone ingrowth was seen. There was, however, some resorption of HA and it was faster when the HA composite was made with low-molecular-weight PDLA (oligomer) compared to high-molecular-weight PDLA (polymer). HA resorption was based on local acidity brought about by lactide acid. The HA used in this study was synthetic, sintered, and granular with a particle size of 44–155 μm.

As a conclusion, studies on HA composites show that bone ingrowth is not hindered by a combination of biodegradable lactides with HA.

2.4.5. Reinforcement of porous hydroxyapatite

The mechanical strength of all porous HA materials is low resulting from both the large pore size and the natural brittleness of the material (Holmes et al. 1983, Tencer et al. 1985, Bucholz et al. 1987, Tencer et al. 1988, Le Huec et al. 1995). When using these materials as defect fillers they must be protected from load-bearing or their mechanical properties should be improved. To reinforce HA, different coating schemes have been suggested. The porous implants can be coated externally or internally. In external coating the principal prerequisite for bone ingrowth may be lost if the coating forms a barrier for tissue ingrowth. Internal coating may alter the surface chemistry of the internal surfaces (Tencer et al. 1985).

Tencer et al. (1985) studied PDLA and polymethyl metacrylate (PMMA) for external and internal coatings in coral-based HA in one-centimetre cubes. External coating may be created on several, but not all surfaces. In their study, Tencer et al. (1985), carried out external coating on three surfaces using either PDLA or PMMA. In the mechanical testing the ultimate compressive load only in the PMMA externally coated specimen was significantly higher compared to that in cancellous bone. In the PMMA internally coated specimen the compressive strength was 2,1 times higher than that of cancellous bone, but the difference was not significant. In the PDLA externally and internally coated specimen the compressive strength was nearly equivalent to that of the cancellous graft. Compressive stiffness (the slope of the deformation curve under compressive force) in the PDLA-coated HA cubes was also approximately of the same magnitude

as in the cancellous grafts. The energy absorption capacity (area under the load-deformation curve), however, was much lower in all coated specimens compared to cancellous bone.

In their other survey, Tencer et al. manufactured coralline HA composite cylinders with an internal PDLA coating to study the compressive and bone ingrowth properties (Tencer et al. 1988). Replamineform HA blocks (pore size 600 μm) were shaped into cylinders which were then dip-coated in PDLA solution in three different solution ratios (at ratios of 30:1, 10:1, and 3:1, acetone/PLA by weight) to produce thin, medium, and thick coatings. The ultimate compressive load was studied in the thin-coated (30:1) HA composite cylinders which showed that their compressive strength was approximately two times greater than that of the uncoated HA cylinders but no better than that of canine cancellous bone. The compressive stiffness was also approximately the same as in cancellous bone, but the energy absorption capacity was significantly lower than that of cancellous bone. Furthermore, the directionality of the microarchitecture of the implants was evident in their mechanical properties. The mechanical tests demonstrated that the compressive strength is highly dependent on the orientation of the longitudinal interconnecting channels inside the HA implant in relation to the compressive force. The best results were achieved when the channel axis orientation was about 0°.

In spite of mechanical testing, Tencer et al. (1988) implanted the PDLA-coated and uncoated cylinders into the rabbit proximal tibiae, inside the anteromedial cortex. Mechanical testing for interfacial shear stress and histomorphometric analyses for bone ingrowth were performed after the follow-up of three, 12, and 24 weeks. The interface shear stresses were identical at three weeks, but at 12 weeks the shear stress of the uncoated HA cylinders increased more than those of the coated cylinders, and the difference was significant among the more thickly (solution ratios 3:1 and 10:1) coated specimens. However, at 24 weeks, identical interface shear stresses were featured in all coated and uncoated implants. Histologically, bone ingrowth was seen in all coated implants, but in some regions the interposing polymer layer was seen between bone and the implant. Histomorphometrically, the average area fractions of bone ingrowth (the area of bone in the total available area for bone ingrowth) were 68-70 % at three weeks for thickly coated specimens, whereas in uncoated or thin coated specimens the values were significantly higher, approximately 82 %. In 24 weeks the ingrowth values were 86–90 % and 93 %, respectively.

Iwano et al. (1991) coated HA with collagen using synthetic HA with a mean pore diameter of 280 μm and with a porosity of 85 %. The HA blocks were infiltrated in type 1 collagen solution so that the porous surfaces were covered by a film of collagen. The ultimate compressive strengths of pure HA- and collagen-coated HA blocks sized 3×4×5 mm were mean 20,6 MPa and 88,2 MPa, respectively; thus the compressive strength of collagen-coated HA was 4,3 times or significantly higher than that of HA. The histological studies using a rabbit femoral metaphyseal defect model showed that bone ingrowth occurred more slowly into collagen-coated implants, but it was equalized with pure HA implants at 12 weeks. Accordingly, the number on multinucleated giant cells in collagen coated implants was

significantly higher at the beginning of the follow-up at four weeks, but no significant difference was seen at 12 weeks.

In summary, by coating the external and internal surfaces of porous HA with PDLA, a significant improvement in compressive strength and stiffness was achieved, and the values were comparable to those of cancellous bone. After cortical implantation of PDLA-coated implants their interfacial shear strengths were significantly lower at 12 weeks than those of uncoated implants, but, at 24 weeks, there were no significant differences in the shear stress between the specimens. The PDLA internal coating of porous HA delays the bone ingrowth, and new bone area fractions were about 10 % lower at three weeks and 5 % lower at 24 weeks compared to uncoated implants. Likewise, collagen coating delays bone ingrowth in synthetic porous HA, but bone ingrowth was equalized in 12 weeks. Both types of coatings, either PDLA or collagen, seem to increase the compressive strength of porous HA approximately four-fold which correspond to the strength of cancellous bone. Furthermore, porous HA increases its strength, as bony penetration progresses after implantation (Piecuch et al. 1984, Sartoris et al. 1986b).

2.5. HYDROXYAPATITE IN CAVITARY AND SEGMENTAL BONE DEFECTS

2.5.1. Experimental studies

Successful experimental use of synthetic or replamineform HA as defect filler in the dog and rabbit tibiae and femora has been reported by Chiroff et al. (1975), Holmes (1979), Hoogendoorn et al. (1984) and Renooij et al. (1985), Holmes et al. (1986), Bucholz et al. (1987), Eggli et al. (1988), Kühne et al. (1994), Pollick et al. (1995), and in articular cartilage defects by Chiroff and White (1977).

Chiroff et al. 1975 made a preliminary study in implanting coralline HA derived from genus *Porites* (pores mean 200 μm) in the distal femur or proximal tibiae in dogs. The finding was that the replamineform implants were all thoroughly ingrown with new and mineralized bone, continuous with the trabeculae of the host cancellous bone. Furthermore, no migration and no encapsulation of the HA implants were observed. The concept of "biological fixation" of coralline HA in the living bone was established. In their further study Chiroff and White (1977) made experimental articular cartilage defects and placed porous HA implants immediately beneath the damaged articular cartilage. What they found was that HA implants enhanced and facilitated regenerative healing also of the hyaline cartilage from the margins of the defect in contrary to aluminium (Al_2O_3) and titanium (TiO_2) implants as well as unimplanted defects where the articular surfaces were reconstituted by fibrocartilage.

A decade later, Bucholz et al. (1987) used *Goniopora*-derived coralline HA with a pore size of 600 μm similarly in the dog tibiae and compared HA with an autologous bone graft. The histomorphometric quantitation of bone demonstrated a bone volume fraction of 13 %, an implant fraction of 35 %, and a soft tissue fraction of 52 %. The number of implant and bone fractions was identical to that of bone in the normal leg, and, thus the fraction of ingrown bone alone was less than the fraction of bone in the normal leg.

Hoogendoorn et al. (1984) studied the tissue compatibility and biodegradation of synthetic porous HA blocks in cortical segmental defects of the dog femora with a follow-up period of up to 3,5 years. The average pore size of the implants was 400 μm and the porosity 56 %. Histologically bone apposition was seen at 20 weeks and its maximum level was achieved at 40 weeks. The maximum bone fraction achieved was 18 % of the cross sectional area. No reduction of HA mass could be observed, i.e. biodegradation was negligible. The authors also verified that bone ingrowth was significantly higher on areas where the extension of the bone-HA contact was largest. They concluded that bone ingrowth depends on the extension of bone-HA contact and the material itself does not induce osteogenesis.

Renooij et al. (1985) used the same implants in the experimental femoral defect model as did Hoogendoorn et al. (1984) and showed that by labelling the implants with radioactive strontium-85 analogs of the calcium phosphate compounds the bioresorption process can be followed. Measuring the radioactivity over the implant sites a clear decline in the radioactivity of strontium-labeled tricalciumphosphate implants was seen, but the radioactivity in the HA implants remained unchanged. In the microradiographic histologic studies a resorption of tricalciumphosphate was also elucidated, and the process was cell-mediated with mononuclear phagocytes or multinuclear osteoclast-like cells carrying ceramic debris inside the cell interior. The HA implants were not affected by the resorption process.

In a preliminary study of Holmes (1979) a reconstruction of a two-centimetre segmental gap in the dog mandible was made with coralline HA with a mean porosity of 200 μm. The author used the scanning electron microscope (SEM) analysis and found that at six months 88 % of the implant area was filled with tissue having a density of that of bone, but the one-year analysis revealed a 29 % loss of the implant area. The author concluded that HA started to biodegrade after the bone had regenerated. In their further study, Holmes et al. (1986) used one-cubic-centimetre coralline HA blocks with a pore diameter of average 600 μm in the proximal tibial metaphyses of dogs. The histologic findings were the direct new bone apposition to the trabeculae of HA and no evidence of chronic inflammation or fibrous encapsulation. In the histomorphometric measurements they found that the bone volume fraction ranged from 8 % at one month to 17 % at one year. Soft tissue and HA fractions at one year were 49 % and 34 %, respectively. Bone was homogenously distributed inside the implant, and no apparent evidence of HA biodegradation was observed. These results were confirmed by Sartoris et al. (1986a) in the identical dog metaphyseal defect model. In their study a 17 % volume fraction of new bone at one year was measured. The implant volume fraction was maintained through the one-year follow-up, and no correlation was observed between the radiographic transmission density and the volume fraction of the implant material. Hence, the primary observation of HA biodegradation by Holmes (1979) remains obscure, since it has not been confirmed by others and it may be related to the inaccuracy in the SEM analysis.

Eggli et al. (1988) made a study comparing HA and TCP with a pore size range of 50–100 μm and 200–400 μm. Synthetic HA and TCP (Geros 80™, Geros 82™) cylinders 3 mm in diameter were implanted in the

distal femora and proximal tibiae in rabbits. Due to implant resorption, less than one sixth of the originally implanted TCP with small pores and one third of TCP with large pores persisted after six weeks. In both HA implants, in contrary to TCP, no or insignificant resorption was detected. Bone ingrowth was more rapid and more voluminous both in TCP and HA with smaller pores, and the rate of degradation of TCP was related to that of tissue penetration. In the synthetic HA, numerous pore interconnections were found only in the HA with a smaller pore size, while there were practically no interconnections in the material with a larger pore size. The authors considered the pore interconnections to be the decisive precondition for bone ingrowth.

The results of Kühne et al. (1994) contradict the preceding findings of Eggli et al. (1988). They compared bone formation in HA implants of two pore sizes with bone allografts and with spontaneous bone repair of empty cavities. Coralline HA cylinders with pore sizes of 200 µm (HA 200) and 500 µm (HA 500) as well as allografts were implanted in the rabbit lateral femoral condyles, and empty holes served as controls. The cavities which were filled with allograft bone and those which were left empty showed nearly complete osseous integration with new bone already at 12 weeks. In the HA 500 implants new bone was formed slowly, but the entire implant was filled in 26 weeks. Instead, the HA 200 implants showed no new bone formation or remodelling even at 26 weeks, and the implants were delineated from the surrounding host bone. According to Kühne et al. (1994), particular attention must be paid not only to the size of the pores, but also to their interconnections. The HA 500 featuring an average interconnecting pore size of 260 µm (Holmes et al. 1986) has apparently distinct advantages over the HA 200 material with interconnections of only a few microns. Moreover, Shimazaki and Mooney (1985) have shown that larger pore sizes of coral-derived HA are associated with better bone ingrowth compared to smaller pore sizes.

Experimental studies in the canine metaphyseal defect model by Sartoris et al. (1986b) showed the HA 500 to be even better than the autogenous bone graft regarding the radiographic and mechanical parameters. They found a greater percentual increase in the radiographic density with bone incorporation in coralline HA material compared to autogenic grafts. Following incorporation of the coralline HA, the compressive strength was better than that of the autograft, but the compressive load-bearing capacity was highly dependent on the channel orientation within HA implants. The maximum compressive strength could be achieved when the implant channel axis was parallel to the direction of the stress. The mechanical stiffness of implants with osseous ingrowth was somewhat lower than that of incorporated autografts. Martin et al. (1989) reported rapid and good bone ingrowth in HA 500 implants in canine cancellous defects, but they found this material unsuitable for cortical defects, due to its low mechanical strength. Ripamonti (1991) made heterotopic implantation of HA 500 and found even new bone formation, indicating some biologic activity of this material. True induction of bone has been studied by combining HA with an osteogenic protein (Miller et al. 1991, Ripamonti et al. 1992).

Coralline HA in segmental diaphyseal cortical defects was studied by Sartoris et al. (1986c, 1987) using a canine diaphyseal

defect model and *Porites*-derived coralline HA 200. Surgically created segmental distal radius defects of two centimetre were replaced by an HA 200 implant or a cortico-cancellous autograft associated with internal fixation. The union rate and failures were evaluated radiographically. There was a 57 % incidence of failed union among the coralline implants. The reasons for failure were implant fractures, instability and/or incomplete bone ingrowth. According to their study, coralline HA implants did not appear as good as autograft bone in the management of diaphyseal defects.

As a conclusion of experimental studies, HA is shown to be bioinert and bioactive favouring bony ingrowth by osteoconduction. Bone ingrowth is dependent on the physical properties of the HA material, especially on the pore size, and their interconnectivity. In HA derived from corals a larger pore size seems essential when expecting bone ingrowth, and in that respect *Goniopora*-derived HA 500 shows distinct advantages over *Porites*-derived HA 200. There might be some degradability of HA which is under dispute and is not completely understood. In the degradation process the role of micropores, the detachment of grain from surfaces, and the possible role of macrophage-type cells are under discussion (Winter et al. 1981, Klein et al. 1983, Holmes et al. 1984, Hoogendoorn et al. 1984, van Blitterswijk et al. 1985, Higashi et al. 1986). Finally, the most important conclusions from the experimental implantation studies are the three prerequisites for the successful use of synthetic and coralline HA in bone defects: rigid stabilization of bone fragments in relation to HA, direct apposition of HA to surrounding bone, and viability of host bone in the proximity of HA. These criteria are called *the triad of osteoconduction*, consisting of stability, proximity, and viability (Shors 1999).

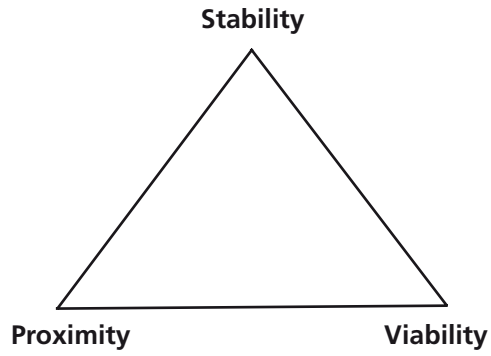

Fig 3. Triad of osteoconduction (Shors 1999).

2.5.2. Heterotopic osteogenesis in porous hydroxyapatite

When bone marrow is transplanted heterotopically (ectopically) into subcutaneous and intramuscular sites, bone formation can be observed. Likewise, marrow cells may give some osteoinductive capacity to porous ceramics when added to these implants. Ohgushi et al. (1989) showed that when HA as well as TCP and BCP implants were combined with marrow cells, osteogenesis was observed in heterotopic sites. In their study osteogenesis started approximately three weeks postimplantation on the surface of the pore region. Bone marrow induced osteogenesis has also been confirmed by Vuola et al. (1996) who found bone ingrowth in natural coral and coralline HA implants in heterotopic intramuscular implantation, but no bone ingrowth was seen without marrow. In natural coral bone formation was more abundant, but through early resorption the implants lost their framework and diminished, whereas coralline HA was not resorbed. Ripamonti (1991, 1996) has

studied heterotopic ossification of coralline HA in the rectus abdominis muscle implantation of primates, rabbits, and dogs and found substantial bone in specimens harvested from primates but no or minimal bone in rabbits or dogs. This finding may be related to adsorption of locally produced bone forming proteins characteristic of the primate species. Furthermore, recombinant osteogenic protein-1 (OP-1) or transforming growth factors (rhTGF) applied exogenously into the HA implants have shown to induce bone formation both in heterotopic and orthotopic sites (Ripamonti et al. 2001) as well as in composite grafts of HA and BMP and collagen (Takaoka et al. 1988).

Heterotopic bone formation in HA implants has also been shown with the autogenous periosteum which was wrapped around the HA implant (Kurashina et al. 1995). Interestingly, according to Kurashina et al., HA alone or the periosteum alone revealed no osteogenesis inside the muscle tissue although Poussa (1980) had found osteogenic activity and vascularization of free periosteal grafts in muscle tissue environment.

2.5.3. Clinical experience

The need for bone grafting is often necessary in impacted or displaced fractures, especially in cancellous metaphyseal areas. Likewise, the filling of bone cysts or defects due to tumour resection is an indication for grafting. The limitations in the volumes of autograft bone and the morbidity, which are related in harvesting procedures have stimulated the use of synthetic bone graft substitutes. The Food and Drug Administration (FDA) in the United States approved porous HA for human investigation in traumatic defects of long bones in 1982 (Bucholz et al. 1987). In spite of this approval, the number of publications of its use in clinical situations has been limited.

Bucholz et al. (1989) compared the use of coralline HA 500 blocks in proximal tibial condylar fractures to fill bone defects as compared to cancellous autograft repair. After open anatomic reduction the HA blocks were contoured with rongeurs to fill the defect press fit with the surrounding cancellous bone. The fractures were stabilized using the standard interfragmental plate and screws fixation technique. During the follow-up of 34 months there were no differences regarding the union of fractures, loss of reduction or maintenance of joint-space height or the complication rate compared to the autograft controls. Inflammatory reactions to the hydroxyapatite implant were not seen. Biopsies of the HA grafts were taken from seven patients at the time of the hardware removal, and bone ingrowth was present in all biopsies. Specimens taken 7,5–18,5 months after implantation showed a bone ingrowth volume fraction of mean 37 %. There was no evidence of implant resorption.

Cornell et al. (1991) and Chapman et al. (1997) used collagen-calcium phosphate material (Collagraft™) for grafting in acute long bone fractures requiring grafting. Collagraft™ includes bovine collagen, sintered hydroxyapatite, and tricalciumphosphate and was available in the granular or strip form. The studies were prospective, randomized multicentre trials comparing autograft to Collagraft™. On the basis of the radiological studies, Collagraft™ and autograft performed equally well, and the complication rates were comparable in both groups, though the infection rate was significantly lower in the fractures treated with Collagraft™. The antibodies to bovine col-

lagen were analysed in 12 % of the patients but no specific allergic reactions were identified. Accordingly, the use of collagen-ceramic material in traumatic defects of long bones appeared justified.

In their retrospective study Wolfe et al. (1999) made a follow-up examination on 18 patients with a distal radius fracture treated with a K-wire or external fixation, using coralline HA implants as bone graft substitute to support reduced articular surface. The average outcome measures of 35 months displayed subjectively, radiologically and clinically scores that were fully comparable with those of other studies reporting the operative management of complex distal radius fractures. Coralline HA grafts maintained articular congruency during fracture healing, and radiographs at the follow-up showed satisfactory results in terms of radial height, inclination, tilt, and ulnar variance. The only complication related to the use of HA was an extrusion of coral through an articular defect; larger blocks, instead of granules, are thus recommendable. The study supports the use of coralline HA in operative management of unstable distal fractures of the distal radius.

Clinical studies on using HA in human cavitary or segmental defects of long bones are limited. In spite of HA there are a great number of other commercial substitutes available or under development such as demineralized bone matrix, bioactive glass (Aho et al. 1993, Heikkilä et al. 1993) or calcium phosphate (carbonated apatite) cement (Ladd and Pliam 1999). So far, the use of coralline HA in metaphyseal defects under non-weight bearing conditions as in fractures of the distal radius and proximal tibiae has proved favourable, provided that some kind of internal or external support is used to assure the stability of the grafted region. Interporous HA has lower capacity to absorb energy compared to cancellous bone, but, due to bone ingrowth, its strength after six months of implantation has shown to equal or surpass that of the cancellous graft.

2.6. HYDROXYAPATITE IN SPINE SURGERY

Spinal fusions are one of the most common clinical settings where bone grafting is performed. Spinal fusion models, in spite of diaphyseal defect models, have been widely used for evaluation and comparison of synthetic graft materials. The pros for using spinal fusion models are that the spine is a very common anatomic site for clinical applications of graft materials, that there are multiple comparable graft sites suitable for mechanical testing, and that the spinal unit is mechanically a demanding site for reliable assessment of bone formation and union presenting no stress-shielding in the area of interest and, thus, not compromising the results. The cons are the difficulties in having reliable radiological studies and mechanical testing methods to quantify the outcome parameters. The radiographic assessment is not reliable due to the overlapping geometry of the facet joints and anterior and posterior osteophytes, which often results in an overestimation of the union rate.

2.6.1. Experimental use of hydroxyapatite in posterolateral fusion

Posterior and posterolateral fusions assess spinal fusion of adjacent posterior bony elements, including laminae, facet joints, and

transverse processes. An autogenous corticocancellous bone graft is the golden standard in achieving a successful fusion. Beyond this, the poorest results using an autograft are found especially in posterolateral lumbar fusion procedures. Autograft bone available for multilevel posterolateral fusions is limited and has led to extensive studies of additional graft extenders or biologic enhancement methods. Studies on the use of coralline HA in posterolateral experimental fusions are numerous (Holmes et al. 1984, Hardouin et al. 1991, Zerwekh et al. 1992, Muschler et al. 1993 and 1996, Guigui et al.1994, Boden et al. 1999, Delecrin et al. 1997, Bozic et al. 1999, Baramki et al. 2000, Steffen et al. 2000, Walsh et al. 2000).

Holmes et al. (1984) were the first to use HA 500 blocks to produce posterior fusion mass in dogs. The clinical results were poor but the histologic analysis showed that bone bridging into the graft had occurred in sites where the implants were in apposition to the surrounding host bone.

Hardouin et al. (1991) compared HA/TCP (65/35) with autogenous bone graft in lateral spinal arthrodesis in sheep. They used concomitant Cotrel-Dubousset instrumentation and found that bone ingrowth inside the implant occurred slowly, filled only part of the porotic areas, and fusion could be obtained later than with autogenous bone, even 27 months were calculated to be needed for 100 % inter-implant osseous apposition.

In the study of Zerwekh et al. (1992) implants made of a mixture of granular HA/TCP (60/40) and highly purified bovine type I collagen were used together with an autogenous bone graft in posterolateral spinal fusion in canines, compared to that of purely autogenous grafting. The mechanical testing showed no significant differences in the bending or compression studies, in angular deformation, in stiffness or in energy adsorption. Histologically, good bone ingrowth into the autogenous bone graft as well as into the implant/autogenous graft sites was seen. Histomorphometry showed a greater percentage of bone for autogenous bone grafting compared to HA/TCP implantation, due to initially greater autogenous bone volume in the control dogs. Microscopically, the union of the implant or graft material with the host bone was seen in all cases.

Muschler et al. (1993) developed a canine segmental spinal fusion model to assess different bone grafting materials. The fusion was performed at posterior interfacet-interlaminar sites, which were decorticated, and with additional internal fixation. In their primary study autogenous bone yielded significantly the best union scores compared to HA/TCP ceramic. However, the addition of the transforming growth factor (TGF-β) and bone morphogenetic protein (BMP) to ceramic improved their union score but was not higher than that of autogenous bone. Collagraft™ (HA/TCP plus collagen) was tested in their further study (Muschler et al. 1996), and it was showed to be clearly inferior to autogenous bone. Collagraft™ even reduced the effectiveness of autogenous bone when the two were combined. The authors cautioned using collagen ceramic composites such as Collagraft™ in the spinal fusion.

Conversely, the results of Walsh et al. (2000) conflict totally with the afore-mentioned results reported by Muschler et al. (1993, 1996). Collagraft™ was used in their study in posterolateral intertransverse fusion combined with pedicle screw stabilization. After six months of follow-up the his-

tology showed that the Collagraft™ strips had fused with the decorticated transverse processes and served as scaffold for new bone formation. Also the radiographies revealed fusions with evidence of new bone formation. Furthermore, the Collagraft™ fusions were mechanically equivalent to the autograft controls. No benefit was shown if Collagraft™ was coated with bone marrow. The authors supported the use of Collagraft ™ in spinal fusion with pedicle screw stabilization.

Guigui et al. (1994) used adjunctive posterior stabilization with Cotrel-Dubousset instrumentation by comparing biphasic porous ceramic (HA/TCP 65/35, pore size 400 μm) to natural coral (pore size 250 μm) in sheep. BCP blocks of 20 × 5 × 5 mm and natural coral blocks of 15 × 5 × 4 mm in size were placed on the decorticated posterior laminar grooves in two rows. Macroscopically bony union was obtained in all cases. The mechanical tests after removal of instrumentation exhibited a large decrease of flexibility in all directions in both treatment groups. However, the mean flexibility was higher in the natural coral arthrodeses, and all natural coral implants resorbed in 12 months. The authors concluded that using posterior instrumentation spinal arthrodesis can be obtained both with natural coral (coral-based calcium carbonate) and BCP, the latter showing a lesser degree of variability.

Delecrin et al. (1997) evaluated the influence of the fusion site on the bone ingrowth into the porous ceramic implants. They performed a posterolateral fusion in canines using biphasic calcium phosphate (HA/TCP 60/40, pore size 400–600 μm) block grafts and implanted them separately on the decorticated transverse site (lateral arthrodesis) and on the decorticated laminar site (posterior arthrodesis). Transpedicular instrumentation was assembled between the implantation sites and cancellous autografts were used in another group for comparison. The results demonstrated significantly more new bone ingrowth at the laminar fusion site, probably because the amount of fresh bleeding bone as a source of osteogenic cells was higher. In the mechanical testing there were no significant differences between the ceramic and autograft groups.

Boden et al. (1999) compared single-level posterolateral fusions in rabbits with HA combined with autogenous bone, autogenous bone marrow or osteoinductive bovine bone protein extract. A fusion rate of 100 % resulted with HA+osteoinductive protein extract, a 50 % fusion rate with HA+autogenous bone, and no fusion occurred when HA was used with bone marrow. Fusions with HA+autograft were generally comparable to those obtained with the autograft alone. The results indicated that HA can be an excellent carrier for osteoinductive agents but also a good graft extender for autologous bone.

Bozic et al. (1999) studied the effects of electrical stimulation when coralline granular HA 500 was used in single level posterolateral spinal fusion. The granular HA was mixed with bone marrow aspirate. The results showed that direct current electrical stimulation significantly increase the fusion success, stiffness, and ultimate compression load of fusions in a dose-dependent manner, and in the group of the highest electrical stimulation of 100 μA they were all even significantly better than in the group of the autogenous bone graft. The role of bone marrow aspirate remained obscure since no osteogenic precursor cells were identified in the haematological analysis.

Baramki et al. (2000) assessed the mechanical strength and radiographic findings of instrumented posterolateral spondylodesis in sheep with particulate coralline HA 500 compared to HA with autogenous bone. According to mechanical testing, the fusion rate was evaluated as 57 % in the group of HA 500 and as 70 % in the HA 500/autogenous bone group. The fusion rates were obtained from the mechanical testing and compared to the group of autologous fusion with radiologically confirmed unions. Radiological studies and grading were based on the CT scans and sagittal and coronal reconstructions were assessed most reliable.

Steffen et al. (2000) used also the sheep model with transpedicular fixation and used HA 500 granules alone or mixed with autogenous bone and resorbable natural coral (mean pore diameter 500 μm) granules mixed with autologous bone. Radiological, mechanical, and histologic studies showed that, in spite of resorption the natural coral granules mixed with the harvested bone had fusion rates similar to those of pure autologous bone. Interestingly, in their study, the results of using non-resorbable HA 500 with autologous bone were inferior not only to autologous bone but also to natural coral/autograft mixture.

In summary, the results of posterolateral fusions with porous ceramic materials are, to some extent, controversial and highly dependent on the animal model, the specific technique, and the ceramic substitute material used in the study. To conclude, the applications of ceramic materials in situ posterolateral spinal fusion without instrumentation demonstrated unreliable fusion rates compared to autograft. Porous HA with additional posterior stabilization revealed improved fusions and was often comparable to autogenous bonegraft; especially rigid adjunctive transpedicular stabilization was advantageous. This finding is in agreement with the human study of Zdeblick (1993) who was the first to show that rigid pedicle screw/rod fixation led to a significantly higher percentage of fusions in degenerative lumbar decease than did the fusion without instrumentation. The supplementation of the HA ceramic matrix with autologous bone, osteoinductive protein or with electrical stimulation also improved the fusion. Generally, HA may function as bone graft substitute combined with an autogenous bone graft or even alone in the well prepared vascular and stabilized bony environment as in posterolateral lumbar spine or in tubular bone metaphysic or diaphysis, but may fail in a less stable and vascular environment (Boden et al. 1999).

The summary of the experimental posterolateral fusion studies with HA is presented in Table 2.

Table 2. Literature review summarizing the experimental posterolateral fusion studies with HA, BCP or natural coral.
HA 200= hydroxyapatite with a mean pore diameter of 200 μm, HA 500= hydroxyapatite with a mean pore diameter of 500 μm, BCP= biphasic calcium phosphate, BMP= bone morphogenetic protein, TGF-α= transforming growth factor- alfa

Authors/ Year	Animal/ Number/ Follow-up	Ceramic/ Ceramic+ autog.bone/ autog. bone	Radio-graphs/ CT/MRI	Histology/ Histomor phometry/ Mechanical testing	Internal fixation/ Biologic enhancement	Fusion rate
Holmes et al. 1984	dog/16 /24 months	HA 200	+/–/–	+/–/–	–/–	no solid fusion inferior to autograft
Harduin et al. 1991	sheep/13/ 12 months	BCP/autog. bone	+/–/–	+/+/–	Cotrel-Dubousset/-	BCP allows later fusion, inferior to autograft
Zerwekh et al. 1992	dog/12 /12 months	BCP+collagen+ autog. bone/autog.bone	+/–/–	+/–/+	Steinman pin around spinous proc. /bone marrow	fusion in all
Muschler et al. 1993	dog/26 /12 weeks	BCP+collagen / BCP+collagen+autog. bone/autog.bone	+/–/–	–/–/+	Cerglage btw. spinous processes +PMMA/TGF-β; BMP	inferior to autograft
Guigui et al. 1994	sheep/20 /12 months	BCP/ natural coral /autog.bone	–/–/–	–/–/+	Cotrel-Dubousset/-	BCP and natural coral equivalent to autograft
Muschler et al. 1996	dog/25 /12 weeks	BCP+collagen/ BCP+collagen+autog. bone/autog.bone	+/–/–	–/–/+	Cerglage btw. spinous processes+PMMA/ bone marrow	inferior to autograft
Delecrin et al. 1997	dog/13 /9 months	BCP/autog. bone	–/–/–	+/+/+	pedicle screws/-	equivalent to autog. bone signif. diff. in ceramic bone ingrowth btw laminar and intertransverse sites
Boden et al. 1999	rabbit/48 /10 weeks	HA500/ HA500+atogenous	+/–/–	+/–/+	-/bone marrow, osteoinductive protein	HA+inductive prot.100% HA+autograft 50% HA+marrow 0%
Bozic et al. 1999	rabbit/53 /8 weeks	HA 500	+/–/–	+/–/+	-/bone marrow, electric stimulation	with electrical stimulation equivalent to autograft
Walsh et al. 2000	sheep/24 /6 months	BCP+collagen	+/–/–	+/–/+	pedicle screws/ bone marrow	equivalent to autograft
Baramki et al. 2000	sheep/ 28 /20 weeks	HA 500/HA 500+autograft/autograft	+/+/–	–/–	pedicle screws/-	HA 500< HA500+autograft<autograft
Steffen et al. 2000	sheep/52 /20wks	HA 500 or natural coral/HA 500+ autograft or natural coral+ autograft/ autograft	+/+/–	+/–	pedicle screws/-	autograft equivalent to natural coral+autograft > HA 500+autograft

2.6.2. Experimental use of hydroxyapatite in anterior interbody fusion

In terms of stability solid lumbar interbody fusion is considered more advantageous compared to posterior or posterolateral fusion. The interbody fusion constructs are stiffer in axial compression than the respective constructs in posterolateral arthrodesis (Evans 1985). Interbody fusion permits high load transmission through the anterior column, restores the disc height and segmental lordosis, and requires a minimal bone graft volume (Fraser 1995, Greenough et al.1998). New techniques with transpedicular posterior stabilization have increased the use of interbody fusions. Discectomy, decortication, and placement of the intervertebral graft accomplished by posterior approach alone (Posterior Lumbar Interbody Fusion, PLIF) or by anterior open transperitoneal approach (Anterior Lumbar Interbody Fusion, ALIF) along with transpedicular instrumentation are seen more frequently indicated. Bi- or tricortical iliac autografts are still the golden standard. A review of literature has demonstrated similar fusion rates with autogenous and allogenous grafts in single level anterior cervical fusions, but the rate of autograft fusion has been higher in multilevel fusions (Hanley et al. 1989, Zhang et al. 1983). However, the use of bone graft substitutes alone or in combination with cages is still enticing due to their availability, mold ability, and lack of patient morbidity due to bone harvesting. Concomitant posterior transpedicular stabilization may also decrease the mechanical demands set upon HA or TCP which are brittle and show a low impact and fracture resistance. Moreover, biologic enhancement may increase the use of bone substitutes in the future.

HA has been studied in the use for anterior intercorporeal fusion alone or in combination with TCP. New methods in the surgical techniques have also led to new types of demands in bone grafting. The use of HA/TCP placed inside the cages has been studied. HA may have a role in the form of injectable bone cement in transpedicular anterior fusions (Blattert et al. 2002) or in the treatment of osteoporotic vertebral fractures (Bai et al. 1999) and in augmenting of the spinal screw fixation strength (Yerby et al. 1998). The use of BMP in combination with HA and other calcium phosphates has been studied. The role of disc replacement with calcium phosphate materials has also been debated.

Most of the experimental anterior spine interbody fusions with HA ceramic have been performed in cervical spine (Shima et al. 1979, Cook et al. 1986, 1994, Pintar et al. 1994, Zdeblick et al. 1994, Toth et al. 1995) or in lumbar spine (Flatley et al. 1983, Ragni and Lindholm 1991, Pintar et al. 1994, Blattert et al. 2002). Because of the great mobility of the cervical and also lumbar regions, the studies have shown a great number of graft extrusions and collapses. Shima et al. (1979) were the first to use ceramic substitutes in interbody cervical fusion in dogs. They introduced synthetic TCP dowels into the discectomized cervical interspaces and found various degrees of compression of implants as well as displacements even in 70 % of the cases. Using the thoracic spine model the inherent stability of that region allows an advantage to evaluate more accurately the biologic capabilities of bone ingrowth into the ceramic implants (Emery et al. 1996, Fuller et al. 1996). Moreover, in-

ternal fixation of the vertebral bodies may provide adequate stability for optimal studies of biologic behaviour of ceramics. Total discectomy prior to implantation tends to make the segment increasingly unstable and in such models, the mechanical evaluation has often failed to demonstrate differences between the different treatment groups. This has been the result from the formation of massive anterior bone bridges, masking the effects of individual treatments.

The next three chapters will summarize the experimental studies on HA and its composites in anterior vertebral fusion studies.

2.6.2.1. Cervical fusion studies

Cook et al. (1986) used two different ceramic implants in cervical intercorporeal fusions in dogs. The ceramic implants were either non-bioresorbable or bioresorbable disc-type blocks which were inserted in one or two of the disc spaces between the C3-C6 vertebral bodies. The authors did not clarify the exact composition of the ceramics used, but being bioresorbable means that the implant, in all likelihood, is either TCP or BCP (biphasic ceramic TCP/HA). The non-bioresorbable block was dense in its core but porous in surface, and the bioresorbable blocks were uniformly porous. Fourteen non-bioresorbable and nine bioresorbable implants were grafted, and the follow-up was from one to 24 weeks. Radiologically, nine out of 23 implants were fractured, and extruded and all 14 implants that remained in the interspace were fractured. By 12 and 24 weeks the radiographic fusion looked evident. The histologic studies of the 12- and 24-week specimens showed that most of the bioresorbable ceramic had disappeared and was replaced by bone and fibrous tissue. In the non-bioresorbable implants the ceramic/bone interface showed excellent bonding of bone. In the non-extruded implants only varying degrees of osseous fusion were demonstrated.

In a further study Cook et al. (1994) performed two-level anterior cervical interbody fusions in 21 dogs using synthetic dense HA blocks of 14×12×5 mm in size and compared them to autogenous corticocancellous grafts of 10×10×4 mm in size. New bone apposition and bonding were observed histologically as early as six weeks postoperatively. The HA blocks were minimally cracked in six cases, but the follow-up did not demonstrate any displacement of the cracked pieces, and there was only one HA block extrusion. Radiology demonstrated HA graft incorporation already from six weeks on, but in the CT studies gap areas were seen in the implant/bone interface from six to 12 weeks and good incorporation at 26 weeks. The MRI was of little value in evaluating the quality of fusion. In the mechanical testing the HA segments were as good as the autograft segments. The autograft sites showed bone bridging already at six weeks and complete fusion at 26 weeks. However, the loss of disc height at 26 weeks was only 5 % for disc spaces with HA implantation and 40 % for disc spaces with autogenous grafting. As a conclusion, the use of dense HA block in anterior cervical fusion was encouraging.

Pintar et al. (1994) compared synthetic, dense HA implants and tricortical iliac crest autografts in the anterior fusion of goat cervical and lumbar spinal units. The six-, 12-, and 24-week preparations were evaluated with CT for fusion and changes in disc space. Two-level fusions were performed

both in the cervical (between C2-C6) and upper lumbar regions; in both regions one disc space was grafted with an HA and another with autogenous tricortical graft. From 12 to 24 weeks, three out of ten cervical HA implantations (30 %) were seen radiologically fused compared to four out of ten (40 %) of the autogenous graft fusions. The corresponding figures for fusion in the lumbar spine were 7/10 (70 %) for both HA and autogenous grafting. The non-fused HA implants demonstrated a wide soft tissue gap around the implant, seen in the CT and in the histological studies. However, the CT images presented from the fused segments showed a clear resorption line around the implants, and the fusion was mainly due to anterior bone bridging. The HA blocks maintained the disc space height better showing a 21 % reduction in all material compared to a 39 % reduction with the bone graft. In the mechanical testing, the fused segments demonstrated no difference between the HA and autogenous bone segments. The cracking of the implants was seen quite often, though exact figures were not presented.

Zdeblick et al. (1994) studied multilevel anterior cervical fusions using coralline HA 500 in a goat model. In the group of HA implantation without an anterior plate the radiographic results showed that 29 % of the implants were collapsed and 14 % extruded. In the group with an anterior plate 24 % collapsed and none extruded. The radiographs could not determine true radiographic fusion. Histologically, 48 % of the HA implants were well incorporated by bone and 10 % showed an intervenous fibrous gap in the bone implant interface. In the group with an anterior plate the figures were 71 % with incorporation and 5% with fibrous gap. According to the authors, coralline HA was considered a promising material for interbody fusion, as approximately one half of the implants showed good incorporation with a tight host bone/implant interface. The authors recommended implants with a smaller pore size and increased rigidity to be used in further studies.

Toth et al. (1995) assessed the effect of percentual porosity in the two-level cervical fusion in goats using biphasic calcium phosphate (50/50 HA/β-TCP) implants with 30 %, 50 % and 70 % porosity. The blocks were $10 \times 10 \times 8$ mm in size. The histologic analysis showed that the union rate was identically 67 % for all three porosities at six months and the results were even better than those for an autograft. The more porous implants had a higher union rate already at an early stage, but they also had a higher incidence of graft fracture. During the follow-up the radiographic fusion scores in all porous implant groups were better than in the autograft groups. The incidence of graft fractures was equal in all groups, in approximately half of the cases. The disc height was better maintained by the ceramic implants compared to the autografts, even when the grafts were broken. There was only partial extrusion of the autograft in three cases.

2.6.2.2. Thoracic spine fusion studies

In 1996 Emery et al. compared biphasic ceramic HA/TCP (60:40), porous HA 200, natural coral, and autogenous tricortical grafts for anterior interbody fusion in a canine model in the thoracic spine. A cube-like defect of $6 \times 6 \times 6$ mm was created over the disc and adjacent end-plates, and a graft

of 6 × 6 × 6 mm was inserted through the left transthoracic anterior approach and additional, internal fixation was used. After eight weeks of follow-up, the biomechanical testing showed that the spines from the autogenous graft group were statistically significantly stiffer in the bending and torsion tests. Histologically the autogenous grafts showed osseous union in 83 % of the cases, in the HA group in 67 %, and no ossification was seen in the natural coral group. The HA/TCP implants had a consistent osseous union at the bone implant junctions in all cases, but the bone ingrowth was incomplete in ¾ of the cases. Three out of six HA implants and two out of four HA/TCP were cracked. To conclude, the fusion rates were histologically and mechanically superior for autogenous bone compared to coralline HA or HA/TCP and natural coral showed no ossification.

To evaluate the effect of internal fixation on porous ceramics in anterior interbody fusion Fuller et al. (1996) performed a study in the canine thoracic spine. An anterior interbody fusion was done using a natural coral (mean pore size 250 μm) or iliac crest autograft with or without intraoperative stabilization using anterior instrumentation. Again, radiographs were not considered sensitive enough to judge osseous union and therefore it was determined microscopically. During the follow-up of eight weeks, the most minimal bone ingrowth was seen in the fusions with natural coral without internal fixation. The most consistent healing was seen in the group of an iliac crest autograft together with a fixator. The cases where natural coral together with a fixator was used there was, however, osseous and fibrocartilaginous ingrowth without an intervenous gap, though the union was not solid. In the mechanical analyses the unions with an autograft together with a fixator showed significantly greater stiffness than the unions with the other treatment modes.

2.6.2.3. Lumbar spine fusion studies

Flatley et al. (1983) used porous ceramic TCP/HA blocks in experimental intervertebral fusions in rabbits. The ceramic blocks were composed of β-TCP and HA with a pore size of 400–600 μm and porosity of 50 % in the ratio of 1:1. The blocks were inserted tightly into the bony mortise that was created across the disc between the two adjacent vertebral bodies. In the control rabbits the disc space was curettaged without implantation. As a result, ingrowth of fibrovascular tissue into the implants was seen from three weeks on, and from six weeks onward immature woven bone was identified within the pores of the ceramic. At 24 weeks the TCP/HA blocks were completely incorporated by new bone histologically seen as mature, lamellar bone, which was, in addition stress-oriented. The control specimens showed no bony fusion either radiologically or histologically.

Ragni and Lindholm (1991) studied the enhancement of HA in the anterior lumbar fusion of rabbits by adding demineralized bone matrix with HA. The purpose was to create osteoinductive activity in the porous HA. In terms of stability and roentgenologic evaluation at six months they found no difference between the HA 200 implants and the HA 200 implants enhanced with demineralized bone matrix, though the latter showed earlier stabilization. Complete incorporation of new bone throughout both

implants was seen identically, but mature bone could be observed only in the peripheral pores. The authors found biologic enhancement promising in developing the bone graft substitutes.

Blattert et al. (2002) performed a study in the sheep lumbar spine using synthetic injectable HA cement with 6–10 % porosity in the posterior lumbar interbody fusion (PLIF). Bone grafting was carried out through the transpedicular canals. The transpedicular instrumentation between L4 and L6 was performed, and subsequently transpedicular canals of L5 were created. Through the canals the disc space L4/L5 was evacuated, the end-plates were decorticated, and the autogenous bone chips, HA alone or HA enhanced with osteogenic protein-1 (OP-1), were inserted into the cavity. The transpedicular path to the intervertebral space was minimally invasive and safe. Radiological fusion was seen in one out of ten in the autograft group, in two out of 12 in the HA group, and in ten out of 12 in the HA+OP-1 group. The histologic examinations confirmed the radiological findings. In both groups of HA cement there were one or more cracks in the HA cement mass, but the cracks were bridged by new bone in the group of HA+OP-1 in contrast to the HA group where early cracks led to gross fragmentation and resorption of the HA cement. The authors considered the combining of the osteoconductive and osteoinductive properties in the HA substance a promising result and an improvement of the original autograft version of the transpedicular interbody grafting.

2.6.3. Biologic enhancement of spinal fusion

The most commonly used strategy for enhancement of healing in spine fusions is that of rigid internal fixation. Internal fixation is reported to increase the fusion rate in posterolateral fusions by approximately 10 % (Zdeblick 1993, Boden and Schimandle 1995, France et al. 1999). Biophysical stimulation is another strategy that has been studied and used in spine surgery. Electrical stimulation and low-intensity ultrasound are examples of physical stimulations which both bear some evidence of enhancing ossification in spine fusions (Mooney 1990, Glazer et al. 1997, France et al. 2001). Generally, bone graft enhancers are substances or devices that when added to bone graft, increase the bone growth or healing (Boden 2002). Theoretically, the enhancers used in combination with autogenous bone grafting should also enhance bone growth and healing used in combination with bone graft substitutes.

Currently, numerous substances are studied as potential enhancers in spinal fusion models, such as demineralized bone matrix DBM which is thought to include osteoinductive proteins or osteoprogenitor cells, as well as bone marrow, or different osteoinductive substitutes, such as BMPs, osteogenic protein-1 (OP-1) and growth factors like TGF-β and platelet-derived growth factor PDGF.

Tay et al. 1998 tested cross-linked collagen latticework coated with HA and specifically developed for use as an alternative to autograft (Healos™). The material was tested in posterolateral lumbar fusion in rabbits by enhancing it either with bone marrow or heparinized bone marrow. They found a clear enhancement with bone marrow in terms of

histologic bone ingrowth and radiographic fusion, and the fusion results were comparable with those of autogenous bone.

2.6.4. Human clinical studies on hydroxyapatite in spine surgery

In spine surgery, the main prerequisite for bone bonding and osteoconduction of HA implants include the creation of viable bone bed such as decortication of posterior elements or roughening or removing the vertebral body endplates. The stabilization of the graft site may often require additional stabilization measures.

Zdeblick (1993) has shown in a prospective, randomized study that rigid pedicle screw instrumentation led to a significantly higher rate of posterolateral fusions in the degenerative lumbar disease compared to fusions without instrumentation. Thus, adjunctive rigid stabilization has become more essential both in posterolateral and intercorporeal fusions. Theoretically, instrumentation is even more essential in using bone substitutes like HA to fulfill the requirement of stability in the triad of osteoconduction (Shors 1999).

2.6.4.1 Hydroxyapatite in scoliosis surgery

A number of clinical series have been published on using ceramics in posterolateral spinal fusion for scoliosis patients (Passuti et al.1989, Pouliquen et al. 1989, Heise et al. 1990, Le Huec et al. 1997, Ransford et al. 1998, Delecrin et al. 2000, Mashoof et al. 2002).

Passuti et al. (1989) used BCP (60 % HA, 40 % β-TCP) in 12 selected patients with severe neurological scoliosis and osteogenesis imperfecta as substitutes to allograft bone due to insufficient bone supplies or BCP alone. The BCP blocks were placed into the posterior interarticular area with adjunctive Cotrel-Dubousset instrumentation. The clinical and roentgenographic data after 15 months of follow-up demonstrated a complete fusion associated with the instrumentation stability and lack of angular loss. The histologic specimens taken in two cases from the vertebral implants showed bone remodellation similar to the haversian system inside the implant pores, and lamellar bone bonded to the BCP material.

Pouliquen et al. (1989) used natural coral as a substitutive element to bone supply in 49 patients with idiopathic scoliosis. Correction was carried out with posterior Cotrel-Dubousset instrumentation, and according to X-rays, scintigraphies, and biopsies, the results were comparable to those of the usual bone grafts. Heise et al. (1990) used HA granules mixed with autologous spongy bone in 12 scoliosis patients, in eight of whom Harrington rod internal fixation was used. They reported a good outcome in their follow-up of mean 21 months in 11 patients. Only in one patient the fusion was not achieved. Le Huec et al. (1997) did not use HA but made a comparison between TCP and allograft bone as substitutes to autogenous bone in a series of 54 consecutive patients. In a minimum of four years of follow-up the clinical results, such as loss of correction or number of complications, were identical in both groups. TCP was totally resorbed in two years.

Ransford et al. published in 1998 a large multicentre series of 341 scoliosis patients comparing prospectively synthetic porous BCP (60 % HA, 40 % β-TCP) to autograft bone in posterior spinal fusion. Adjunctive posterior instrumentation with porous BCP alone or with autograft bone was carried out

either with Cotrel-Dubousset or Harrington-Lugue implants. The corrections achieved were identical in the BCP and autograft groups. In 18 months the losses of correction were 3 % in the BCP group and 8 % in the autograft group. Donor site pain was registered in 15 patients in the autograft group. Biopsies taken from six patients showed osteoconduction inside the ceramic pores and gradual resorption of the material into new bone. The authors report no adverse effects related to the synthetic ceramic graft material and find this material a safe and effective substitute for autografts in scoliosis patients.

Delecrin et al. (2000) reported good clinical results in a prospective randomized study of 58 scoliosis patients using BCP combined with local autogenous bone or autogenous bone graft harvested from iliac bone along with Cotrel-Dubousset instrumentation. In the follow-up of mean of four years the radiological correction was maintained similarly in both groups. Local complications related to bone harvesting and preoperative blood loss were considerably higher if the iliac bone autograft was taken. Finally, Mashoof et al. (2002) mixed coralline HA 200 with iliac autograft bone (70/30 ratio of HA to autograft) in a series of 27 scoliosis patients and reported solid fusion in all at a follow-up of two years.

2.6.4.2 Hydroxyapatite in cervical spine surgery

Most human studies on ceramics in anterior spinal fusion have been made in the cervical spine (Senter et al. 1989, Kim et al. 1998, Thalgott et al. 1999, Suetsuna et al. 2001, Ito et al. 2002, and McConnell et al.2003). The aim of the anterior cervical fusion is decompression of the neural structures, restoration on stability, and maintenance of physiological alignment. The autogenous tricortical iliac graft is the standard method. The use of HA or its derivates is supposed to have their mechanical strength sufficiently high enough to withstand mechanical stresses. Wittenberg et al. (1990) measured the best axial compressive strength for tricortical iliac grafts which was 1,5–2,3 times higher than the strength of bicortical grafts. HA 200 grafts had a compressive strength comparable to the bicortical grafts, and it was significantly higher than that of the HA 500 grafts. Theoretically, after bone incorporation the mechanical strength would even exceed the autograft and HA implant would serve as a permanent spacer while fusing the nearby vertebrates.

Senter et al. (1989) compared the results of their non-randomized, retrospective trial of cervical interbody fusion with a synthetic, dense, non-resorptive HA block to those with the use of autologous iliac crest bone. Discectomy and fusion were performed due to radiculopathy and myelopathy. There were 75 patients in the autograft group with a follow-up of 4–6 years and 84 patients in the HA group with a follow-up from six months to three years. Only 5 % of the HA blocks fractured or dislodged, but complete fusion could not be confirmed by plain x-rays due to radiodensity of the material. The clinical outcomes were equivalent between the two groups.

Kim et al. (1998) used synthetic porous HA implants with a pore size of 200–500 μm. The implants were specifically designed and their upper and lower surfaces were convex to withstand compression, to prevent dislodgement and to maintain the lordotic curve of the cervical spine. When the discus and cartilaginous end-plates were removed the cortical end-plates of

both the upper and lower vertebral bodies were drilled to create a concave shape to be equivalent to the implant. The authors did not use any adjunctive intervertebral fixation. In 70 patients there were three early dislocations, no collapse of implants or vertebral bodies were observed, and formation of bridging bone was observed in the tomograms and CT scans within six months after surgery. Preservation of lordosis could be verified in 22 out of 23 patients, and formation of kyphosis was detected in six out of 55 cases. Neurological deterioration related to fusion was not observed. The authors report to continue the use of HA implants in single-level cervical interbody fusions.

Thalgott et al. (1999) made a retrospective radiological and clinical review of 26 patients who underwent anterior cervical fusion using coralline HA 200 blocks sawed into the size of disc space dimensions and an adjunctive AO spine locking plate anteriorly. The minimum follow-up period was two years. According to their studies, total incorporation was achieved in all patients, though conclusions on the basis of the radiographic analysis cannot be regarded as definitive. There were five non-propagating cracks of implants, and no implant collapses were encountered. The authors evaluated the use of HA 200 with rigid anterior plating promising in anterior fusion of the cervical spine.

McConnell et al. (2003) made a further study comparing the identical coralline HA 200, as did Thalgott et al. (1999), prospectively to the tricortical iliac crest autograft in cervical interbody fusion. The HA group consisted of 13 patients and the autograft group of 16 patients, and, like Thalgott et al., an anterior cervical spine locking plate was used in both groups. Within three months 89 % of the HA grafts fragmented, compared to 11% of the tricortical autografts. In spite of fragmentation, non-union was encountered only in four intercorporeal spaces in the HA group, while all three fragmented autografts led to non-union. Therefore, there was no significant difference in the fusion rate between the HA group (78%) and the autograft group (79%). No significant difference was found in the clinical outcomes, either. However, due to HA 200 block fragmentations and collapse this clinical trial was terminated.

Suetsuna et al. (1999, 2001) have reported the use of synthetic porous HA (pores 100–500 μm) in anterior fusion in the treatment of cervical disc herniation since 1992. The authors also used a wide decompression method to remove the end-plates completely. Operated with this technique Suetsuna et al. (2001) made a retrospective study of anterior fusion with synthetic porous rectangular HA implants in the treatment of 36 cervical disc herniation patients. The height of the HA implants ranged between nine and 15 millimetres, and the recipient surfaces of the implants were grooved. The method includes the resection of both end-plates as well as a wide decompression to achieve a rectangular slot corresponding the size of the HA insert. No additional internal fixation was used. Clinical and plain radiographic studies including flexion-extension views and CT were made from two to seven years postoperatively. No motion of the fused segment was observed in any of the cases, and bony union was assessed in all cases, though radiolucent zones were seen in four (11 %) cases. A loss of height of fused segments was observed in 29 cases (81 %) with an average of 1,6 mm, and a decrease of lordosis was measured in six cases (17 %). Cracks of the inserts were seen in four (11 %) cases, but also in those

cases the bony fusion was good and no collapse or displacements were seen. The clinical outcome was assessed good or excellent, and there was no need for re-operations. The authors conclude that their method using rectangular porous HA blocks and complete resection of end-plates is viable and can replace the use of autogenous bone.

Ito et al. (2002) reported complications related to the use of HA spacers in anterior cervical surgery in seven patients. The patients were referred to the authors from other institutions, and the rate on complications remains obscure. All the patients had a radiolucent clear zone around the spacer. Four had breakage of the HA spacer, two had spinal cord compression due to retropulsed HA fragments, and in one case an obliteration of the oesophagus emerged due to HA block instability and anterior plate loosening. Most of the broken HA blocks were less than ten millimetres thick. Grooves on the outside of the recipient area of the block cause an additional stress concentration and should be avoided. The authors also stress creation of sufficient room for insertion and gentle insertion techniques.

In summary, the most promising results in the clinical series of cervical surgery were seen in the study of Kim et al. (1998) and Suetsuna et al. (2001). In both of these studies, specifically designed porotic HA blocks with a pore size of 100–500 μm were used along with a widely created recipient area. Accordingly, the resection of the end-plates and sufficient exposure of vertebral cancellous bone were reported to result in improved adaptation and osteoconduction. The implants were high, 10–15 mm in height. Although the rigid anterior plating was not used, it seems to be advantageous according to other studies.

2.7. HYDROXYAPATITE IN MANDIBULAR AUGMENTATION

Alveolar resorption follows the loss of tooth. When resorption is excessive, the replacement of missing teeth by removable or even fixed prosthetics can result in poor stability and in a poor appearance. The ideal treatment would be to correct the ridge deficiency with a bone graft or its substitutes and to insert a removable prosthesis and/or implants onto it. Autogenos bone or its substitutes can be applied to any vertical augmentation or to increase the width of a narrow, sharp-pointed ridge.

Autogenous bone grafts harvested from the ileum or rib as well as freeze-dried allografts have been used in oral and maxillofacial surgery to restore bone defects, augment alveolar ridges, and re-establish the periodontium. However, these grafts have showed a tendency to resorb, especially with the onlay techniques (Kent et al. 1982, Maletta et al. 1983). The placement of dentures over autogenous bone grafts has commonly required a delay of four to six months postopratively for revascularization and remodelling before the placement of the dentures. When traditional bone grafting has proved ineffective in maintaining the defective alveolar ridge, ceramics have been widely investigated and used in repairing and maintaining the alveolar bone. The studies of HA as bone graft substitute have, in fact, had their origin and scope of applications in oral and maxillofacial surgery. Dense non-resorbable HA has been used clinically in dentistry already from the 1970's to fill periodontal defects (Rabalais et al. 1981), to augment edentulous resorbed alveolar ridges (Kent et al. 1982) or to repair local alveolar de-

fects (Brook et al. 1987). Histologic studies in human patients have showed new bone formation in association with implanted HA (Moskow and Lubarr 1983, Beirne and Greenspan 1985, Page and Laskin 1987). Modifying and augmenting the atrophic alveolar profile for prosthetic reasons have become the most important clinical applications of HA in oral and maxillofacial surgery. In clinical practice HA implant materials have been shown to be especially suitable as bone substitutes in areas where only small compressive forces are applied. Compared to the autogenous bone graft, HA has also many advantages in mandibular ridge augmentation. The surgical procedure is less complicated, it can be more easily performed in an outpatient setting, and it allowes a decreased postoperative period before the placement of dentures. Common problems with HA as well as with all materials used for ridge augmentation are their failure to bond with the underlying bone, migration or extrusion of the implants, and failures to form union with the other surrounding tissues. Generally, the biomaterial to be successfully used as onlay graft on the alveolar ridge should fulfil the following criteria (Frame 1987):

- easely carved and molded
- adequate mechanical properties to support denture
- biocompatible and stable
- firm bonding with bone and soft tissues
- no interference with soft tissue healing
- resistance to infection
- no adverse effects

The problem in the mandibular contour augmentation is related to the difficulty in confining the material, especially the HA granules to the augmented ridge. The graft material in this environment should therefore show great stability, since the regeneration capacity of the atrophying ridge is relatively low. In using a resorbing material such as TCP, the underlying bone should have a capacity for new bone ingrowth at the rate similar to that of the resorption of the graft. In clinical practice the use of TCP is disregarded, because its degradation rate varies greatly and overcomes the ridge generation capacity. Hence, HA has gained popularity as a stable, practically non-resorptive augmenting material in oral and maxillofacial surgery.

2.7.1. Use of hydroxyapatite blocks

The block forms of HA, either dense or porous, have their advantages in controlling the placement of the restoring or augmenting material of the alveolar ridge. Theoretically, the HA block can be placed more accurately and it can produce a greater ridge height. Furthermore, dense HA is known to have good compressive strength withstanding masticatory forces. Porous blocks exhibit poorer compressive strength, but they have better bone and tissue ingrowth properties and may thus form more stable "synthetic bone". Dense HA has been used for total and partial alveolar ridge correction by utilizing a tunnelling submucous vestibuloplasty approach (Kent et al. 1982, Rothstein et al. 1984). In interpositional grafting the HA block is positioned between the mobilized alveolus and the remaining alveolus to increase the mandibular vertical dimension, so-called "sandwich-type" implantation (Frame and Brady 1984).

Holmes (1979) made an experimental study in dogs using coralline HA blocks in

the surgical two-centimetre segmental defects of the dog mandible. He extracted the teeth from the premolar region four weeks before the surgery and then created a two-centimetre gap and replaced it with the coralline HA 200 block which was held in place by a metal tray fixed with screws in the mandible. After the follow-up of two, four and six months, he found that the implants were filled with regenerated bone in 11 %, 46 %, and 88%, respectively. However, the alveolar defects which were left open without an implant were also found to be completely filled with regenerated bone in six moths.

Generally, porous coralline blocks have been investigated as an onlay graft on the alveolar ridge. Piecuch et al. (1983) have studied coralline HA implants as onlay grafts on the dog alveolar ridge. They found bony ingrowth already at six weeks after implantation, and after one year the regenerated bone was seen in most of the pores. Furthermore, due to the tissue and bone ingrowth, after two years the compressive strength of these subperiosteally placed coralline implants was measured 3,5 to 10 times higher than that of the unimplanted porous HA (Piecuch et al. 1984, Piecuch 1986). The authors concluded that the strength of the coralline HA after tissue ingrowth might be sufficient to withstand masticatory forces.

Early tissue ingrowth seems to be crucial for osteointegration of the onlay blocks. In the experimental study of El Deep and Holmes (1989) dense HA blocks implanted in the subperiosteal pocket on the mandible were surrouded by fibrous encapsulation, whereas similar porous HA blocks were attached to the cortex by bone ingrowth. Surface-texturing has shown, however, to improve the bone bonding of dense HA (Block and Kent 1988).

The titanium screw fixation of the coralline HA blocks has also been tested experimentally in surgically created defects in minipig mandibles by Schliephake and Neukam (1991). The study showed a high rate of implant fractures, though the bone ingrowth was seen throughout the implant, even in close contact with titanium.

When placed between the bone and periosteum, coralline blocks have been shown to become rapidly fixed into the ridge and surrounding tissues. They are also easier to shape during surgery, and bone and connective tissue ingrowth has been confirmed radiologically and histologically, the bone ingrowth occurring mainly at the HA/ridge interface. Furthermore, after tissue ingrowth porous blocks have showed sufficient strength to withstand masticatory forces and support to denture (Piecuch et al. 1983, Frame and Laird 1987). However, the placement of HA blocks has serious limitations (Frame et al. 1987). Despite their initial success, later clinical studies of porous blocks encountered problems with wound dehiscence and mucosal ulceration that led to exposure of the implants, often necessitating their removal. In a clinical series of Rooney et al. (1988) mandibular and maxillary augmentation was performed with porous HA blocks in 29 alveolar ridges. Within one year 65 % of the implants had to be partially or totally removed. Due to delayed healing, 38 % of the patients had a significant delay in receiving their dentures. The most common complications were dehiscence (79 %) and infection (21 %). Stable blocks which were removed due to infection showed bone ingrowth into the HA block only in areas of immediate contact with alveolar bone to a depth of 1–2 mm. The finding was similar to that of Piecuch et al. (1983) who found

in their experimental model only partial filling of the upper HA block with follow-up of two years. Hupp and McKenna (1988) and El Deep et al. (1988) reported further poor results. Finally, in their later clinical report, Piecuch et al. (1990) recommended that porous HA blocks should not be used for alveolar ridge augmentation. Most of the complications are related to thin overlying mucosa. The resorbed, atrophic mandible or maxilla is covered with thin atrophic mucosa and, thus, is not usually suitable for interpositional or onlay grafting with HA blocks. Actually, due to the reasons above, the use of dense and porous HA blocks in mandibular ridge augmentation is abandoned.

2.7.2. Use of particulate hydroxyapatite

Due to a lower complication rate and a simple insertion technique into the mucoperiosteal pocket, the use of HA particles in alveolar augmentation became a standard treatment. No signs of resorption of either dense or porous particles have been found, but there has been some evidence of better bone ingrowth into the porous particles which stabilizes the particles better. However, problems have arisen in obtaining an adequate ridge form because of displacement or migration of particles in the early phase of their tissue integration. Several technical modifications have been suggested to control the particle placement. One of the first methods was an open splint for retaining the particles (Pham 1986). Thereafter, several open perioperative techniques such as creation of the pocket, mucosal flaps, and vestibuloplasties, have been described (Barsan and Kent 1985, Lew 1985, Kent et al. 1986). Most of the studies in this field are, however, focused on investigations of various vehicles to maintain the particles and to facilitate their manipulation into the desired form. Studies on collagen tubes, biodegradable meshes, various binding substances or glues, and soft tissue expanders have been performed. To improve HA particle containment with a special biodegradable implant is also the scope of the present study.

2.7.2.1. Experimental studies on particulate HA augmentation

In clinical practice the HA particles are placed into the subperiosteal pocket between soft tissues and alveolar bone through one vertical bilateral mucoperiosteal incision for mandibular augmentation and through one vertical midline incision for maxillary augmentation (Kent et al. 1982). In this technique by Kent, HA particles are delivered to the created subperiosteal pockets from the plastic syringe. The results were initially satisfactory, but with widespread use problems emerged with the migration of particles, loss of adequate ridge formation, low vestibular depth, and mental nerve disturbances. For hindering these problems the container concept was developed. The experimental studies on using dense or porous HA particles for onlay augmentation are mainly focused on different methods of particulate HA containment.

A number of containment methods have been studied experimentally, e.g. with collagen tubes (Gongloff and Montgomery 1985, Gongloff et al. 1985, Shen and Gongloff 1986), polyglycolic acid (PGA) mesh tubes (Silverberg et al. 1986), and polyglycolide suturing matrix (Cook 1994). Likewise, different binding substances such as fibrin sealant (Tisseel™) (Hotz 1991, Meijer

et al.1997) have been added to HA particles to avoid dispersion.

Gongloff and Montgomery (1985) were the first to study the use of collagen tubes for containment of particulate HA augmentation. They compared free and collagen-contained HA particles on the rat mandible and concluded that the collagen tube facilitated the placement of the particulate HA and prevented its displacement and migration in subperiosteal implantation. However, the study revealed no bone ingrowth in any implant. In a further study Gongloff et al. (1985) used collagen-contained HA in the transosseous mandibular defects of rats. Likewise, the implants showed less migration, a dense consolidation with primarily fibroblastic cellular response with macrophages and giant cells, but no bone ingrowth. Finally, according to the study of Shen and Gongloff (1986), HA encased in collagen film helped to shape and contain the particulate HA for as long as four weeks, and the collagen did not seem to interfere with the normal tissue response.

Identically to collagen tubes, a polyglycolic acid (PGA) mesh tube was developed and studied for HA augmentation by Silverberg et al. (1986). They studied the fate of HA contained within a PGA mesh tube when placed in a surgically created subperiosteal pocket against the tibia in rats. Similarly to collagen tubes, the PGA mesh tube prevented displacement of particles, allowed connective tissue ingrowth, but, in all likelihood, hindered the bone ingrowth. Absorption of the PGA mesh took place in six to twelve weeks.

Fibrin sealant is a resorbable, biological binding agent and can be used by mixing with HA particles for obtaining mouldable implants. Hotz (1991) studied fibrin-HA implants in an experimental alveolar ridge augmentation. The sealant prevented HA migration and dislocation and retained the shape of the implant until sufficient connective tissue ingrowth. The authors did not report any bone ingrowth.

Cook (1994) made an experimental study using specially fabricated torous-shaped dense HA particles with a hole, through which an absorbable PGA suture was threaded. These kinds of "HA-ropes" were further bound together with PGA sutures to form a bundle, which was implanted on the extracted site of the mandibular ridge in dogs. They found neither complications nor migration, and the implants maintained the initial augmentation height as compared to free HA particle implantation which lost 25 % of its height. The histologic studies showed better bone ingrowth into the augmentation with the HA-rope as compared to the HA particles alone.

Tominaga et al. (1994) studied the utility of the subperiosteal tissue expansion for mandibular augmentation with particulate HA in dogs. After the subperiosteal tissue expansion a firm fibrous capsule was formed inside the expanded tissues. The study showed that this capsule allowed consolidation of the HA particles and prevented their migration, though the bone ingrowth was delayed and was not seen until after one year. The capsule maintained well the desired contour of the HA augmentation. If the firm capsule overlying the mandibular bone was removed before the implantation of HA, marked resorption of the mandibular cortex resulted, though the bony union of the HA particles was soon evident.

2.7.2.2. Augmentation with a combination of HA and osteoinductive material

By combining particulate HA and autogenous bone more active bony ingrowth has been revealed throughout the implant when compared to HA alone (Block and Kent 1985). The assessments of augmentation in clinical series have, however, been similar to augmentation with particulate HA alone, and the combination has been considered useless. Likewise, the induction of osteogenesis with demineralized bone was sparse and delayed (Block et al. 1987).

Although different purified collagens, fibrin glues or resorbable agents are used as binding vehicles or carriers for particulate HA, the HA-carrier combination may also be reverse. HA can be used as carrier material for osteoinductive proteins to obtain new composite osteoinductive materials. Under the influence of bone morphogenetic protein BMP, the primary non-osteogenic cells can be transformed into osteoblasts. Thus, theoretically, the delivery system for contour augmentation should maintain the shape until new bone ingrowth retains the reconstruction. HA and TCP in their different forms (Urist et al. 1984, Kawamura et al. 1987) and HA composites such as HA/collagen and HA/fibrin glue, have been studied as carriers for BMP. Hotz and Herr (1994) studied different kinds of HA as delivery and osteoconduction system for BMP for ectopic bone formation in rats. All tested ceramic materials showed a good incidence of bone formation ranging from 75 % to 100 %. They also reported clinical cases for facial contour augmentation with the BMP/HA bone substitute. Likewise, Kusumoto et al. (1997) studied recombinant BMP in combination with porous HA in the rat muscle pouch and found new trabecular bone in all porous HA implants immersed with BMP. In the experimental ridge augmentation by Barbosa et al. (2000) the combination of recombinant BMP and particulate HA exhibited some bone growth among HA and clinically relevant augmentation. Further studies would, in all probability, widen the range of applications of HA as carrier and conductor for bone tissue engineering.

2.7.2.3. Clinical studies on particulate hydroxyapatite augmentation

The clinical studies on mandibular augmentation with particulate HA are mainly focused on applying different containment methods for HA, e.g. using collagen tubes (Gongloff 1988, 1992), polyglactin (Vicryl™) tubes (Sugar et al. 1995, Härle and Kreusch 1991, Brown et al. 1992), and polyglycolide suturing matrix (Cook 1994). Various binding substances such as purified fibrillar collagen (PFC) (Marouf et al. 1990, Marshall 1989, Mehlisch1989, Mercier et al. 1992, Mercier et al. 1996) or fibrin sealant (Tisseel™) (Meijer et al. 1997) have also been added to HA particles and used in clinical series to avoid dispersion.

In their experimental studies Gongloff and Montgomery (1985) found collagen tubes successful as containers for particulate HA augmentation. Histologically, however, at the osseous interface the bony contact was intervened with connective tissue, and there was no osseous ingrowth in any implant. Later on, in a clinical series of 20 patients, collagen-contained HA implants with vestibuloplasties improved the patient function and aesthetics in 95 % of the cases

(Gongloff 1988). Likewise, when using contained HA and autogenous bone composite in a small series of six patients the clinical results were good, though ingrowth of bone within the implants was not seen in every sample (Gongloff 1992).

In the studies with Vicryl™ mesh tubes Sugar et al. (1995) reported 11 maxillary augmentations together with open vestibuloplasty and found the result acceptable for prosthetics in nine of the 11 patients. Early postoperative dehiscence was found in three cases. The vertical augmentation height decreased gradually over the follow-up time of three years. The authors made no histologic studies. In the study of Härle and Kreusch (1991) augmentation with a Vicryl™ tube and simultaneous submucous vestibuloplasty were performed in 118 patients. Healing was seen as uneventful in 92 cases, and the complications were dehiscence in 12 cases and local necrosis in 14 cases. Mental nerve hypestesia was detected in 11 % of the patients. The authors had not performed any histologic studies, but no gross instabilities of the HA grafts were reported. In a small series of six patients Brown et al. (1992) reported one postoperative infection and one graft migration, but five of the six patients showed improved ridge form and function. No histologic studies were performed.

Several studies have been performed on particulate HA with resorbable composites as control medium, e.g. fibrin glue (FG) and purified fibrillar collagen (PFC). In addition to binding particles together, the role of the substances added to HA is to evoke fibrovascular reaction at the interface of bone and graft and to promote bone formation (Mercier et al. 1995). Meijer et al. (1997) used prefabricated composite implants made of porous particulate hydroxyapatite and fibrin glue (HA-FG) in a clinical series of 22 patients with 44 augmentation sites. There was a 20 % infection rate postoperatively, and the mean loss of height during the two-year follow-up was marked. Furthermore, the implants showed instability still after a two-year observation period. In the study of Marouf et al. (1990) using HA-PFC composite implants, separation of the HA particles and loss of the preformed shape have been shown to be related to the expansion of collagen on hydration. The histologic analyses in the clinical series showed only slight evidence of new bone formation at the interface of the implant and host bone (Marshall 1989) and, moreover, the only experimental histologic study of these implants showed bone ingrowth only in three out of five specimens (Mehlisch 1989).

3. OBJECTIVES OF THE PRESENT STUDY

3.1. Concepts

The particulate form of HA has been used as bone graft substitute for contour augmentation in oral and maxillofacial surgery, mostly in alveolar ridge augmentation. Particulate HA is prone to dispersion and migration and as a result the augmentation effect is lost. Theoretically, a curved biodegradable containment might better retain the HA graft still allowing direct contact between HA and host bone that is essential for tissue and bone ingrowth. To obtain even more predictable augmentation, the HA containment was further developed by filling the curved implant beforehand with particulate HA using a fast resorbable polymer as adhesive.

The applications of porous HA blocks are limited due to their mechanical fragility. To strengthen the implants the blocks should be coated internally or externally using resorbable materials, which would, however, jeopardize tissue ingrowth. Resorbable fibre reinforcement, however leaves porous HA partially open for intimate tissue contact. The bone bonding, ingrowth, and ingrowth pattern of the PDLLA or PGA fibre reinforced porous HA implants were studied in cortical and cancellous bone implantation. The potential and alluring applications of reinforced HA blocks might be used as bone graft substitutes in vertebral interbody implantation. The mechanical properties, the retainment of the disc height, and the interbody fusion properties of the implants were studied in the lumbar spine in minipigs and growing pigs.

3.2. Aims

Based on the basic concepts, the studies were planned to find answers to the following questions:

1. Can a biodegradable polyglycolide (PGA) curved implant hinder the HA particle migration and allow tissue and bone ingrowth in experimental mandibular ridge augmentation in the sheep mandible diastema model? (I)
2. Can HA particle migration be further diminished by the use of implants made of curved biodegradable container and particulate HA bound together with a fast resorbing PGA/PLA copolymer? Can fast resorbing PGA/PLA copolymer have any harmful effects on tissue and bone ingrowth? (II)
3. What are the bone ingrowth properties and growth patterns in PGA and PDLLA fibre reinforced HA implants when used as bone void fillers in cancellous and cortical bone defects of the rabbit tibiae? (III).
4. What are the fusion properties in plain radiographic, CT, and MRI studies as well as histologically of the porous synthetic and coralline HA implants reinforced either with PGA or PDLLA fibres in anterior lumbar interbody implantation in minipigs? (IV)
5. Can coralline HA implants reinforced with PDLLA fibres maintain the disc space after discectomy and end plate roughening and start a fusion in anterior lumbar interbody implantation in growing pigs? (V)

4. MATERIALS AND METHODS

4.1. IMPLANTS

4.1.1. Hydroxyapatite

Coralline hydroxyapatite used in all experiments in the present study was porous with a mean pore diameter of 200 μm (Interpore International, Irvin, CA, USA). The inner matrix of this HA is coralline derived from the mineral skeleton of the marine coral, reef building coral genus *Porites* with the replamineform process (Roy and Linnehan 1974, Chiroff et al. 1975). The pore sizes ranged from 190 to 230 μm as reported by the manufacturer. The pores were interconnected by channels, forming a continuum without dead ends. Its porosity is 40 % and it has no micropores. Additionally, synthetic sintered porous HA developed by Dr. Petr Patka from the Free University of Amsterdam was used in the block form in lumbar interbody implantation in minipigs (*Study IV*). In this form of HA the interconnected pores were also approximately 200 μm in size varying from 150 μm to 250 μm and the Ca/P ratio was found 2,2 (Patka 1984, Klein et al. 1989). The total porosity of these implants is 45 % and the microporosity 0–5% (pore size 1–2μm).

In the *Studies I* and *II* HA was used in the granular form delivered in 5.00 cc vials. The nominal size of the granules was 425–600 μm in diameter, and each granule had a unique interconnected porous matrix described above (Interpore International, Catalog No. 4210, June 1986). The granular HA was further handled in one-millilitre syringes for placement into the subperiosteal tunnel on the alveolar ridge.

For the *Studies III, IV,* and *V* coralline HA was delivered in rectangular blocks of 30 mm in length, 8 mm in width, and 8 mm in height (Catalog No. 3340, 1986). The synthetic HA of Patka was delivered as cylinders with a diameter of 10 mm and length of 15 mm.

4.1.2. Curved implants for particulate hydroxyapatite containment

For contour augmentation, bioabsorbable groove-like curved implants for HA particle containment were manufactured (Fig 4.). PGA polymer was used as raw material (Dexon® "S", Davis & Geck Inc., USA). The PGA threads were knitted to form a net-like structure, 20 mm x 7 mm in size, which was then molded under high pressure (630 MPa) and temperature (240 °C) to form a curved 2 cm long and 0.5 cm deep implant. This manufacturing process made the implant somewhat flexible facilitating its placement into the submucous tunnel above the alveolar ridge. The curved biodegradable implant was used separately for HA particle containment in the *Study I*.

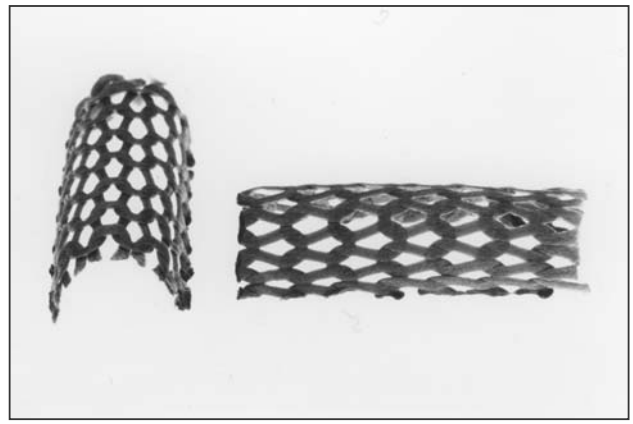

Fig 4. Curved biodegradable implant made of coloured polyglycolide (PGA) threads for coralline HA 200 particle containment (*Study I*, J Oral Maxillofac Surg 1991; 49:1191).

Aiming to improve the biodegradable containment for particulate HA augmentation, a curved biodegradable PGA implant which was prefilled with HA granules bound together with resorbing adhesive was developed, and these implants were used in the *Study II.* Non-coloured PGA threads (Dexon® 2, beige, Davis & Geck, U.S.A.) were used in the implants and knitted to form a net-like structure, which was then molded under high temperature and pressure (240 °C, 630 MPa) to form a curved 2 cm long and 0.5 cm deep implant. The particulate HA used in filling the curved implants was the same porous material used in the *Study I* (Interpore 200, Interpore Inc., CA, USA). The filling was accomplished using polyglycolide/polylactide copolymer (50 % PGA/50 % PLA, Resomer RG 503, Boehringer Ingelheim, Germany) as adhesive agent. According to the manufacturer, this PGA/PLA copolymer degrades in a reasonably short period, in approximately four weeks. The molding process was carried out at 110 °C with a mixture of the particulate HA and PGA/PLA copolymer in the ratio of 4:1. The size and shape of the curved implants were the same as in the *Study I* and the loading volume of HA – PGA/PLA mixture was 2 ml (Fig 5.).

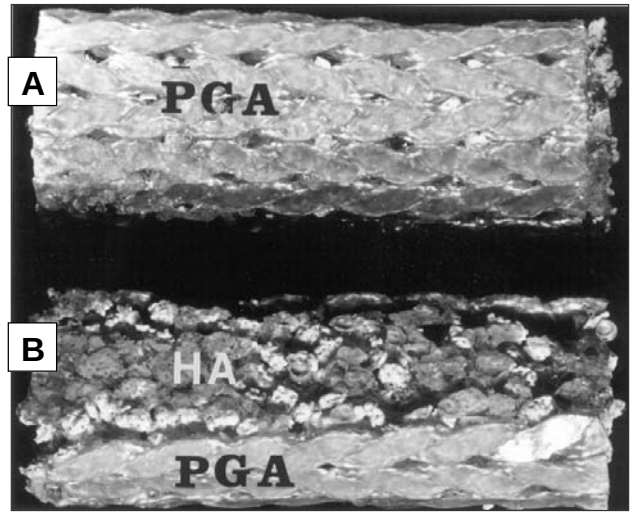

Fig 5. Curved containment device made of non-coloured polyglycolide (PGA) threads prefilled with hydroxyapatite (HA) and fast biodegrading PGA/PLA copolymer as adhesive. A) seen from the convex side, B) seen from the concave side (*Study II,* Int J Oral Maxillofax Surg 2002; 31:405).

4.1.3. Reinforced hydroxyapatite blocks

In the *Study III* smaller blocks from coralline HA of 2 × 3 × 4 mm in size were sawn, and 0.3 mm deep grooves were made on the outer large surfaces, one groove created lengthwise and the other crosswise in the centre of the implant (Fig 6.). Two kinds of these smaller blocks were made by wrapping reinforcing fibres of either poly-DL/L-lactide (PDLLA) of 0.3 mm in diameter or polyglycolide (PGA) (Dexon ® "S" 4–0) into the prefabricated grooves.

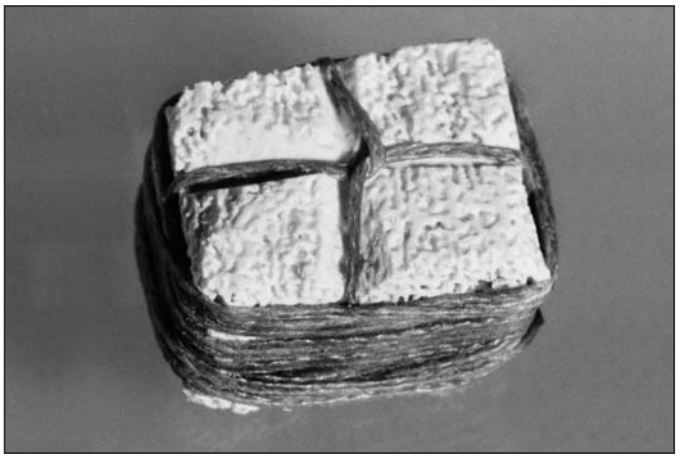

Fig 6. Coralline HA 200 block reinforced with polyglycolide (PGA; Dexon "S" 4–0) fibres. Blocks sized 2 × 3 × 4 mm were used to study the bone ingrowth properties in the implant and tissue reactions against reinforcing fibres (*Study III,* J Mater Sci Mater med 1994; 5: 522).

In the *Study IV* two kinds of HA were used, synthetic sintered porous HA delivered by Patka (Patka 1984, Klein et al. 1989) and synthetic coralline HA (Interpore™). The blocks were sawn as 3 × 8 × 12 in size, and for fibre reinforcement 0, 5 mm deep grooves were made lengthwise and crosswise on the largest surfaces 2 mm apart from each other. The measurements of the implant were similar to those of the disc space of a growing pig weighing 15–20 kg and being equivalent to that of a minipig. For fibre reinforcement PLLA was melt-spun, and the fibre was drawn through DL-PLA solution to coat the fibre and improve its adhesion to the ceramic. The final coated composite fibre was on an average 0, 3 mm in diameter and it was wound into the grooves and around the block so that, for the most part, the ceramic surface was left open to get in contact with the host bone (Fig 7. A and 7. B). Three fibers were wound in each groove. Correspondingly, for PGA reinforcement Dexon® "S" threads were wound in the grooves, and, finally, the blocks were pressed against a hot surface (135 °C) to melt poly-l-lactide and fuse the fibres into each other and into the HA block. The reinforced HA implants were sterilized by gamma radiation. For this study HA blocks were cut randomly without attention to channel axis.

The reinforced blocks used in the *Study V* were all PDLLA-reinforced coralline blocks of 3 × 8 × 12 mm in size.

Fig 7. A) Poly-dl/l-lactide fibre-reinforced coralline HA 200 block sized 2 × 3 × 8 mm. The reinforcing fibres are placed in 0,5 mm deep grooves which were made lengthwise and crosswise on the largest surfaces 2 mm apart from each other. *Studies IV–V.*

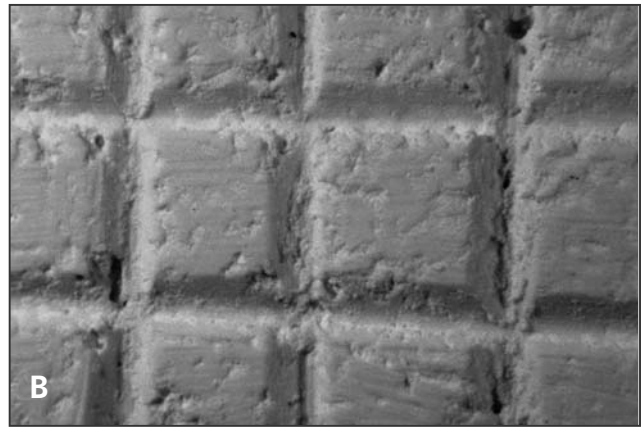

Fig 7. B) Magnification of the grooved HA block reveals that intimate contact between host bone and HA is in large areas possible.

4.2. EXPERIMENTAL ANIMALS

4.2.1. Mandibular augmentation in sheep

Sheep of both sexes were used for experimental contour augmentation on the mandible ridge. A total of 28 sheep were operated on, 20 sheep in the preliminary study of porous HA containment with a curved biodegradable implant in the sheep mandible (*Study I*). Sequels to this study, eight sheep were further operated on using porous HA/polymer-composite prefilled into the biodegradable curved implants (*Study II*). In the *Study I* the sheep weighed mean 35,5 kg (range 15–58,5 kg) and in the *Study II* mean 59 kg (range 48–78 kg). In both studies the follow-up times were three, six, 12, and 24 weeks. No sheep were lost from the follow-up.

4.2.2. Filling of bone defect in rabbits

The Study III was arranged to examine reinforced HA blocks as bone filler. Histologic reactions of reinforcing fibers and their possible impact on bone ingrowth were studied. Twenty-three adult rabbits weighing 3100–4200 g (mean 3620 g) were operated on. In 21 rabbits both hind legs were operated on, and the right tibia was implanted with PDLLA-reinforced HA in created diaphyseal and metaphyseal defects and correspondingly the left tibia with PGA-reinforced HA (Fig 8.). As a pilot study, only one hind leg was operated in two rabbits using PGA-reinforced implants in one rabbit and PDLLA-reinforced implants in the other rabbit and these were also included in the study. The follow-up times were six, 12, 16, and 24 weeks. One rabbit with bilateral operation was sacrificed because of postoperative fracture of the other tibiae.

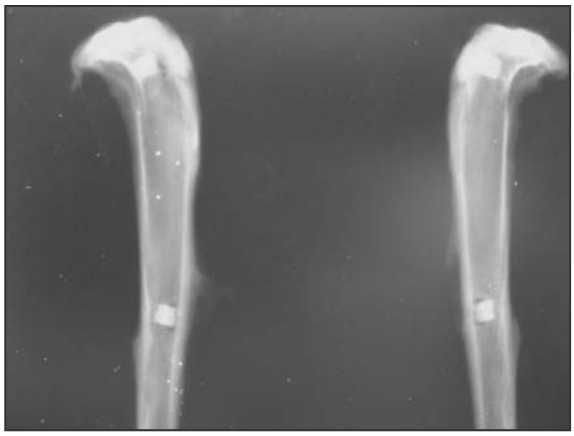

Fig 8. Radiograph of both tibiae of the rabbit after sacrifice after six-week follow-up. Reinforced coralline HA blocks sized 2 × 3 × 4 mm were implanted in both tibiae of the rabbit in created metaphyseal and diaphyseal defects. Right tibia was implanted with PDLLA- and left tibia with PGA-reinforced HA blocks. *Study III*.

4.2.3. Lumbar interbody implantation in pigs

In the *Studies IV–V* the HA blocks reinforced with either PDLLA or PGA threads were further studied in lumbar interbody implantation in minipigs and growing pigs. In the *Study IV* five minipigs aged 10–15 months and weighing mean 43,3 kg were operated on, and the three adjoining lumbar intervertebral disc spaces (L4–L7) were implanted through laparotomy, i.e. 15 disc spaces in all. In this study three different reinforced HA blocks were compared, PDLLA- or PGA-reinforced coralline HA and PDLLA-reinforced synthetic porous HA delivered by Patka. In the *Study V* 27 growing pigs weighing on an average 16,6 kg (range 13,5–20,5 kg) were operated on also through laparotomy, and in 23 pigs the disc space was replaced by the PDLLA-reinforced coralline HA implant. In four growing pigs two non-adjacent disc spaces were identically exposed, but they were left open without implantation to serve as control. In this series two implanted pigs were lost, one due to intraoperative vascular lesion and the other due to postoperative ventricular dilatation and perforation. Table 3 presents the scheme and summary of the experimental studies on the applications for HA grafting in different experimental animals.

Table 3. Scheme of experiments in (A) HA contour augmentation in sheep and (B) of using reinforced HA blocks as bone filler in the rabbit tibiae, and (C) in lumbar interbody implantation in minipigs and growing pigs.

Study	Experimental animal (no.)	Number of experimental animals followed up weeks				
		3	6	12	16	24
I (A)	Sheep (20)	5	5	5	–	5
II (A)	Sheep (8)	2	2	2	–	2
III (B)	Rabbit (23)[1)]	–	5	5	5	7
IV (C)	Minipig (5)	1	1	1	1	1
V (C)	Pig (27)[2)]	6	6	6	7	–

[1)] one rabbit was sacrificed because of postoperative tibial fracture
[2)] two pigs died because of postoperative complications, one due to the intraoperative vascular lesion and the other due to ventricular perforation

4.3. ANAESTHESIA AND OPERATIVE TECHNIQUES

4.3.1. Augmentation procedure

In the sheep the edentulous region of the mandible between the molar and incisor teeth, the diastema, was operated on both sides. For premedication atropin 0,01 mg/kg (Atropine 1 mg/ml injection, Orion, Finland) and for infection prophylaxis procaine penicillin 1,2 million IU (Procain-Penicillin Novo for veterinary use 300 000IU/ml, Novo Industri AS, Copenhagen, Denmark) were administered i.m. preoperatively. In the preliminary series (*Study I*) acepromatzin 0,1 mg/kg was also used for premedication (Plegicil for veterinary use 10 mg/ml, Agrivet, Uppsala, Sweden) and the anaesthesia was accomplished by i.v. administration of thiopental 15 mg/kg (Penthotal Natrium 2,5 %, Abbot S.p.A., Italy) and maintained by oxygen-halothane inhalation with a 1,5 % concentration of halothane. No muscle relaxants were used. A nasogastric tube was used to diminish abdominal distension. In the latter series of eight sheep (*Study II*) the anaesthesia was accomplished by i.m. administration of medetomidine 0,025 ml/kg (Domitor 1 mg/ml, Lääkefarmos, Finland) and ketamine hydrochloride 0,02 ml/kg (Ketalar 50 mg/ml, Parke-Davis, Spain). Half of the original amounts of medetomidine and ketamine were additionally injected i.v. after 20–30 min. if necessary. In this series the nasogastric tube was not needed.

The operative procedure was a modification of the technique described by Kent et al. (1982) and is described in Fig 9. A–D. The oral mucosa was flushed with chlorhexidine gluconate (Klorheksidos® 5 mg/ml). A vertical incision was made through the mucosa and the periosteum mesially in the edentulous part of the mandible. A subperiosteal tunnel, approximately two centimetres in length, was created along the ridge. The periosteum was incised longitudinally and its sides were reflected. A slight decortication of the ridge was done with the help of an elevator. In the *Study I* 0,7 ml of porous particulate HA (Interpore 200™) was injected with a one-millilitre syringe into the subperiosteal tunnel on the right diastema. On the left diastema the curved PGA implant (Fig.

4) was first inserted into the tunnel with the help of the forceps, and thereafter the same quantity of particulate HA was injected under it. The mucosal incision was closed with interrupted resorbable sutures (Dexon® "S" 3–0). Postoperatively the sheep received liquid food for two days.

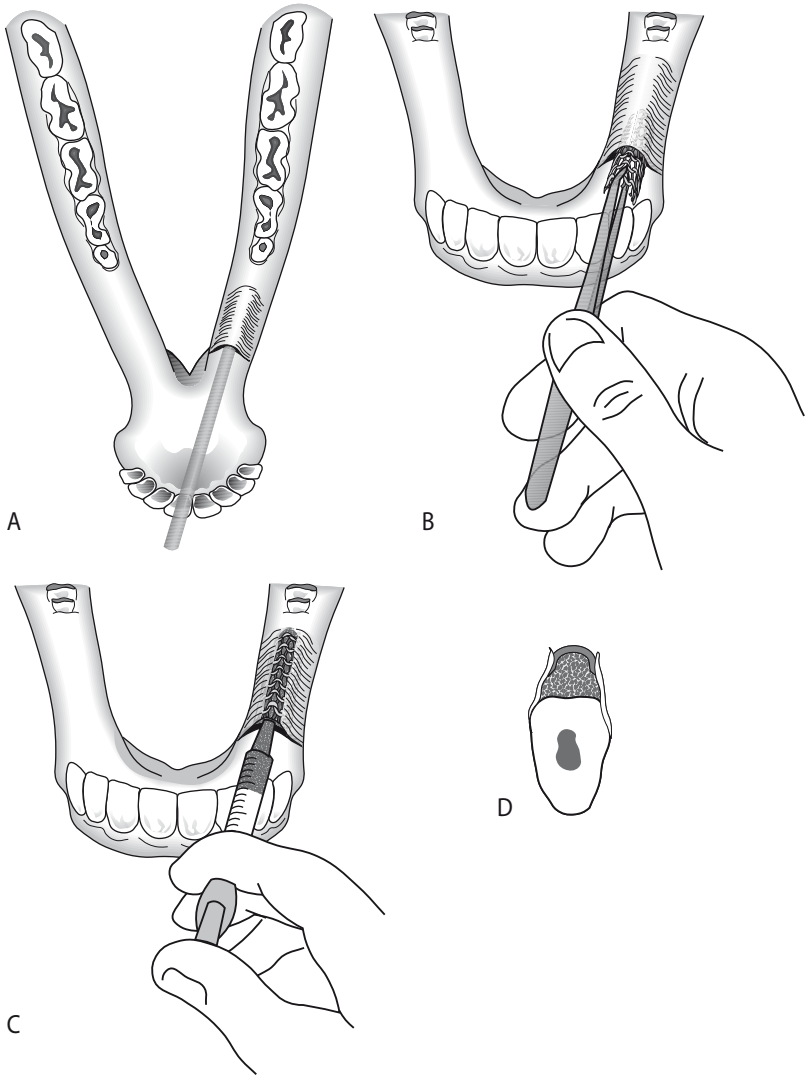

Fig 9. Semantic presentation of the mandibular augmentation using curved PGA containment.
A. a subperiosteal tunnel, approximately two centimetres in length, was created along the diastema of the sheep mandible. The periosteum was also incised longitudinally and its sides were reflected
B. on the left diastema the curved PGA implant was inserted into the subperiosteal tunnel with the help of the forceps
C. coralline particulate HA 0,7 ml was injected with a one-millilitre syringe into the tunnel under the PGA implant
D. frontally cut presentation of the mandible after HA augmentation. HA layer is lined by the curved implant on the top and by the periosteal flaps on the sides

In the further series (*Study II*) a curved PGA implant with prefilled particulate HA (Fig 5.) was introduced into the tunnel so that the implant was lying on the decorticated ridge, HA facing the ridge. The stability of the implant was tested manually and if it was found grossly unstable, an additional suture was inserted through the mucosa either buccally or lingually. Both sides of the mandible were implanted by this method in eight sheep, i.e. 16 augmentation procedures were carried out in this series. Postoperatively the sheep were maintained on a soft diet for two to five days, and antibiotic prophylaxis with procaine penicillin 1,2 million IU/day i.m. was administered for three postoperative days.

4.3.2. Filling of bone defects

In the *Study III* 23 adult rabbits were operated on. Atropin (Atropin 1 mg/ml, Orion, Finland) 0.5 ml/kg and diazepam (Diapam 5 mg/ml, Orion, Finland) 0.3 ml/kg were injected s.c. for premedication. The anaesthesia was accomplished with subcutaneous medetomidine (Domitor 1 mg/ml, Lääkefarmos, Finland) 0.3 millilitres per kilogram and ketamine (Ketalar 50 mg/kg, Parke-Davis, Spain) 0.5 ml/kg (Mero et al. 1989). As an infection prophylaxis procaine penicillin (Procapen 300 000 I.U./ml, Orion, Finland) was given 150 000 I.U. subcutaneously.

Both hind limbs in 21 rabbits and one hind limb in two rabbits were shaved and scrubbed with antimicrobial solvent (Neo-Amisept®). The proximal tibia was exposed through a medial incision, starting at the joint level and extending three centimetres distally. The medial collateral ligament was identified, and the level of implant application was prepared to be between the joint level and the distal insertion of the ligament, i.e. from three to five millimeters below the joint level. Anterior to the medial collateral ligament a drill hole, 2.0 millimeters in diameter and 4.0 millimeters in length, was drilled transversely to the tibial shaft using physiologic saline (NaCl) for flushing. The drill hole was enlarged with the drill bit so that the metaphyseal defect created was about $2 \times 3 \times 4$ millimetres in size. Finally, the drilled cavity was copiously irrigated with saline to remove all debris before implant insertion. A similar bone cavity was created through a lateral incision into the cortical bone region of the middle part of the tibia. The reinforced HA blocks were plugged in so that the implants were flush with the tibial bone cortex. The fascia and cutis were sutured with interrupted 3–0 Dexon® sutures. In 21 rabbits both hind legs were operated on; the right leg was implanted with PDLLA-reinforced HA and the left leg with PGA-reinforced HA, respectively (Fig 8.). As a pilot study, only one hind leg of two rabbits was operated, the one implanted with a PDLLA-HA and the other with PGA-HA implant, both followed up for 24 weeks and they were also included in the study.

4.3.3. Lumbar interbody implantation

Five minipigs (*Study IV*) and 27 growing pigs (*Study V*) were operated on through laparotomy. The pigs fasted for one preoperative day. Atropine 0,1 mg/kg (Atropin® 1 mg/ml, Orion, Finland) and diazepam 0,2 mg/ml (Diapam® 5 mg/ml, Orion, Finland) were injected intramuscularly (i.m.) as premedication approximately 30 minutes before the anaesthesia was inducted. Ketamine

hydrochloride (Ketalar® 50 mg/ml, Parke-Davis, Spain) was administered 10 mg/kg i.m. The induction of the anaesthesia was accomplished by i.v. administration of thiopental until effect (approximately 8 mg/kg) (Penthotal® Natrium 2,5%, Abbot S.p.A., Italy). After induction topical anaesthetics (Xylocain®) were sprayed into the pharynx to eliminate pharyngeal and tracheal reflexes and laryngospasm during laryngoscopy and intubation. The pig was intubated, and the anaesthesia was maintained by oxygen-halothane inhalation with a 1,5 % concentration of halothane. Before starting the operation, the prophylactic antibiotic of one million units of procaine penicillin (Procain-Penicillin Novo® 300,000 IU/ml, Novo Industri AS, Denmark) was injected i.m. The pig was placed in a supine position, and a sand bolster was settled under the lumbar vertebrae to accentuate lumbar lordosis. The operation area was scrubbed with betadine solution (Betadine® 100 mg/ml, Leiras Oy, Finland). The abdomen was opened by a paramedic left-sided rectus muscle-splitting incision. The small intestine, the greater omentum, and the transverse colon were kept in the upper abdomen with the help of a flexible tampon and by placing the pig in the Trendelenburg position. Using deep wound retractors an adequate exposure to the retroperitoneum was achieved. The retroperitoneum was incised left to the lower lumbar vertebral bodies and to the abdominal aorta, carefully preserving the great vessels of the mesocolon and the left ureter. Leaving the aorta and vena cava on the right side of the incision, the finger was smoothly introduced retroperitoneally freeing the attachments of the peritoneum and exposing the long anterior ligament. The ligamental attachments were then freed from the vertebrae with a knife at the area of the two to three lower segments. With the help of two blunt retractors the freed ligament, along with the great vessels, was reflected to the right to get an exposure to the intervertebral disc. The disc spaces were then exposed, in the mipigs three adjoining lumbar disc spaces L4-L7 and in the growing pigs L4-L5 or L3-L4. The disc material was removed, and the cartilaginous end-plates were freshed down to the bleeding bone using a scalpel, rongeurs, and curettes (Fig 10. A). The posterior annulus was kept intact. Before implantation the disc space was irrigated with saline. The reinforced HA block of $2 \times 3 \times 8$ mm in size was introduced into the disc space (Fig 10. B). Care was taken to get it in deep enough so that the eight-millimetre-long edge of the implant was flush with the anterior lumbar bodies. In order to minimize the risk of further slippage, the long anterior ligament was replaced onto the vertebral bodies. The ligament was refixed into the vertebral bodies with a few sutures driven through the vertebral bone after restoring the normal lordosis. No drainage was used. Flunixin meglumine (2mg/kg, Finadyne®, 50 mg/ml, Orion, Finland) was used as analgesic. Five minipigs and 27 growing pigs were operated on. In the minipigs three different reinforced HA implants were inserted according to the code known by the surgeon: PDLLA-reinforced synthetic HA (Patka) implant, PDLLA-reinforced coralline HA (Interpore™) implant, and PGA-reinforced coralline HA (Interpore™) implant. The follow-up times were three, six, 12, and 16 weeks and in the minipigs also 24 weeks. In four growing pigs two non-adjacent disc spaces were identically exposed, but they were left open without implantation to serve as control.

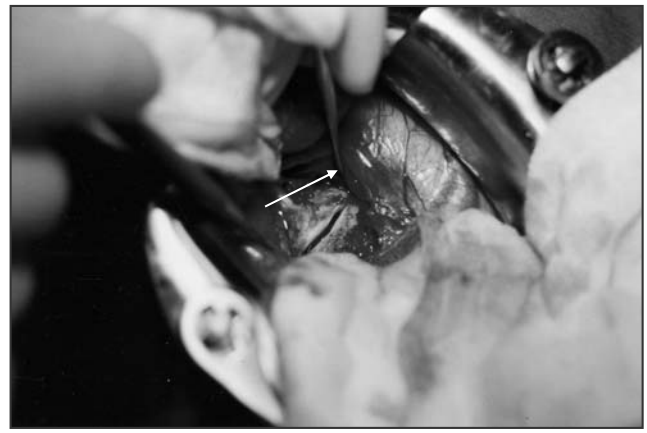

Fig 10. A) Anterior exposure of the lumbar disc space in a growing pig. Disc material has been removed, and the vertebral end-plates are roughened. Anterior longitudinal ligament (arrow) has been detached and reflected on the right side with the help of a small retractor.

Fig 10. B) Insertion of the reinforced coralline HA 200 block into the evacuated lumbar disc space in a growing pig.

4.4. TISSUE SAMPLING TECHNIQUES

4.4.1. Radiographs

Postoperative radiographs were taken of the pigs during the anaesthesia in antero-posterior and lateral projections from the lumbar spine with the focus object distance of 120 cm. From seven to ten days before sacrifice OTC (Terramycin® 100 mg/ml, Pfizer, Brussels, Belgium) was given i.m.to all animals to visualize newly-formed bone in the OTC labelling studies (Milch et al. 1958). The sheep and the pigs were sacrificed in the slaughterhouse. The mandible of sheep was exarticulated, and radiographs were taken on its both sides in lateral and occlusal projections at a focus object distance of 120 cm. The lumbar vertebrae of the pigs with psoas muscles were detached, and radiographs were taken in antero-posterior and lateral projections at a distance of 120 cm. In the minipigs the sagittal CT images as well as the MRI studies were performed of the retrieved lumbar spine block (Philips Tomoscan 60/TX, slice thickness 2 mm, Magneton 42 SP, Siemens AG operating at 1,0T). The CT evaluation included scoring of vertebral sclerosis as grades 0–4. The evaluation of the MRI studies comprised scoring (grades 0–4) of the intervertebral disc height, roughness of the end-plates, thickening of the anterior longitudinal liga-

ment, and signal intensities of the discs and of the vertebral bodies.

The rabbits were sacrificed with a subcutaneous overdose injection of medetomidine and ketamine, and after that an air embolus was given i.v. The operated tibiae were retrieved and radiographs in standardized lateral projections were taken of them (Fig 8.).

4.4.2. Bone sampling

All samples were fixed in a series of ethanol immersions of increasing concentrations of 70 %–99 % and then embedded in methylmetacrylate. Five micrometer-thick sections were cut using a microtome Jung Polycat S for histologic and histomorphometric studies. For microradiographic and oxytetracycline (OTC) fluorescence studies 80-µm-thick sections were cut with a saw microtome Leitz 1600. The histologic specimens were stained by the Masson-Goldner method (Goldner 1938). In microradiography, the Faxitron cabinet x-ray system (Hewlett-Packard 43855A) and Kodak Spectroscopic plates were used.

HA augmentation (Studies I–II). From the mandibular bone the augmented part was devided into two pieces for sampling. The specimens were cut in cross-section starting at the mesial end as follows: a five-µm-thick section for histologic and histomorphometric studies, a 80-µm-thick sample for microradiographic studies, a discarded section of two millimetres and finally another five-µm-thick and an 80-µm-thick specimen. Accordingly, for histomorphometric and histologic studies there were four samples from both sides of the mandible and eight samples from each sheep. This provided a total of 224 samples for histologic/histomorphometric and for microradiographic/OTC studies (Table 4).

Filling of bone defects (Study III). The proximal and middle parts of the tibiae, including the implants, were sawn for sampling. The five-µm-thick sections were sawn transversally, the first section when the implant was displayed totally, and, from that level, the deep section at 1 mm depth. Between the two histologic sections an 80-µm-thick section was taken for microradiography and OTC labelling studies. In all, there were 84 histologic/histomorphometric and 42 microradiographic/OTC specimens available for both types of implants (Table 4).

Lumbar interbody implantation (Studies IV–V). From the spine block the samples including the operated disc were sawn sagittally in the middle of the adjoining vertebrae. The corresponding facet joints were also detached for sampling. In the growing pigs the samples were taken from the upper unoperated spine unit, respectively. Accordingly, the specimens from the non-implanted control spines were taken from the evacuated disc segments, from the intact disc segments, and from the facet joints, respectively.

The blocks with disc spaces were sawn sagittally in the middle of the block for histologic and histomorphometric and for microradiographic and OTC fluorescence studies. In the facet joint specimens the cutting level for histologic and 80-µm-thick sections was determined as transverse in the middle of the joint line. In all, there were 15 implanted histologic and microradiographic disc specimens in the minipig series *(Study IV)* and 25 implanted and eight evacuated control disc specimens in the growing pig series, respectively (Table 4).

Table 4. Scheme of specimens made for histologic, microradiographic and OTC studies. *Studies I–II* include HA augmentation, *Study III* filling of bone defects in the rabbit tibiae, and *Studies IV–V* lumbar interbody implantations in minipigs and growing pigs.

	Follow up, weeks					
	3	6	12	16	24	
	Number of specimens					total no.
HA augmentation (I–II)						
Histologic specimens:						
With curved implant	20	20	20		20	80
Without implant	20	20	20		20	80
With prefilled implant	16	16	16		16	64
Microradiographs + OTC:						
With curved implant	20	20	20		20	80
Without implant	20	20	20		20	80
With prefilled implant	16	16	16		16	64
Filling of bone defects (III)						
Histologic specimens:						
PDLLA-reinforced coralline HA implants		20	20	20	24	84
PGA-reinforced coralline HA implants		20	20	20	24	84
Microradiographs + OTC:						
PDLLA-reinforced coralline HA implants		10	10	10	12*	42
PGA-reinforced coralline HA implants		10	10	10	12*	42
Lumbar interbody implantation, minipigs (IV)						
Histologic specimens:						
PDLLA-reinforced synthetic HA-implants					5	5
PDLLA-reinforced coralline HA implants					5	5
PGA-reinforced coralline HA implants					5	5
Microradiographs						
PDLLA-reinforced synthetic HA implants					5	5
PDLLA-reinforced coralline HA implants					5	5
PGA-reinforced coralline HA implants					5	5
Lumbar interbody implantation, growing pigs (V)						
Histologic specimens:						
PDLLA-reinforced coralline HA implants	6	6	6	7		25
Facet joints of the unoperated disc unit	12	12	12	14		50
Microradiographs + OTC:						
PDLLA-reinforced coralline HA implants	6	6	6	7		25
Facet joints	12	12	12	14		50
Control discs	2	2	2	2		8
Control facets	4	4	4	4		16
			Total no. of specimens			904

*OTC fluorescence studies not done

4.4.3. Histomorphometric and X-ray studies

The histomorphometric studies were performed with the Kontron Videoplan Image Processing System (Kontron, Munich, Germany). A Leitz microscope was connected to the computer, and the microscopic field was displayed on the screen. The lens used in the augmentation studies was 1,6 × (*Studies I–II*) and in the implantation studies 2,5 × (*Studies III–V*). The measuring fields were determined separately in studying HA augmentation (*Studies I–II*), HA implantation (*Study III*) or lumbar interbody implantation (*Study V*). In each specimens the areas of HA, ingrown connective tissue, and ingrown new bone were measured.

In the first studies on HA augmentation the planimetric measurements were performed on radiographs (*Study I*) and on microradiographs (*Studies I–II*) using transparent millimeter paper and a Peak magnification loupe (Tohkai Sangyo Ltd, Tokyo, Japan). In the further studies X-ray analyses were performed using a Videoplan and a digitizing board by coupling the radiographs from the light board with a video camera to the screen. Using a Videoplan in X-ray planimetry the final result was the mean of five successive measurements (*Study II*). In the *Study V*, in measuring the height of the disc space the result was considered the mean of the measurements, both from the lateral and antero-posterior projections, one measurement from each margin of the disc space.

4.5. STATISTICAL METHODS

The strength of association between the variables of the histomorphometric measurements in the *Study I* were analysed using the Pearson correlation coefficient. The planimetric measurements on radiographs and microradiographs between the two augmentation methods (*Study I*) were compared applying the Student's t test. In the *Study II* statistical comparisons of expectations were performed using the one-way analysis of variance (ANOVA), and a further analysis was made by grouping the means (Tukey method). In the *Study III* the Pearson correlation coefficient and the Student's t-test were used in comparing histomorphometric measurements. In the *Study IV*, using the Kruskal-Wallis test, comparisons were made between the three kinds of implants as well as between the operated and non-operated discs. The imaging modalities were compared using the Wilcoxon signed-rank test, and the correlations were calculated by the Spearman rank correlation test. Finally, in the *Study V* the histomorphometric and radiological measurements were analysed by using the one-way analysis of variance (ANOVA). A further analysis was made by grouping the means between the follow-up groups (Tukey method) or by using the regression analysis. All the statistical comparisons of expectations were performed at the risk level of 0,05.

4.6. ETHICAL CONSIDERATIONS

The complete study plan was presented and accepted in the Ethical Committee of the Helsinki University Central Hospital. Permissions for experimental mandibular operations in sheep and for anterior lumbar interbody fusion in minipigs and growing pigs were further presented and accepted in the Ethical Committee of the Department of Surgery, Faculty of Veterenary Medicine, University of Helsinki. The permission to the author to perform lumbar interbody fusion operations in minipigs (Dno 16259 972 88 170) and growing pigs (Dno 26385 972 87 170) was given by the Department of Social and Health Affairs of the State Provincial Office of Southern Finland.

5. RESULTS

5.1. ALVEOLAR RIDGE AUGMENTATION WITH PARTICULATE HYDROXYAPATITE

Clinically there were seven out of 20 augmentations with dehiscence in the series where the curved augmentation device was used separately with particulate HA, i.e. in 35 % of the cases. Three fistulae still existed at sacrifice; the others were healed during the follow-up period. When HA was preimpacted with the help of the resorbing adhesive prior to surgery inside the curved implant, the fistulous tract was seen in three out of 16 augmentations at three weeks (19 %). All these dehiscences in both series occurred at the suture line. No local complications were seen when the augmentation was performed with particulate HA alone.

5.1.1. Radiology

In the planimetric measures the HA migration showed to be significantly greater when using conventional method compared to HA augmentation with PGA containment ($p<0{,}002$, t-test, *Study I*), Fig 11. However, the augmenting effect of HA, i.e. the height of the HA layer remaining on the ridge, did not differ significantly ($p<0{,}2$, t-test). By both methods of the *Study I* the augmentation effect seemed to decrease toward the end of the 24-week period, the height being only 1–2 mm at 24 weeks. In the cases of the impacted HA/polymer-composite the measured augmenting areas of HA varied greatly showing according to regression analysis no correlation to the follow-up time.

On the microradiographs the HA remaining on the ridge (Fig 12.) was significantly

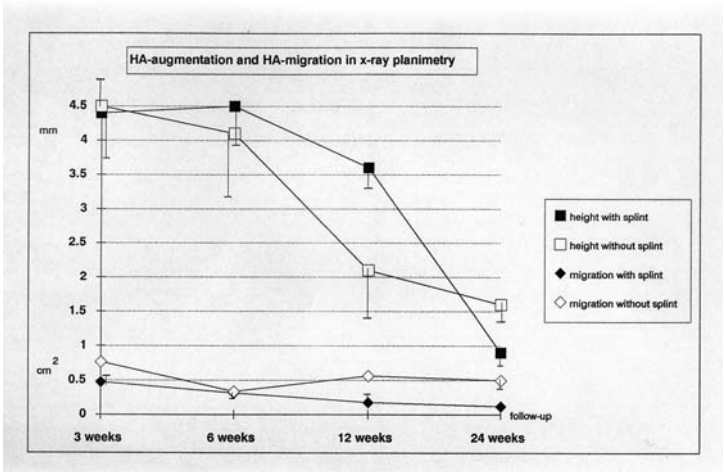

Fig 11. Results of the planimetric studies in experimental alveolar augmentation when the conventional method was compared to the use of a curved PGA containment (*Study I*). HA migration measured in sq cm was significantly greater without containment. The heights of the HA layer did not differ, and the augmentation was partly lost towards the end of the follow-up. (J Oral Maxillofac Surg 1991; 49: 1191).

greater in the cases with HA augmentation without containment compared to those with the PGA containment (p<0,001, t-test, *Study I*). In the cases of HA/polymer composite the amounts of HA on the mandible remained fairly unchanged, that is the mean values were not statistically different (ANOVA, p=077, *Study II*) in contrast to the two other methods where there was a tendency of HA to decline during the follow-up period. The actual HA areas in the two studies are not, however, fully comparable due to e.g. the slightly different loading volumes of HA.

5.1.2. Histology

HA did not stain and was seen histologically as void spaces. Fine collagen fibres were ingrown inside the interconnected porous matrix of HA from three weeks on, and in that respect there seemed to be no difference between the two methods in the *Study I*, i.e. HA augmentation with or without biodegradable coverage, Fig 13. A–E. At the beginning, the connective tissue was loose and poorly organized. During the follow-up time the collagen fibres became richer

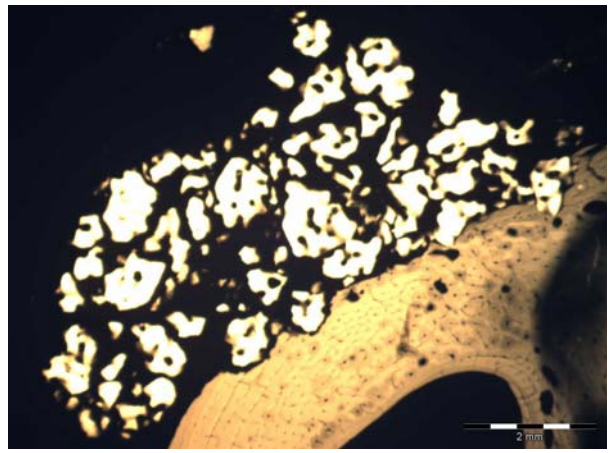

Fig 12. Microradiograph of the alveolar augmentation with HA/polymer composite, *Study II*. Follow-up of 24 weeks. By this method HA remained on the ridge fairly well and showed to be in close contact with the host bone, though histologically no bone ingrowth was encountered. Scale bar 2 mm.

in number and were intensively staining. In some parts of the collagen tissue projections the numbers of osteoblasts and multinucleated osteoclast-like cells were seen lining the HA matrix, also already at three weeks. Later on, these areas started to calcify forming new bone, Fig 13. D–E. Osteoclasts and osteoblasts were numerous towards the end of the follow-up, indicating that an active process of bone formation and remodelling was continuing. Contrary to that, a lower number of osteoblasts and even multinucleated osteoclast-type cells were seen at the beginning of the follow-up when the preimpacted HA/polymer-composite was tested (*Study II*), and towards the end of the 24-week follow-up their number still decreased. Thus, some bone ingrowth into the HA layer was often seen when HA augmentation was performed without or with the separate PGA containment. In the case of bone ingrowth, new bone was formed against the HA layer without any intervening tissue reaction, and at the end of the follow-up the connective tissue seemed again looser, and the number of active osteoblast cells was smaller, Fig 13. E. However, the findings of new bone formation were ab-

sent or very rare in the study of prefilled HA/polymer-composite implants. Moreover, multinucleated foreign-body-type cells were encountered in the interface between the alveolar ridge and HA when the HA/polymer composite was used, Fig 14. A–C.

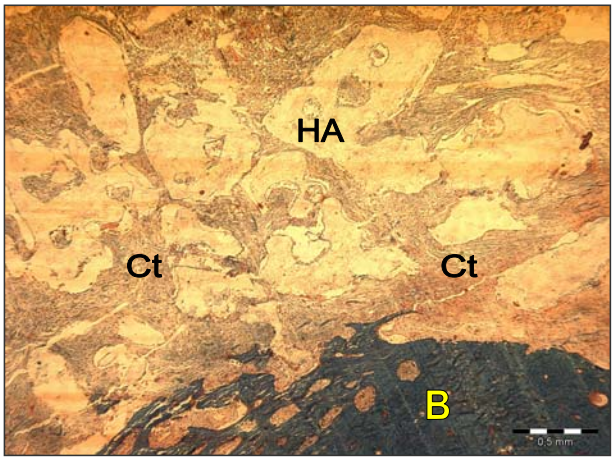

Fig 13. A) Frontally cut histologic specimen of alveolar augmentation using a containment device, *Study I*. Three weeks of follow-up. No bone ingrowth is seen, but connective tissue has grown abundantly into the HA graft. B= alveolar bone, HA= hydroxyapatite, Ct= connective tissue. Masson-Goldner stain. Scale bar 0,5 mm.

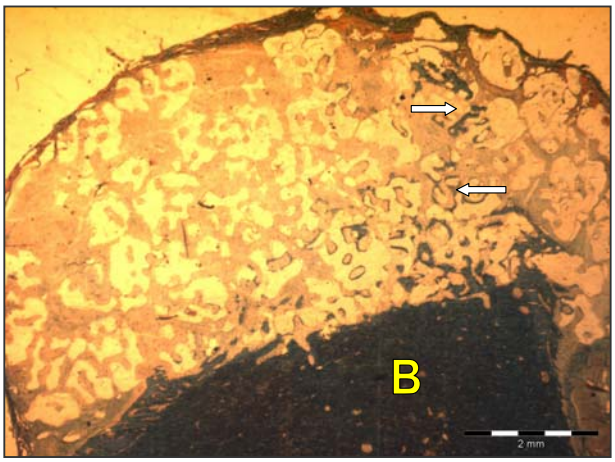

Fig 13. B) Frontally cut histologic specimen after six weeks of follow-up of HA augmentation of the alveolar ridge in sheep without biodegradable curved containment, *Study I*. Some bone ingrowth into the HA graft is seen (arrows). B= alveolar bone. Masson-Goldner stain. Scale bar 2 mm.

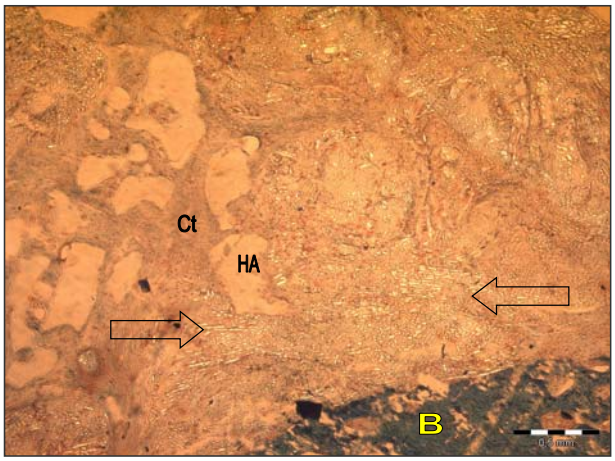

Fig 13. C) Frontally cut histologic specimen of alveolar augmentation with PGA containment, *Study I*. Connective tissue is abundant, and PGA degradation products (open arrows) are seen being birefringent. No bone ingrowth. Follow-up of six weeks. B= alveolar bone, HA= hydroxyapatite, Ct= connective tissue. Masson-Goldner stain. Scale bar 0,5 mm.

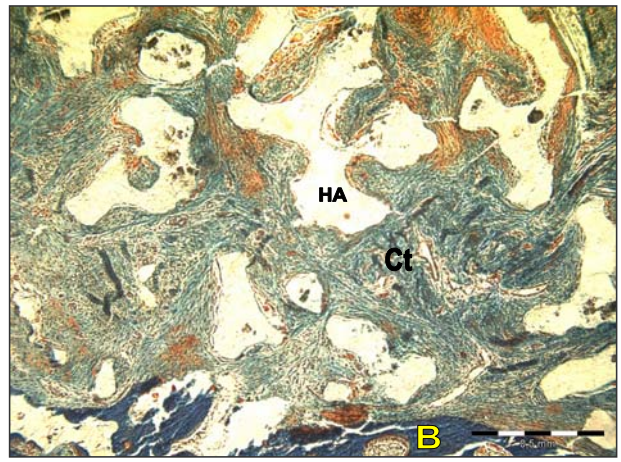

Fig 13. D) Frontally cut specimen of alveolar augmentation with PGA containment after 24 weeks, *Study I*. Connective tissue ingrowth inside the HA graft is very intensive, but no bone ingrowth is seen. B= alveolar bone, Ct= connective tissue, HA= hydroxyapatite. Masson-Goldner stain. Scale bar 0,5 mm.

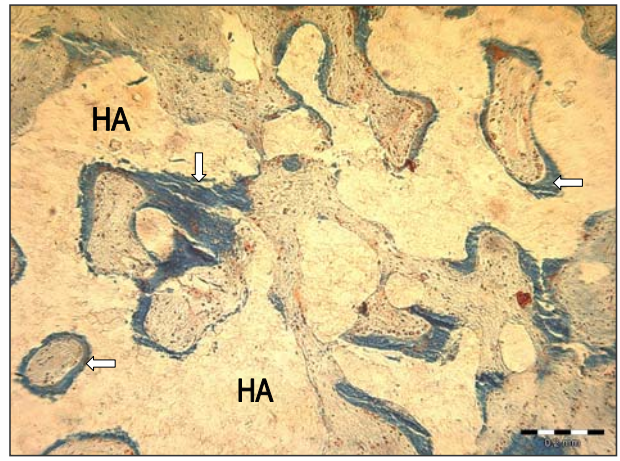

Fig 13. E) Frontally cut histologic specimen of alveolar augmentation without containment at 24 weeks, *Study I*. New bone formation (arrows) can be seen against HA without any intervening tissue reaction. In areas of good bone ingrowth the structure of connective tissue is looser, and the number of active osteoblasts is lower. HA= hydroxyapatite. Masson-Goldner stain. Scale bar 0,2 mm.

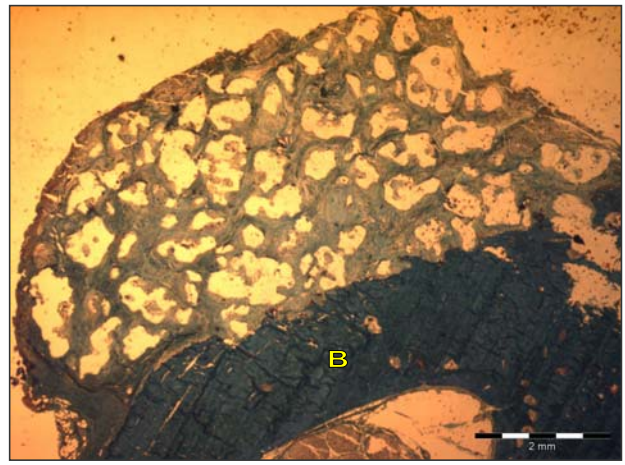

Fig 14. A) Frontally cut histologic specimen of alveolar augmentation with prefilled HA/polymer containment, *Study II*. Connective tissue reaction inside the HA layer is rich and intensively staining. Follow-up of 24 weeks. B= alveolar bone. Masson-Goldner stain. Scale bar 2 mm.

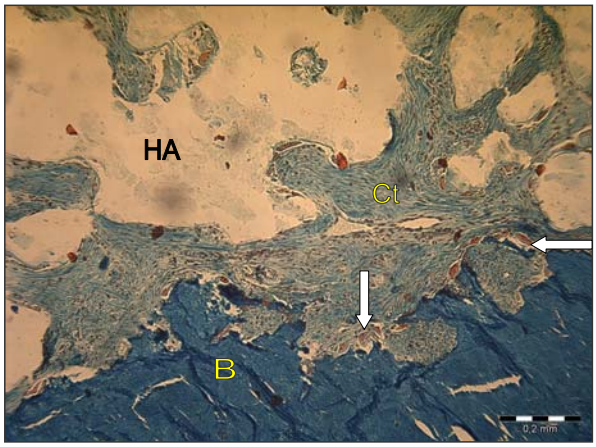

Fig 14. B) Magnification of the histologic specimen in Fig. 14 A. Augmentation with prefilled HA/polymer containment, follow-up of 24 weeks, *Study II*. Magnification reveals no bone ingrowth; some multinucleated foreign-body-type cells (arrows) are seen in the border of the alveolar bone and HA graft. HA= hydroxyapatite, B= alveolar bone, Ct= connective tissue. Masson-Goldner stain. Scale bar 0,2 mm.

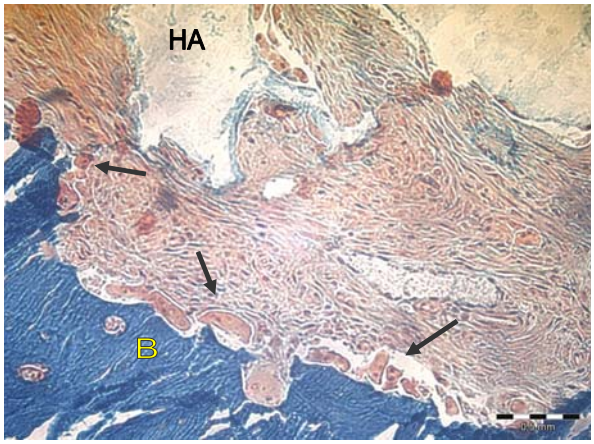

Fig 14. C) Frontally cut specimen of alveolar augmentation with prefilled HA/polymer containment, *Study I*. Follow-up of 24 weeks. Several multinucleated foreign-body-type cells (arrows) inside the connective tissue (Ct) are seen between the alveolar bone (B) and HA. Masson-Goldner stain. Scale bar 0,5 mm.

5.1.3. Histomorphometry

The surface areas of connective tissue, new bone, and HA were measured and the amount of them in relation to the total measuring fields was calculated. The results showed that in the *Study I* the connective tissue reaction inside the porous HA augmentation was more abundant when the biodegradable PGA containment was used ($p< 0{,}001$, Pearsson correlation test). The percent area of connective tissue varied around 60 % in uncontained and around 70 % in PGA contained augmentations, thus the difference was around 10 % all along the 24-week follow-up. Likewise, the connective tissue reaction was abundant from the beginning with the use of the pre-impacted HA/polymer-composite augmentation device, and percent areas around 75 % were measured in the *Study II*. In that study the percentual rates were approximately of the same order – or even slightly higher – than when using containment alone. Statistically according to two-way analysis of variance, in both studies and in all three methods, the measured percent amounts of connective tissue varied only slightly, i.e. they were not time-dependent.

The new bone measured inside HA increased during the follow-up in contained and uncontained HA augmentation (*Study I*).

In augmentation without containment the new bone ingrowth was significantly greater (P<0.05, ANOVA) and exceeded 12 % of the total measuring area at 24 weeks compared to 10 % with biodegradable containment. Contrary to these findings, the new bone ingrowth was virtually inconsiderable in the cases of the HA/polymer-composite devices (*Study II*). Most of the cases in the latter series showed no bone ingrowth.

Finally, percentual amount of HA seemed to decrease in all methods during the 24-week follow up but the decrease with time was not significant. In the *Study I* the percent areas of HA were more dependent on the type of augmentation than on follow-up time (p< 0,001, two way analysis of variance) but, instead, the absolute measure of HA decreased with time (p< 0,001, Pearsson correlation test). In the series with the HA/polymer composite devices the HA amounts remained statistically constant although, in appearance, HA seemed to decrease slightly.

The results of histomorphometric measurements are summarized in Fig 15. A–C.

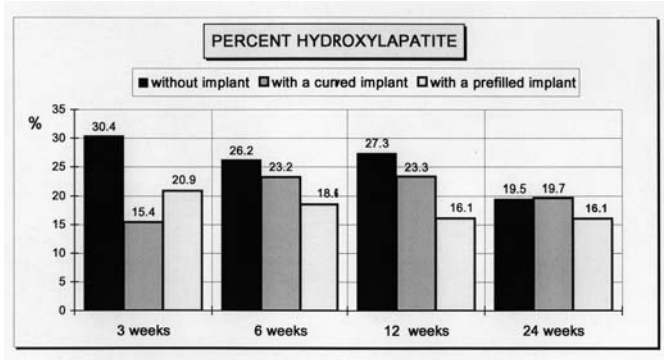

Fig 15. Results of histomorphometric measurements in particulate HA augmentation combined from the two studies (*Studies I–II*). Basically, the latter study is not fully comparable due to slightly different volumes of HA used in the augmentation.
A) although the percent amounts of HA seem to decrease in all three methods in 24 weeks the decrease was not significant and the percent amount of HA was more dependent on type of the implant than on follow-up time (p< 0,001, two way analysis of variance). Absolute measures of HA decreased with time (p< 0,001, Pearsson correlation test).

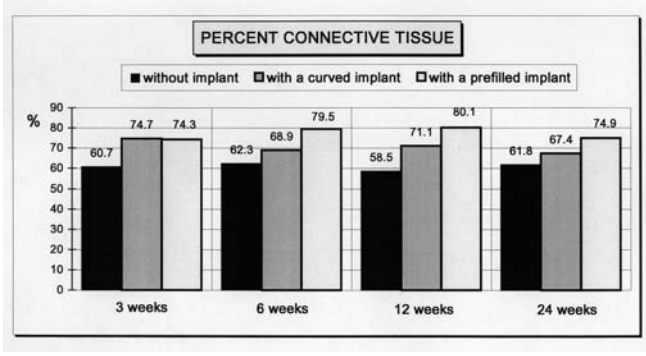

Fig 15. B) Percent areas of connective tissue were higher when separate or prefilled biodegradable splints were used. The difference between uncontained and contained augmentations was significant in the *Study I* (p< 0,001, Pearsson correlation test).

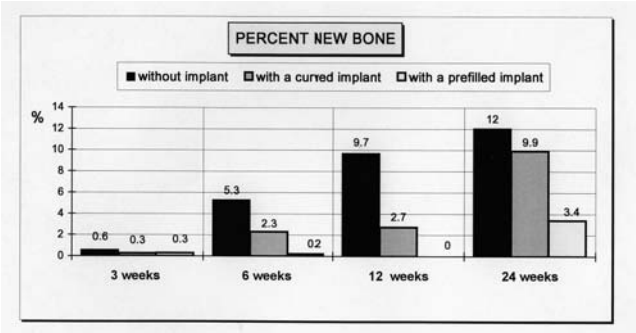

Fig 15. C) Percent areas of new bone in the *Study I* were significantly greater in conventional augmentation without containment ($p < 0,05$, Pearsson correlation test). Specificly, the new bone ingrowth was neglibible when augmentation was done with prefilled HA/polymer containment *(Study II)*.

5.1.4. Oxytetracycline fluorescence studies

The OTC studies were in accordance with histology showing a slightly greater uptake in the HA layer on the side operated without containment. Fluorescence was as it highest at six weeks extending approximately one third of the height of the HA deposit.

5.1.5. Comments

The comparison of the histologic and histomorphometric studies revealed that connective tissue grew into the particulate HA no later than at three weeks. The connective tissue was more abundant if the augmentation was carried out with curved PGA containment compared to using particulate HA alone in the submucosal pocket. In addition, by mixing a fast degrading PGA/PLA copolymer with particulate HA the fibrous connective tissue reaction inside the HA graft was still increased. Contrary to that, the new bone ingrowth was best in augmentations carried out without any containment and exceeded 12 % of total augmentation area at 24 weeks and it was significantly lower with the PGA containment exceeding 10 % at 24 weeks. Interestingly, actually no bone ingrowth was shown in the cases where the polymer composite was used for binding the HA particles together and, related to that, foreign-body-type cells were seen in the interface between the HA and alveolar bone. The PGA degradation products of the curved implant were visible until 24 weeks, but no adverse reactions related to them were seen.

There are studies which have shown some osteogenic potential of PGA. Hollinger (1983) showed accelerated rate of bone healing of osseous wounds with PGA/PLA implants. According to Ashammakhi et al. (1994, 1995) the use of the PGA membrane around the cortical and metaphyseal bone enhanced bone formation. Those findings were thought to be an early osseous inductive response to the PGA degradation products, on the condition that they are acting on the pluripotential or osteogenic cells. The present study shows, however, that when covering the HA graft the PGA degradation products have no inherent bone inductive potential. Instead, they stimulated the fibrous tissue reaction being more abundant throughout the 24-week follow-up. The degradation time of PGA has been shown to be around 30 weeks (Ashammakhi et al. 1995)

5.2. FILLING OF BONE DEFECTS WITH REINFORCED HYDROXYAPATITE BLOCKS

One rabbit operated bilaterally had a postoperative diaphyseal fracture of the tibia on the site of implantation and was sacrificed two days after the operation. Another postoperative diaphyseal fracture was identified at sacrifice at six weeks, but it was healed in a good position and was included in the study. Macroscopically the sites of implantation healed normally, and no infections encountered. Radiologically the implants were visible through the study at 24 weeks. Radiologically no resorption or implant fractures were seen.

5.2.1. Histology

HA did not stain and presented as voids bordering the ingrown tissue. Histologically it seemed that all porous spaces were filled with ingrown connective tissue from six weeks onwards.

Reactions against reinforcing fibres were found from six weeks to the end of the study. In the case of PGA foreign-body-type giant cells and foam cells clustered in the vicinity of the PGA threads, mostly at the ends of the blocks where a number of threads were wrapped. There were also some occasional foreign-body granulomas. The reactions against PDLLA also included several multinucleated giant cells, but the areas around the PDLLA fibres were smaller in size and showed a fibrous-type connective tissue reaction. Accordingly, both types of reinforced HA blocks were surrounded by a fibrous tissue layer being more remarkable at the sites where the reactions against the reinforcing fibres were increased.

In spite of the reactions around the biodegradable reinforcing fibres, the connective tissue ingrowth as well as the bone ingrowth inside the implants was abundant already at six weeks, which was the shortest follow-up period in this series. Considering the ingrown tissue, no difference was histologically seen between the two implants. There were also sites where no intervening tissue was seen between the host bone and the implant, and in those regions the new bone formation was the most advanced also inside the implant. Histologically, bone ingrowth seemed to start at the cortical end of the implants and at sites where a tight contact was accomplished (Fig 16. A).

At six weeks the connective tissue within the porosities of HA was structurally loose and had a remarkable vascular component. New bone was formed between the connective tissue protrusions and the HA. On the areas of new bone formation there was a deposition of osteoblasts and osteoclasts as an expression of bone forming and a remodelling process. At later stages vascularity of the connective tissue decreased and, instead, more fat cells and marrow cells emerged. In the 16- and 24-weeks specimens the connective tissue appeared more fibrous with intense collagen staining, and the nuclei of the collagen fibres seemed small and inactive. On the areas of abundant bone formation the amount of connective tissue was marginal. Accordingly, inactive osteoblasts or "lining cells" without an osteoid layer were seen correspondingly with interrupted bone formation. According to the histologic expression, new bone formation seemed to be more active on the most loaded areas, i.e. at the cortical end of the implant and, moreover, at the tubular bone region (Fig 16. B–C).

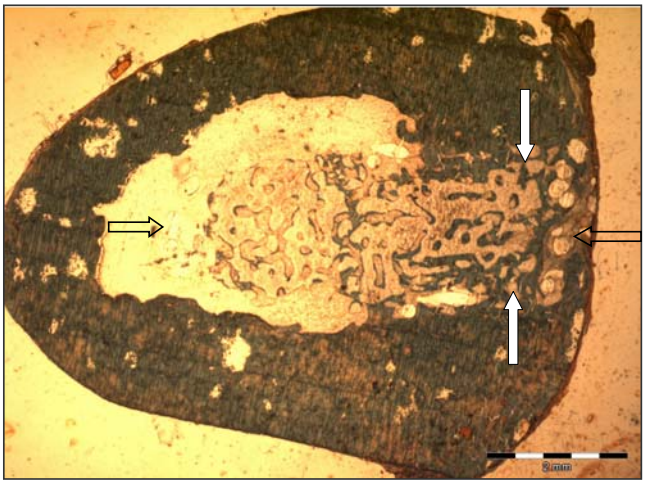

Fig 16. A) Transversely cut histologic specimen from the rabbit tibial diaphysis at six weeks through the poly-dl/l-lactide reinforced HA implant, *Study III*. Good bone ingrowth is seen in the most loaded cortical region (white arrows). Due to lactide fibres only a moderate fibrous-type connective tissue reaction is seen (open arrows). Masson-Goldner stain. Scale bar 2 mm.

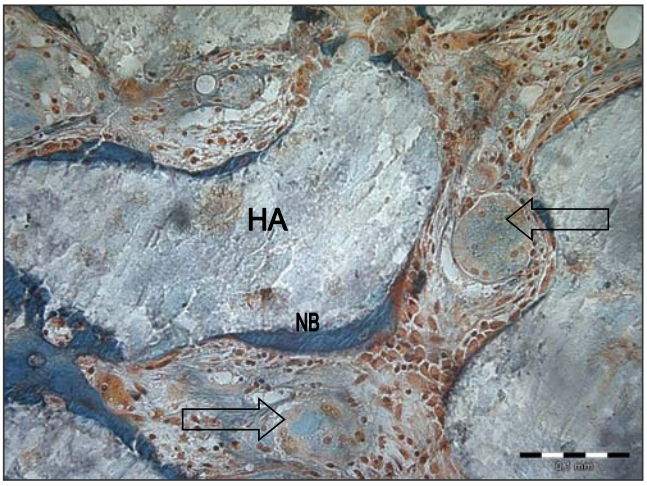

Fig 16. B) Magnification of the bone-forming process from the six-week specimen in Fig. 16A, *Study III*. Osteoblasts and osteoclasts are lining against HA, and new bone (NB) is formed against HA without intervening tissue. Among the ingrown connective tissue some multinucleated giant cells (arrows) are seen, propably as a sign of a foreign-body reaction. Masson-Goldner stain. Scale bar 0,1 mm.

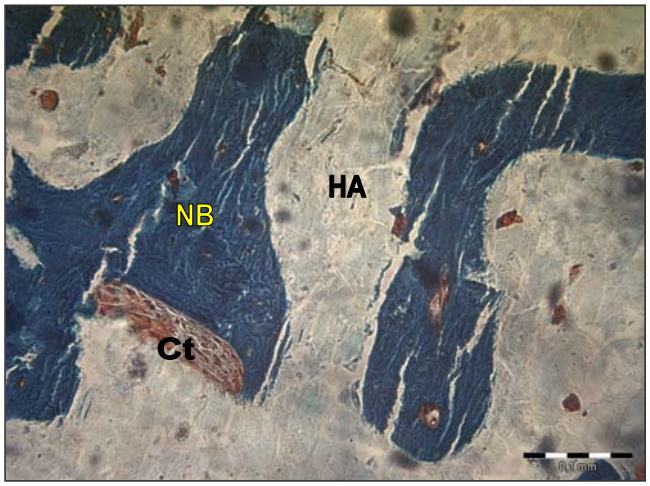

Fig 16. C) Histologic specimen at 24 weeks after poly-dl/l-lactide reinforced coralline HA block implantation in the rabbit tibial diaphysis, *Study III*. New bone (NB) ingrowth is rich and has ended, and there is only some active connective tissue (Ct) left in the region. Masson-Goldner stain. Scale bar 0,1 mm.

5.2.2. Histomorphometry

The mean area fractions of new bone and connective tissue ingrowth did not correlate with the duration of in vivo implantation. For the new bone ingrowth the Pearson correlation coefficient (r) in relation to the follow-up time was in the surface-sectioned specimens r = 0,056 and in the deep-sectioned specimens r = 0,196, thus p> 0,05. Likewise, the means of connective tissue ingrowth in both section levels at different follow-up times was of the same order with the significance level of p<0,05 using Student's t-test. Furthermore, the correlations of tissue ingrowth with the reinforcing fibres (PGA vs. PDLLA) were non-significant in both section levels, p>0,05 (ANOVA). Consequently, the variations between the two implants and over the course of the follow-up were nonsignificant, i.e. new bone formation was nearly accomplished already at six weeks and it remained fairly constant thereafter.

A difference was found when tissue ingrowth between the cancellous and cortical implantation was compared. The percentual mean area of new bone ingrowth into the reinforced HA blocks implanted cortically was 17,1 % compared to that of 12,9 % in the cancellous implantation, and the difference was found significant (p<0,01, Student's t-test). A consistent correlation was found when the percentual mean areas of connective tissue ingrowth were compared, the ingrowth being smaller in the cortical (24,1 %) than in the cancellous (27,8 %) implantation, but a statistical significance was found only in the deep-sectioned samples (p<0,001, Student's t-test). Furthermore, considering the bone ingrowth, a statistical significance was found in favour of the PDLLA-reinforced implants in the cortical implantation (p<0,001, Students t-test), but in cancellous implantation no significant difference was found between the reinforced implants.

When the histomorphometric measurements were focused separately on the cortical end and on the medullar end of the implants, a difference was found in the tissue ingrowth. The new bone area fractions were greater and the connective tissue fractions smaller when measured at the end of the implant facing cortically compared to those measured at the opposite end facing medullarly (p<0,001, Student's t-test). Finally, no differences were found in the percentage amounts of HA between the implants and implantations.

5.2.3. Microradiographies and oxytetracycline fluorescence studies

In microradiographies a close contact between the implant and host bone starting at six weeks was seen in some areas. Typically, the contact was best along the long side of the implant starting at six weeks near by the cortex of the bone. At the ends of the implants where most of the reinforcing fibres were located the bony contact was mostly lacking. No fractures of the implants were registered (Fig 17. A–B).

OTC-fluorescence index was scored according to the number of fluorescing spots in the samples and graded from gr0 to grVI. The fluorescence indexes were evaluated as follows: grade IV at six weeks, grade II at 12 weeks, and grade I at 16 weeks. In the 24 week samples the OTC studies were not performed. Fluorescence was greater at six weeks vs. 12 weeks and at 12 weeks vs. 16 weeks (p<0,001, p<001, respectively, Student's t-test), Fig 17. C. Fluorescence was most intensive at six weeks.

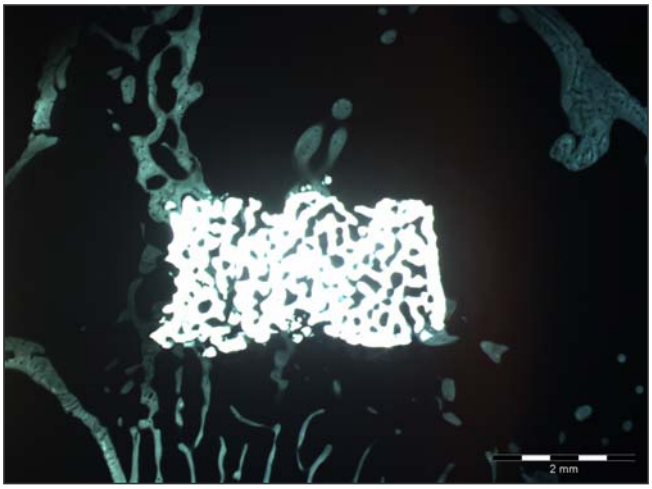

Fig 17. A) Microradiograph of the reinforced HA block after 16-week implantation in the rabbit proximal tibiae, *Study III*. Close contact with the host bone is seen even at the end of the implant despite the reinforcing PGA fibres. Scale bar 2 mm.

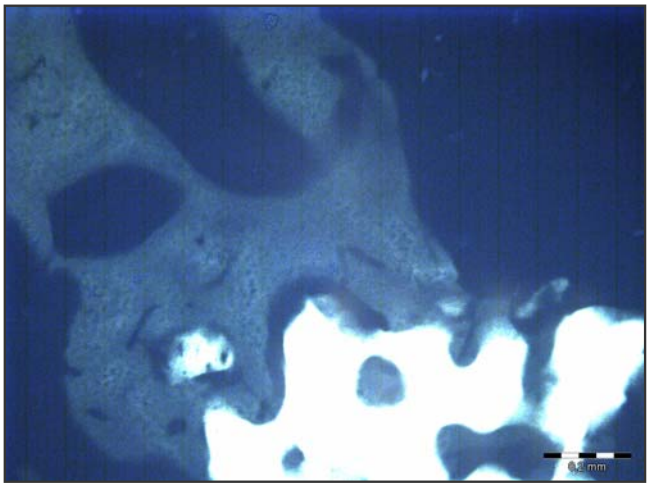

Fig 17. B) Magnification of the microradiograph of the specimen in Fig. 17A reveals close contact between the host bone and HA and even bone ingrowth, *Study III*. Scale bar 0,2 mm.

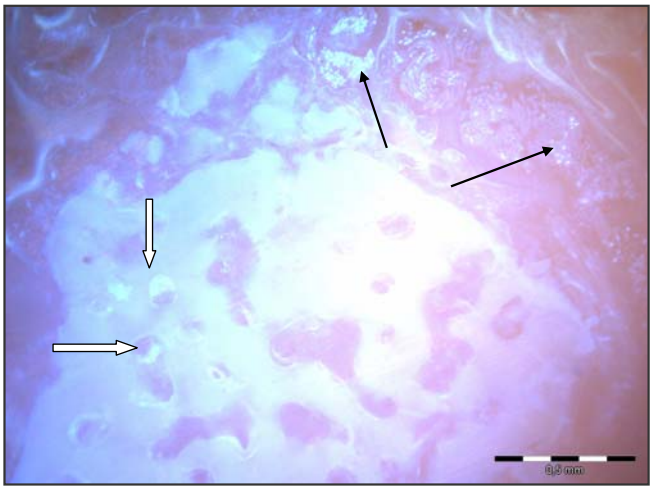

Fig 17. C) OTC fluorescence, PGA-reinforced HA block implanted in cancellous bone, follow-up of six weeks, *Study III*. In the reinforced coralline HA implantation study most of the OTC fluorescing areas were seen at the six-week specimens. Fluorescing areas are seen inside the implant (white arrows) as a sign of new bone formation. PGA degradation products are birefringent and fluorescing (black arrows). Scale bar 0,5 mm.

5.2.4. Comments

Klawitter and Hulbert (1971) reported that a minimum pore size of 100 μm is necessary for bone ingrowth. It has also been shown that bone formed within the pores is faster in implants with the smaller pore size compared to those with the larger pores (Eggli et al.1988), probably due to the larger surface for invading tissue. Quantitatively the bone ingrowth in the present study exceeded nearly its final stage already at six weeks which is in accordance with the study of Eggli et al. where the bone ingrowth exceeded nearly its final state in four weeks using TCP implants with 50–100 μm pore size. Probably, in rabbits the bone regenerates rapidly and the porous capacity of the small sized HA 200 implants is fulfilled early. There were no differences regarding bone and connective tissue ingrowth between the implants reinforced with PGA or PDLLA threads. Both PGA and PDLLA threads induce a local inflammatory fibrous reaction around themselves but it did not seem to hinder the bone ingrowth. However, histologically the bone ingrowth seems to be more rapid in sites where a close contact with HA and the host bone exists. A significant finding was that bone ingrowth was directed according to the loading conditions so that the load carrying cortical ends of the implants were the most ossified. Similarly, implants in the cortical region were more ossified than those in the metaphyseal region.

5.3. LUMBAR INTERBODY IMPLANTATION WITH REINFORCED HYDROXYAPATITE BLOCKS

The study consisted of two series of pigs. In the first series two kinds of reinforcement and two kinds of porous HA blocks were compared in lumbar interbody implantation in minipigs followed up with radiological, microradiological and histological studies. In the second series the survival of PDLLA-reinforced HA blocks were followed up radiologically and microradiologically and with histological, histomorphometrical and OTC-fluorescence studies in lumbar interbody implantation in growing pigs.

5.3.1. Comparison of reinforced sintered and coralline porous hydroxyapatite blocks in lumbar interbody implantation in minipigs

Of the five operated minipigs one suffered transient paraparesis. In the whole series the initial position of the implants was radiologically good: lateral deviation of mean 2 mm (0–6 mm) and anterior deviation of mean 1 mm (0–2,5 mm) was measured. None of the implants was registered to be situated too posteriorly.

5.3.1.1. Radiology

During the first six weeks some migration was observed. Five implants in two minipigs migrated sagittally, two PDLLA-reinforced synthetic implants (Patka) 1,5 and 3,0 mm anteriorly, one PDLLA-reinforced coralline (Interpore™) implant 3,0 mm anteriorly, and two PGA reinforced coralline implants anteriorly 2,5 and 6,0 mm.

The ossification of the implanted disc spaces was evaluated on the plain films and on the CT films taken at 24 weeks, Fig. 18 A–C. The conclusions of the imaging studies and histologic studies are presented in Table 5. On the plain films moderate ossification of grade 3 (51 %–75 %, see Materials and Methods, *Study IV*) was seen only in one implantation (PDLLA-reinforced synthetic HA block), all the other implantation scoring less. In three implantations no ossification was seen (one PDLLA-reinforced synthetic HA and two PGA-reinforced coralline HA blocks). In all, the observed ossification surrounded the implants and was marginal. On the CT scan films some ossification was identified only in two implantations (one PDLLA-reinforced coralline and the other PGA-reinforced coralline HA block), scoring 1 and 2 respectively. Subsequently, the findings on the plain films and CT did not correlate well which each other. In relation to the evaluated ossification there was a negative correlative trend to implant migration (σ= -0,44, p<0,10) and a positive correlative trend to the residual height of the implant (σ= 0,45, p<0,09) (Spearman rank correlation test).

The mean residual heights of the implants after the 24-week follow-up were in accordance with each other: in those of the PDLLA-reinforced synthetic HA implants the heights were 68±29 %, in those of the PDLLA-reinforced coralline HA implants 70±27 %, and in those of the PGA-reinforced coralline HA implants 64±26 %. The fragmentation was scored grade 4 in 11, grade 3 in one and grade 1 in three implants being equal in the different kinds of implants. All implants lost up to 51–71 % of their original height, and, accordingly, the volumes of the implants were heavily reduced so that < 25 % of residual volume was measured in ten implants.

The height of all the intervertebral discs was distinctly narrowed. An over 50 % narrowing was seen in 3/5 of each type of implantation on the plain films: on MRI 1/5 in PDLLA-reinforced implantation of synthetic HA block, 2/5 in PDLLA-reinforced implantation of coralline HA blocks and 1/5 in PGA-reinforced implantation of coralline HA blocks. The results according to CT and MRI did not differ significantly. The narrowing correlated significantly with the decrease of the signal intensity in MRI (σ=0,77, p<0,0003).

The development of kyphosis was determined in four minipigs in which discs between L4–L7 were operated. The initial postoperative kyphosis was 10,5° (range 2–17°), the mean increase was 9° (range 4–13°), and as an end-result kyphosis was mean 19° (range 15–21°). In the non-operated region no kyphosis or lordosis was identified (Fig 18. A–C).

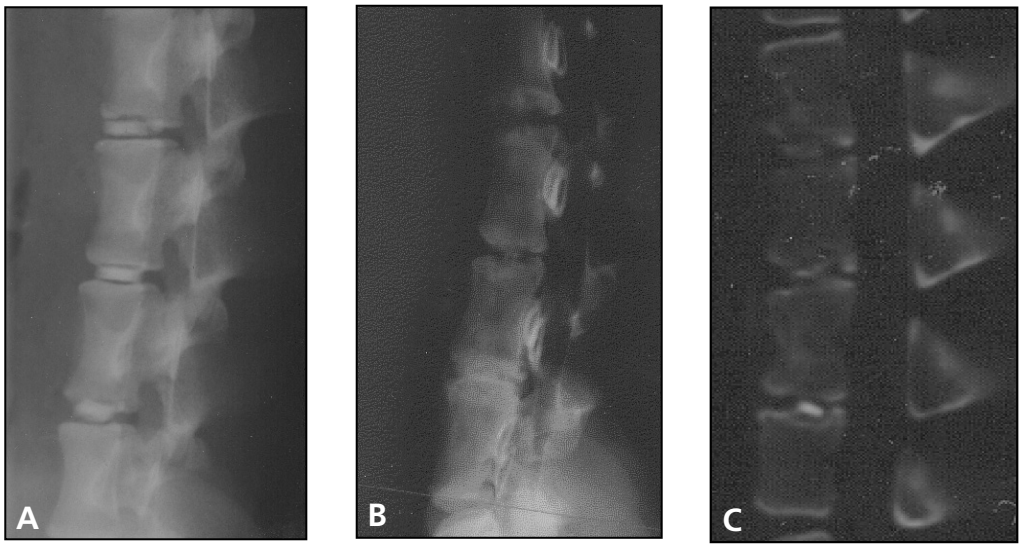

Fig 18. Radiographs after HA implantation in minipigs, Study IV.
A) postoperative radiographs where the upmost implantion was done with PDLLA reinforced synthetic HA, the middle one with PDLLA reinforced coralline HA and the lowest with PGA reinforced coralline HA.
B) radiographs after sacrifice show a small kyphosis developed during 24 weeks follow-up. Both discs implanted with coralline HA show some signs of ossification in these native radiographs but CT
C) confirmed that no ossification had occurred in any of these three operated discs.

All the end-plates showed roughness and osteolytic changes from six weeks onwards. At 24 weeks marked roughness was demonstrated on the plain films and on MRI in 60 % of the operated discs compared to CT which demonstrated marked roughness in 40 % ($p<0,03$). Osteolysis scored >1 was identified in half of the operated discs and fragmentation of the end-plates in 12 out of 15 operated discs. There was no difference regarding roughness, osteolysis or fragmentation between the different implants. Four complete epiphyseolyses were demonstrated in three minipigs. In all operated areas well-marked anterior longitudinal ligamentous ossification was seen by all imaging methods. Anterior and posterior osteophytes were seen significantly more commonly on the plain films than on the CT. Slight to moderate anterior osteophytosis was found in three operated disc spaces and posterior osteophytosis in all disc spaces, respectively, and they were detected significantly more commonly on the plain films than on the CT ($p<0,0003$). Both anterior and posterior osteophytes were detected as commonly also on the non-operated disc spaces nearby. Slight spinal stenosis was demonstrated owing to posterior osteophytes. Increased vertebral signal intensity on MRI was seen in 17 operated and in two non-operated vertebrae.

5.3.1.2. Histology and microradiographies

Small areas of new bone ingrowth were seen in all PGA-reinforced and in three PDLLA-reinforced coralline HA implants, Table 5 and Fig 19. A. However, no bone ingrowth was seen in the PDLLA reinforced synthetic HA implants. All the reinforced synthetic HA implants were surrounded by dense connective tissue with collagen fibres and a few fibrocytes. The connective tissue around and inside these implants was more thickly grown and stained more intensively compared to that of both reinforced coralline HA implants, Fig 19. B–C. Regarding ossification, no correlation was found between the histological and plain films excepting in the respect that no ossification was found by either method in the reinforced synthetic HA implants.

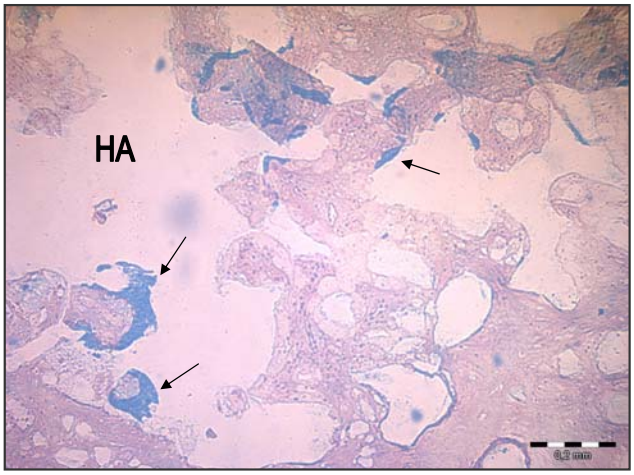

Fig 19. A) PGA-reinforced coralline HA block implanted in the minipigs lumbar disc space, followed for 24 weeks, *Study IV*. Small islets of new bone (arrows) inside the HA implant can be seen. Scale bar 0,2 mm.

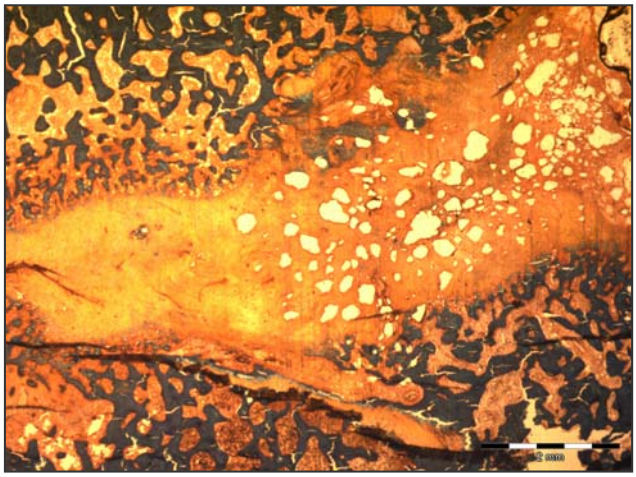

Fig 19. B) Sagittally cut histologic specimen of a minipig lumbar disc space replaced by PDLLA-reinforced synthetic HA implant, *Study IV.* Follow-up of 24 weeks. The quantity of HA is low, but extensive and intensively staining connective tissue has been ingrown. No bone ingrowth. Scale bar 2 mm.

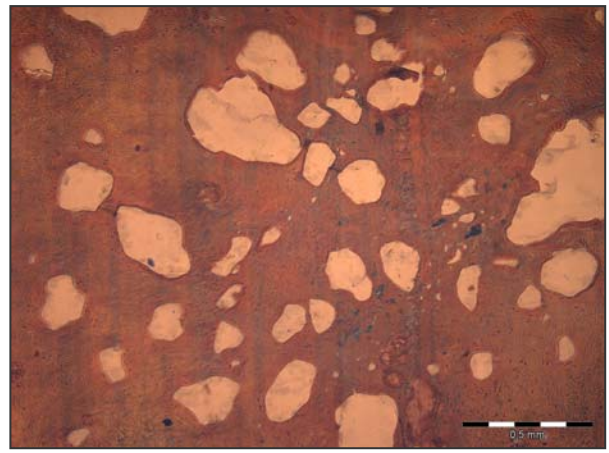

Fig 19. C) Magnification of the operated disc space in Fig 19. A, *Study IV.* Synthetic HA implant has lost its inner structure and integrity. Extensive inactive, acellular connective tissue growth has replaced the HA. No bone ingrowth is seen. Masson-Goldner stain. Original magnification × 25. Scale bar 0,5 mm.

In microradiographs the inner structure of all the reinforced synthetic and coralline implants were lost. Because of dispersion of the HA no bone ingrowth could be identified in microradiographs. Often, particles of dispersed HA were incorporated inside the vertebrae, Fig 20.

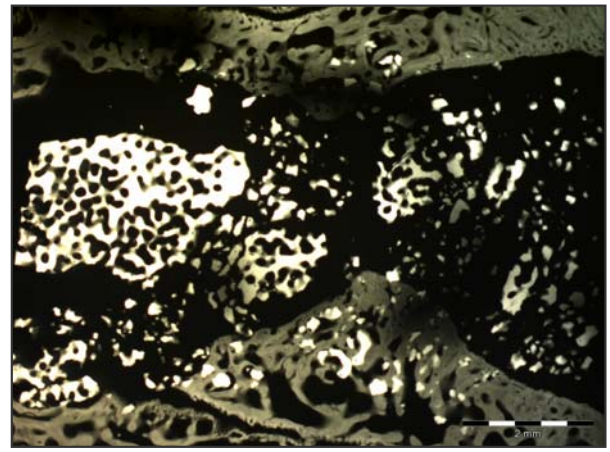

Fig 20. Microradiograph of the implanted disc space in a minipig after 24 weeks follow-up, *Study IV*. The PDLLA-reinforced coralline implant is broken, and dispersion of HA has occurred. Close contact to vertebral endplates is unfulfilled. Scale bar 2 mm.

Table 5. Ossification of implanted disc spaces visualized on plain films and on histological sections at 24 weeks (*Study IV*). Scoring 0–4, see Materials and methods, *Study IV* (Arch Orthop Trauma Surg 110:250–256, 1991).

Minipig	PDLLA-synthetic HA		PDLLA-coralline HA		PGA-coralline HA	
	Plain	Histol	Plain	Histol	Plain	Histol
A	0	0	2	2	1	1
B	2	0	2	0	0	1
C	2	0	2	0	1	1
D	3	0	1	1	1**	1
E	1	0	1*	1	0	3
Sum of scores	8	0	8	4	3	7

*, ** Disc spaces where CT also showed ossification, * score 1, ** score 2

5.3.1.3. Comments

Migration of implants, the lowering of the implanted disc spaces and of the implants as well as the fracturation of the three different kinds of implants were similar. Ossification showed by the plain films was confined to the peripheral areas around the implants. When the results of ossification were related to the histologic findings, CT was markedly better to show ossification inside and through the implant. Histologically, the ossification was marginal including only small islets of new bone formation inside the implants and was seen merely in two reinforced implants, one in the PDLLA-reinforced and the other in the PGA-reinforced coralline HA implant. The synthetic sintered porous HA implants showed histologically no ossification. In his own study Patka (1984) showed, however, that this kind of synthetic porous HA implants were filled with ingrown bone in load-bearing conditions in femoral defects in dogs. There is no clear explanation for the observation in the present study, but the impurities of the material may have a role.

In all minipigs ossification was also seen in the anterior intervertebral ligaments in combination with kyphotic formation in the operated region. All except one of the implants were fragmented already at six weeks. Four implants migrated slightly, and there was a negative correlation between the implant ossification visible in plain films and the migration. Furthermore, a positive correlation was found between the residual height of the implant and the ossification seen in the plain films. Hence, the ossification which was confined to the periphery and was seen in the plain films was more reactive due to instability.

The roughness and fragmentation of the end-plates and even osteolysis were frequent findings and visible from six weeks on. They were not dependent on the implant used. No signs of infection were encountered. In all likelihood, these findings were related both to the operation method, i.e. roughening of the end-plates, and to the instability of the operated lumbar vertebral segment.

5.3.2. Coralline hydroxyapatite reinforced with polylactide fibres in lumbar interbody implantation in growing pigs

Based on the studies of tibial implantation in rabbits (*Study III*) and of lumbar interbody implantation in minipigs (*Study IV*) the PDLLA-reinforced coralline HA blocks were further studied in lumbar interbody implantation in growing pigs. Poly-dl/l-lactide reinforced coralline HA blocks, such as those used in minipig lumbar implantations and tested in rabbit tibiae, were used in 23 growing pigs through laparotomy. In four pigs the emptied disc spaces were left open to serve as control. From 27 operated pigs six had postoperative complications, all belonging to the HA implanted study group. Two pigs died; one because the intraoperative vascular lesion and the other beause of ventricular dilatation and perforation. Two pigs had mild paraparesis, but they were able to move and could be followed up, one for three weeks and the other for six weeks. Two pigs had a postoperative incisional hernia without evidence of symptoms relating to it. After the follow-up of three, six, 12, and 16 weeks the disc blocks were studied radiologically, histomorphometrically, histologically, microradiographically, and with the OTC fluorescence techniques.

5.3.2.1. Radiology

In the plain films the radiological findings with a special reference to implant displacement, fragmentation, resorbtion, and ossification are presented in Table 6. No dislocation or major displacements of the implants were shown. At three weeks the implants were not fragmented, at six weeks some yielding was seen in the implants but none was fragmented, at 12 weeks 3/5 of the implants were fragmented, and at 16 weeks 5/6 of the implants were fragmented.

Local kyphosis was a frequent finding of the implanted disc segment along with anterior bone bridging or ligament ossification (Fig. 21). Kyphosis measured at three weeks was mean 13,4° (range 6°–17,4°), at six weeks mean 10,7° (range 3,1°–13,8°), at 12 weeks mean 15,9° (range 9,3°–26,8°), and, finally at 16 weeks mean 18° (range 6,5°–28,3°).

Among the pigs followed for the longest 16-week period the mean original kyphosis measured postoperatively was 0,5° (range from 5° lordosis to 5,4° kyphosis) and the mean increase of kyphosis was 17,6° (range 6,6°–25,4°), the mean resulting kyphosis thus being 18°. The figures show that the local kyphosis increased from 12 weeks onwards. In spite of implant fragmentation and collapse, kyphosis increased due to epiphyseolysis in the operated vertebrae which was frequently seen in one or both epiphyses anteriorly (Table 6).

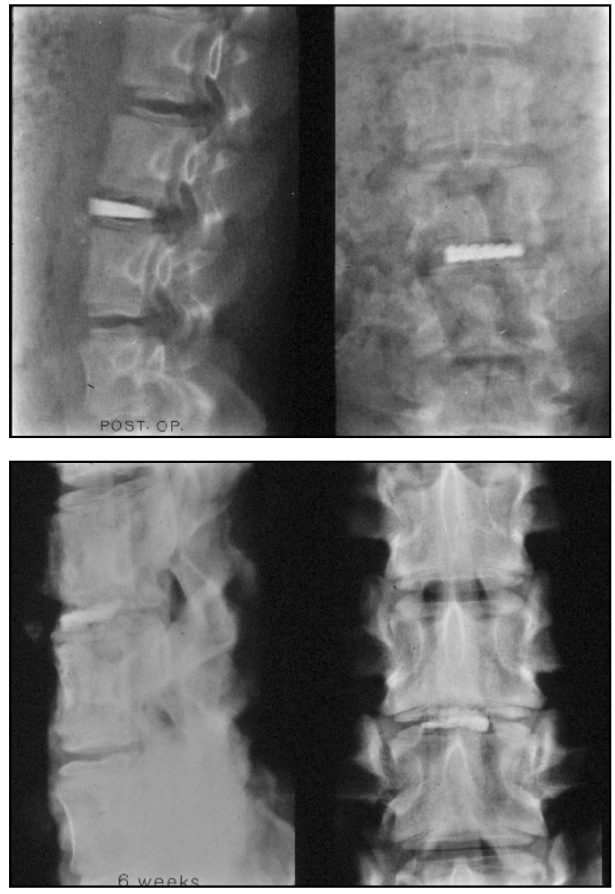

Fig 21. PDLLA reinforced coralline HA implanted in LV/LVI disc space in a growing pig, *Study V*. **A**) postoperative radiographs and **B**) radiographs after 6 weeks of follow-up. In six weeks radiographs no displacement or fracturation of the implant can be seen but the disc space is lowered and lordosis is changed to a mild kyphosis. The ap-view confirms that no solid bone bridge across the implant is developed (J Mater Sci Mater Med 16:325–331, 2005).

Anterior ligament ossification was seen already from three weeks onward. Some ossification was visible in plain films of every pig implanted with reinforced HA. The mean score of anterior ligament ossification was evaluated moderate (gr 2) after every follow-up period though some increase was seen at twelve and sixteen weeks follow-up. Instead, anterior bone bridge was identified in 3/5 pigs at three weeks, 1/4 at six weeks, 3/5 at twelve weeks and in 6/6 at sixteen weeks.

Table 6. Findings in plain films after lumbar interbody implantation in growing pigs at different follow-up times (J Mater Sci Mater Med 16:325–331, 2005).

	Follow-up time (weeks)			
	3	6	12	16
Findings*	Grade of changes			
Implant displacement	0	0	0	1
Implant fragmentation	0	0	2	3
Implant resorption	1	1	2	3
Disc space ossification	0	0	1	2
Anterior ligament ossification	2	2	2	2
Anterior bone bridge	1	1	2	3
Local kyphosis, mean	13,4°	10,7°	15,9°	18,0°
Epiphyseolysis	1	1	1	2
Total no. of implants	5	4**	5	6

** The grade of changes is classified as follows: 0 none, 1 slight, 2 moderate,3 plenty, 4 complete see Materials and Methods, Study V.*

*** The plain radiographs of one pig were missed after slaughtery.*

The changes in the disc height were measured from the radiographs. The results showed a gradual loss of height in the implanted disc spaces during the follow-up in distinction to the above non-operated disc spaces (Fig 22.).

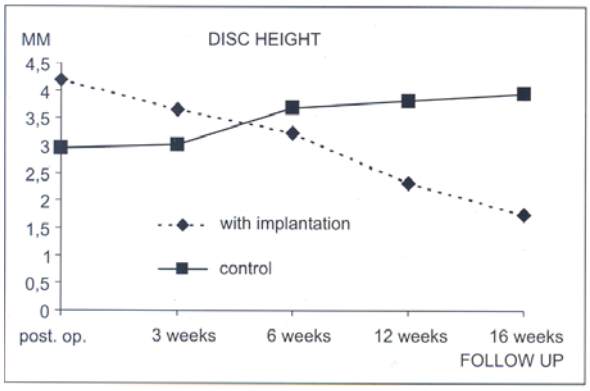

Fig 22. Disc height of the HA-implanted disc space and that of the non-operated upper next one were measured from the postoperative and follow-up radiographs of the growing pigs, *Study V*. The results show that the implanted discs lost their height gradually but the non-operated discs gained their height during the growth period (J Mater Sci Mater Med 16:325–331, 2005).

In microradiography the implants remained unbroken until six weeks, but disintegration of the coralline structure was seen, starting from the implant surface at three weeks and being propagated throughout the implant at six weeks. At its worst the porotic scaffolding inner structure was totally lost (Fig 23. A–B). The implants were seen as uniform at three to six weeks' follow-up, but at 12 and 16 weeks all the implants were broken, first in pieces through the lines of the reinforcing fibres and later as totally collapsed. In the 16-week specimens even migration of the HA granules into the vertebral bodies was seen.

Microradiography demonstrated no bone ingrowth. A microradiographic void zone surrounding the implant lost the intimate contact between the implant and the bone. The facet joints were detected radiologically and in microradiographs as normal both in the implanted and non-operated lumbar segments (Fig 24. B).

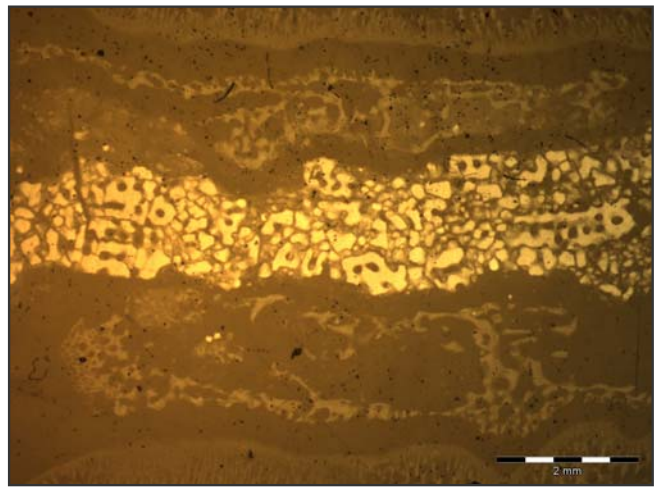

Fig 23. A) Microradiograph of the interbody implantation in the growing pig at 3 weeks, *Study V.* The inner structure of the coralline implant is preserved. There seems to be a small gap between the implant and vertebral end plate. Scale bar 2 mm.

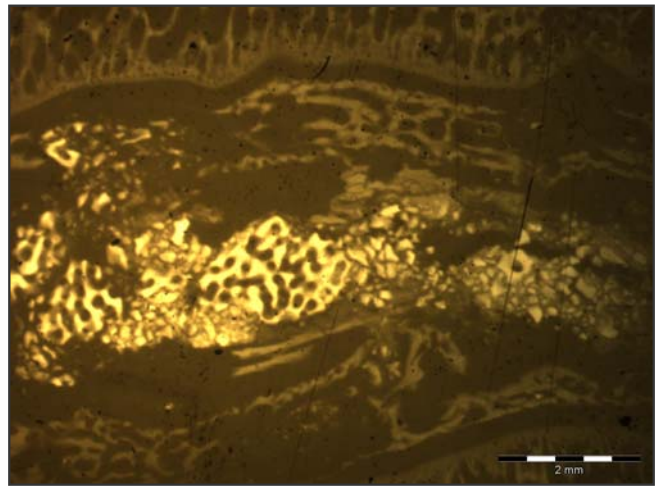

Fig 23. B) Microradiograph at six weeks after interbody HA implantation in growing pigs, *Study V*. Typically, the implant is broken and also the inner coralline structure is mainly lost. Scale bar 2 mm.

5.3.2.2. Histology

From three weeks on the ingrown connective tissue was seen filling the porotic spaces and their interconnections of the implant. At the beginning it was formed of loose bundles of collagen fibres and, later on, at six and at 12 weeks it emerged in increasing numbers of bundles which stained partly greener as a sign of collagen activity. New bone formation was not seen until at 12 weeks at which time a few small islets of bone formation inside the implant were noticed. At 16 weeks the connective tissue was very thickly stained, and some more new bone was seen. The bone was formed between HA and connective tissue showing no interfering reactions.

The reinforcing PDLLA fibres were seen histologically and in polarized light, Fig 24. A. Their inner structure started to fragment at 12 weeks, but no foreign-body reactions against them were seen. The fractures of the HA implant at 12 weeks and additional fragmentation later on were also seen in the histological specimens. Destruction of the vertebral epiphysis was a frequent finding, which, together with implant fracturation, led to the collapse of the disc spaces. To stabilize the collapsed disc space, anterior bone bridge formation was developed.

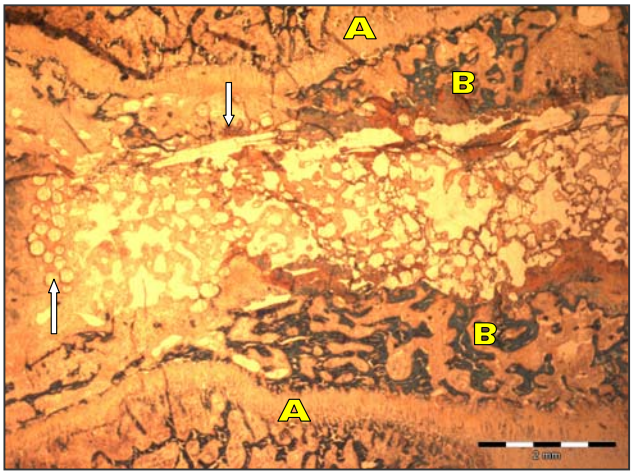

Fig 24. A) Sagittally cut histologic specimen of the implanted disc space in the growing pig followed for 3 weeks, *Study V*. The inner structure of the coralline HA block is preserved, and connective tissue ingrowth is rich. Reinforcing poly-dl/l-lactide fibers are seen (arrows), but there is no adverse reaction in their surroundings. Also bony endplate (B) and vertebral growth line (A) are seen. Masson-Goldner stain. Scale bar 2 mm.

Fig 24. B) Histologic specimen of the facet joint of the implanted segment in the growing pig after 6-week follow-up, *Study V*. Histologic specimens revealed no degenerative changes, and the joints in both the unoperated and operated segments were similar until the end of the 24-week follow-up. Scale bar 2 mm.

In the control specimens the collapsed disc space was mainly filled with unorganized collagen fibres from three weeks onwards. Bone formation anteriorly between the adjacent vertebrae was present already at three weeks becoming more expansive during the follow-up. At the beginning, some void spaces were seen, but later the disc space was filled with more organized and thickly grown collagen fibres. Finally, at 12 weeks and especially at 16 weeks the evacuated control space was mainly ossified, and only some islets of collagen tissue were left.

5.3.2.3. Histomorphometry

The connective tissue reaction inside the implant was marked already at three weeks. The rate of connective tissue varied from mean 65,6 % at three weeks to mean 79,4 % at 16 weeks, but the means were significantly different ($p<0,02$, ANOVA). However, by using the Tukey comparison of means the percentual rates of connective tissue were equal at three, six and 12 weeks ($p<0,05$) and, consequently, they did not correlate to the follow-up time. Respectively, the means of the percentual rate of HA varied from 33,7 % at three weeks to 19,2 % at 16 weeks and they were identically statistically different ($p<0,03$, ANOVA). Again, by grouping the means by the Tukey method the mean rates of HA were identical at three, six and 12 weeks, in accordance with those of connective tissue. Finally, the bone ingrowth was minimal in every follow-up period, but rates of only 0,7–1,7% could be measured. Statistically the expectations of the new bone rates did not differ from each other at different follow-up times ($p<0,6$, ANOVA) and, according to the regression analysis they did not correspond to the follow-up time, either.

5.3.2.4. Oxytetracycline fluorescence studies

Slight OTC uptake was seen in the majority of specimens in every follow-up group. Even at three weeks in three out of five specimens some uptake inside the implant was seen. Three out of four, four out of five, and three out of six specimens showed some uptake at six, 12, and 16 weeks, respectively. The amount and intensity of the uptake did not increase with the follow-up time. The more the implant was fragmented, the less, or no, uptake was detected.

In facet joints some OTC uptake was seen in the subchondral bone, but there was no difference between the facets joining the operated or non-operated discs. The activity was similar throughout the follow-up period.

5.3.2.5. Comments

Alitalo (1979) have made identical study in growing pigs using high density porous polyethylene (Plasti-Pore®, pore size 250 µm) and polytetrafluoroethylene-carbon-fiber (Proplast®, pore size 100–500 µm) implants. Both implants were displaced in the majority of the cases. Microradiographically radiodense calcified tissue was seen inside the implants in seven out of 21 (33 %) Plasti-Pore implants and two out of seven (29 %) Proplast implants, and histologically the findings were confirmed as bone ingrowth which appeared at the earliest eight weeks postoperatively. No quantitative analysis was performed but the bone formation across the implanted spaces was slight and

it was not seen in all implanted disc spaces, and opposite to that, radiolucency was often seen around the implant. Histologically the implanted disc spaces displayed fibrous union.

In the present study, only one implant followed for 16 weeks was displaced but all were broken and fragmented after six weeks. According to the radiological and histologic studies, some ossification was seen only in the 12- and 16-week specimens. Instead, histomorphometry showed some bone ingrowth even in the three- and six week specimens. The OTC labeling studies advocated histomorphometric findings because OTC uptake was seen in most of the specimens at three to six weeks. Probably, the increased collagen activity was registered as new bone in histomorphometry due to the use of special software with colour thresholds indicating connective tissue and new bone. In all likelihood, this collagen activity was, however, a sign of a starting bone formation indicated by the OTC uptake which was also seen in some of the three- and six week specimens.

6. GENERAL DISCUSSION

Due to its bioactivity, stability, and osteoconductivity the porotic HA has been the most studied material for bone graft substitute. It has also been in clinical use, first in oral and maxillofacial surgery, and later on, in orthopaedics, e.g. in the cavitary and segmental bone defects and in spinal posterolateral fusions. However, most of the commercial preparations of HA are actually not pure HA, which can essentially modify their biologic properties, especially the resorption. Except being chemically and biologically compatible, the artificial bone substitute can be successfully used only in the circumstances where the triad of osteoconduction will be fulfilled (Shors 1999): 1) The graft must be in intimate contact with the host bone, 2) the contact must keep stable, and 3) the host bone must be viable.

One of the main reasons for using a bone-graft substitute is to avoid the surgical morbidity associated with autologous bone harvesting. In iliac crest harvesting the rate of minor complications not affecting functionally has been reported even 20–39% and the overall major complication rate 8–10% (Younger and Chapman 1989, Banwart et al. 1995). Moreover, the supplies for autogenous bone are not always adequate, and the need for bone grafting procedures is continuously increasing.

Alveolar ridge augmentation with porous particulate HA

Particle migration, mental nerve neuropathy, infections, and dehiscense are the major complications related to the alveolar ridge augmentation with HA (Butts et al. 1989, El Deep et al. 1988). Trials of using the block form of HA are based on its theoretically more predictable placement, as well as on possibilities for achieving greater augmentation. In the clinical series of Rooney et al. 1988, dehiscenses and infections occurred in more than two thirds of the patients, and in over half of the cases the blocks had to be removed. The removed blocks showed that the bone ingrowth occurred only in a limited area near to the alveolar bone contact. A similar finding of partial tissue ingrowth into the HA blocks has also been shown experimentally in a dog model (Piecuch et al. 1983). The lack of tissue ingrowth in the upper parts of the blocks probably impairs the tissue nutrition nearby and predisposes to dehiscense and infection. Moreover, the alveolar ridge is very demanding for any type of graft material due to dense cortical bone, thin periosteum, and repetitive loading from the denture.

The particulate HA has been used clinically since the 1970's but today the dental endosseal titanium implants are the treatment of choice. The HA still provides an option, either alone or in combination with the endosseal implants (Marshall 1989, McGrath et al. 1996) and as sinus lift grafts (Wheeler et al. 1996). The major problem in HA augmentation has been the dispersion and migration of the particulate HA, and, thus the incorporation of the graft and its augmentation effect are not optimal. According to Mercier (1995), problems may also arise if HA has been grafted improperly on the buccal side and the ridge has been poorly prepared and defined. The HA grafting should often be combined with adjunc-

tive vestibuloplasty or lowering of the floor of the mouth. Quite recently, Mercier and Bellavance (1999) showed a low incidence of adverse effects and a high percentage (97 %) of patient satisfaction when HA augmentation was combined with vestibuloplasty as second-stage operation. Of paramount importance for incorporation of HA is the tissue ingrowth, either fibrovascular or bony ingrowth or both. The aim in the present study was to make the bone ingrowth possible into the particulate HA graft using curved biodegradable containment, which allows a direct contact of HA to alveolar bone. Furthermore, a fast degrading binding substance, polyglycolide/polylactide (PGA/PLA) copolymer, was used together with particulate HA and the curved PGA containment to ascertain the desired profile to the graft. By binding the particles together, the role of substances added to HA is to evoke a fibrovascular reaction at the interface of the host bone and the HA graft and, thus, to promote tissue ingrowth and bone formation (Mercier et al. 1996).

Ideally, the HA container should be easy to handle and it should preserve the desired contour, promote the tissue ingrowth, and resorb while being replaced by the ingrowing new tissue. As far as augmentation with the Vicryl tube is concerned, the studies have been mainly clinical without histologic evidence of the bone ingrowth (Härle and Kreusch 1991, Brown 1992, Sugar 1995). The use of the collagen tube revealed histologically great variation in the bone ingrowth and it was mainly sporadic (Gongloff 1992). Also various binding substances such as purified fibrillar collagen (PFC) (Marshall 1989, Mehlisch 1989, Marouf et al. 1990, Mercier et al. 1992, 1996) have been added to the particulate HA graft to avoid dispersion and to give rise to connective tissue ingrowth. Likewise, Meijer et al. (1997) mixed the HA particles with a fibrin sealant (Tisseel™). All these additives have shown to evoke pronounced fibrovascular reaction, but the bone ingrowth has not been registered. Accordingly, the collagen-based or biodegradable materials used as containers of the particulate HA or mixed with the HA probably counteract the bone ingrowth when temporarily acting at the interface of the HA and the bone. These substances may favour the connective tissue response, but one of the essential prerequisites for osteoconduction, the direct, and sufficiently extensive contact between the HA and the viable host bone, might be jeopardized.

The histologic and histomorphometric studies (*Study I*) revealed that connective tissue was grown both into the uncontained and contained HA graft already in three weeks and the amount of connective tissue grown in six weeks remained relatively constant up to the end of the 24-week follow-up period. There was more ingrown connective tissue if the augmentation was carried out with the curved PGA containment compared to the conventional augmentation without containment. The fibrous reaction around the PGA degradation products is, in all likelihood, the main reason for formation of additional fibrous tissue. When the HA particles were bound together inside the curved containment with the fast degrading PGA/PLA polymer (*Study II*), the fibrous connective tissue reaction was the greatest. Likewise, the studies of HA-PFC composite by Marouf et al. (1990) have shown more pronounced fibrous reaction inside the HA graft. According to the authors, the rapid expansion of the composite as a result of water uptake leads to an increased space between

the HA particles, and the larger space allows more mobility of the HA particles favouring fibrous tissue ingrowth. This theory may also partly explain the greatest fibrous connective tissue reaction in the present study, when the particulate HA and PGA/PLA composite were used, though the local reaction against the PGA degradation products is also one evident cause.

The best bone ingrowth in the present study was seen in the conventional augmentations without any resorbable containment. At 24 weeks the new bone ingrowth exceeded 10-12% of the total augmentation area. The augmentation with PGA-containment did not prevent the bone ingrowth but was significantly lower compared to the non-contained augmentations. In the literature there are few histologic studies regarding bone ingrowth. Mehlisch (1989) made a few histologic specimens of human patients using HA-PFC composite and found in three out of five specimens host-bone-infiltrated regions of the implant and classified the bone both as woven and lamellar.

In the present study the fibrous connective tissue grew up to the curved implant, though new bone was mainly seen at the lower part of the HA graft. In fact, the biodegradation of the curved implant gave rise to a fibrous reaction which was seen as additional connective tissue ingrowth when measured histomorphometrically and compared to non-contained augmentation. Notwithstanding good tissue ingrowth, the rate of infections or dehiscenses was high; 35 % in the series of curved PGA implants and 19 % in the series of prefilled, curved PGA implants. The high incidence of dehiscenses in the preliminary series was probably due to aseptic, inflammatory sinus formation related to PGA degradation shown in the bone tissue (Böstman et al. 1992) and with subcutaneous sutures. The use of quinine dye in the PGA implants of the preliminary series has shown to correlate with this kind of sinus formation. The mechanical strain of submucous tissue created by the rigid PGA implants predisposes, of course, to dehiscenses.

To conclude, the present study has shown that the curved biodegradable PGA implant can improve confinement of the particulate HA on the alveolar ridge. The curved implant itself did not hinder the tissue ingrowth and bone formation inside the HA graft. The fibrous connective tissue ingrowth inside the curved implant was even higher when compared to that in the non-contained, conventional HA augmentation. Contrary to that, bone ingrowth was lower as compared to the augmentation without any implants. Moreover, when a fast degrading binding substance of the HA particles together with PGA containment was used, fibrous connective tissue ingrowth was at its highest, but the bone ingrowth was negligible. The present study showed that agents added to the particulate HA may strengthen the fibrous connective reaction inside and between the HA particles, but it often intervenes also in the osteoconduction and may even hinder it. In the future studies, the issue of additives cannot be disregarded when agents added to HA as carriers for improving or inducing bone ingrowth are ivestigated, as the carrier itself may hinder the bone formation (Hotz and Herr 1994, Isobe M et al. 1996, Omura et al. 1998). For lowering the frequency of local dehiscenses the PGA material is recommended to be quinone-free (Böstman et al. 1992); also faster resorbing and more flexible materials should be considered.

Reinforced HA implants and their use in experimental anterior interbody fusion.

Spinal fusion is the most common reason for bone grafting. Autograft bone is the gold standard, but there has been considerable interest in the use of calcium phosphate ceramics as substitute material. Besides the lack of donor site morbidity, the reduced operating time and unlimited shelf life have been considered advantageous (van Heest and Swiontkowski 1999). According to a recent estimation, no more than 10 % of bone graft procedures currently involve synthetic materials (Lewandrowski et al. 2000). In all likelihood, the use of synthetic bone void fillers and graft extenders in spinal surgery is to become more frequent.

The mean pore diameter of 200 μm of the coralline HA has shown to be adequate for bone ingrowth (Chiroff et al. 1975, Holmes 1979, Sartoris et al. 1986b, Eggli et al. 1988, Kühne et al 1994). Its inherent structure is more resistant against mechanical loads than those with larger pores. Tencer et al. (1985, 1988) reinforced porous HA both internally and externally by creating a composite with biodegradable DL-polylactide (PDLA) and achieved compressive strength which was on average 3,76 times as strong as for uncoated implants. Iwano et al. (1991) coated porous HA blocks with collagen, which improved the compression strength by 4.3 times. Histologically, however, the delay of the bone ingrowth and the appearance of the tissue reactions with multinucleated giant cells were observed in both types of the coated implants. Moreover, in the implants with thicker PDLA coatings the bone ingrowth was shown to be partly inhibited (Tencer et al. 1988). To overcome blockading the bone ingrowth, the present study utilized absorbable fibre coating on the prefabricated grooves of the porous HA block by which a direct contact between HA and the host bone can be achieved. According to the mechanical studies by Taurio et al. (1992), this reinforcing method improves the tensile and fatigue properties and maintains the compactness of the implants longer, though the compression strength proves to be only slightly superior.

The fibre reinforcement did not hinder the bone ingrowth into the coralline HA blocks. When tested in the rabbit tibia, an almost complete bone remodelling inside the implants occurred in 16-24 weeks. Actually, histomorphometrically the maximal amount of new bone inside the porosities was exceeded already at six weeks. Histologically, both the PGA and PDLLA reinforcing fibres caused foreign-body-type tissue reactions with the occurrence of multinucleated giant cells, but the reactions were local and not disadvantageous to the bone response. Likewise, in the diaphyseal defect model by Sartoris et al. (1987) as well as in the metaphyseal defect model by Holmes et al. (1986) the bone ingrowth occurred rapidly from three weeks to two months, respectively. The present study showed, however, that the bone response is dependent on the mechanical loading acting on the implant, i.e. the bone ingrowth follows Wolff's law showing an adaptive pattern according to the load transmitted through the implant. Contrary to that, Tencer et al. (1988), after having performed a nearly identical operative procedure, concluded that the location of the implant did not affect the resultant growth of the bone into it, and in the study of Sartoris et al. (1987) the quantity of ingrown bone increased over the follow-up time.

The mean area fractions of the new bone in the present study were 12,9 % in cancellous and 17,1 % in cortical implantation. The average area fractions for the implant itself were 59,4 % and 58,9 %, respectively. In the literature the figures for bone ingrowth vary from 13 % (Holmes et al. 1986) to 45 % (Tencer et al. 1988) and seem to depend both on the pore size and porosity of the implant. According to the literature, the higher the pore size and porosity are, the more bone ingrowth can be expected, and, probably, the implants with a 200-micrometer pore size exceed their bone ingrowth capacity earlier, the bone amount remaining constant thereafter. The finding of spatial distribution of bone ingrowth as being more abundant in the cortical than in the cancellous area was, to the author's knowledge, the first time demonstrated in the present study. It is, however, the logical consequence of Wolff's law following the stress pattern of the tubular bone and the osteoconductivity of the porous HA implant.

In spinal surgery, porous HA has been thoroughly studied in the experimental posterolateral fusions as bone substitute or as graft extender (Holmes et al. 1984, Hardouin et al. 1991, Zerwekh et al. 1992, Muschler et al. 1993, and 1996, Guigui 1994, Boden et al. 1999, Delecrin et al. 1997, Bozic et al. 1999, Baramki et al. 2000, Steffen et al. 2000, Walsh et al. 2000). To conclude, the porous HA with additional posterior stabilization has revealed fusions comparable to those with an autogenous bone graft, especially if both are done with adjunctive rigid transpedicular stabilization. Transpedicular posterior stabilization has also increased the use of interbody fusions with the aim to restore the disc height and the column equilibrium, and to minimize the bone graft volume. Histological bone ingrowth in the porous HA implants was shown in experimental lumbar interbody fusions by Flatley et al. (1983) and Ragni and Lindholm (1991), and a fusion rate of 70 % was found when dense HA was used by Pintar et al. (1994). Experimental models of ceramic implantation in the cervical spine have demonstrated graft cracking and extrusion from 8 % (Toth et al. 1995) to 70 % (Shima et al. 1979) which has, in all likelihood, contributed to the higher cervical mobility. The importance of stability was revealed in the study of Fuller et al. (1996), when they found essentially no bone ingrowth in the porous ceramic implants in canine thoracic spine used without internal fixation, whereas with adjunctive fixation a mixture of osseous, fibro cartilaginous, and fibrous union was seen in eight weeks.

In the present study, when synthetic and coralline porotic HA implants were compared in lumbar interbody fusion in minipigs (*Study IV*), the results were promising in terms of osteoconductivity and favoured reinforced coralline implants. Histologically, the synthetic porous HA showed no bone ingrowth in any of the disc spaces implanted with it, whereas in coralline implants some ossification was seen in three out of five and in all five implants reinforced with PDLLA and PGA, respectively. However, the macroscopic radiological picture differed greatly from the histological one. In plain films the ossification scores were judged too high because of marginal new bone formation, which was mainly due to anterior and posterior osteophytes caused by the instability of the implanted vertebral segment. CT-cuts through the implanted discs were consistent with the histological picture. Sev-

enteen out of 20 operated vertebrae showed increased signal intensity on MRI. This is a finding which has been found in association with disc degeneration, consistent with similar consequences after HA implantation in the present study (Modic et al. 1988).

In the successive series of lumbar interbody HA implantation in the growing pigs (*Study V*) the radiological, local kyphosis was a constant finding of the implanted disc segment along with anterior bone bridging or ligament ossification. A moderate change to local kyphosis was seen in every follow-up group, but the means were not statistically different. The disc height measured from the radiographs showed a gradual height loss of the implanted disc spaces. Contrary to that, the non-operated adjacent disc spaces gained their height in accordance with pig growth, whereas the emptied control disc spaces reacted similarly to the implanted disc spaces and a slight kyphosis of mean 13,7° was developed at 12 weeks. The formation of an anterior bone bridge, at its different stages, was found in all cases but was marked from six weeks onwards. These observations show that the implantation method of the present study did not guarantee stability for the implanted segment but gave rise to implant collapse and secondary changes. Vertebral column mobility in growing pigs is also more prone to instability compared to mobility of grown-up minipigs, which is probably one reason for better and promising findings of bone ingrowth into HA implants in the minipig series; in growing pigs the bone ingrowth was minimal and most implants showed no bone ingrowth at all, but in some pigs only 0,7–1,7% ingrowth could be measured. Although no displacements were seen, the fragmentation and the signs of dispersion of implants were marked. Microradiographs showed fracturation of implants constantly from six weeks on.

In the literature there are reports of implant adaptation showing an improved fusion rate in anterior interbody fusions (Kim et al. 1998, Suetsuna et al. 2001). The studies show jointly that the resection of the endplates results in improved osteoconduction into the HA implant due to sufficient bone exposure of cancellous bone. The HA itself does not have an ability to induct bone, and, therefore, it is important to insert HA in full contact with cancellous bone. If there is a gap between the HA and the vertebral bodies, e.g. sclerotic end-plate bone, it takes longer for bone to be formed on HA, and instead, fibrous tissue will be formed, impeding bone formation and predisposing to implant cracks. Biomechanical studies also show that implant breakages were fewer when HA implants were impeded into the vertebral spongiosa (Suetsuna et al. 1999).

Studies in the literature do not show exactly what degree of porosity is the most suitable for HA as graft material in anterior fusion, and the reports are somewhat contradictory (Zdeblick et al. 1994, Toth et al. 1995, Senter et al.1989, Cook et al. 1994). Generally, the more porous the material is, the more fragile it becomes (Wittenberg et al.1990, Tencer et al. 1985, 1988). However, cracks, slippage, and displacement are as highly related to the grafting method and to the possible adjunctive internal fixation method used. At present it seems that porosity up to 40 % is suitable as substitute. The height or thickness of the implants has proved to be essential as well, and, according to the studies of Kim et al. (1998) and Suetsuna et al. (2001), a minimum thickness of 10 mm should be preconditioned. One of

the shortcomings in this study was that the implants were too thin.

As a summary, interbody implantation of HA blocks without adjunctive internal fixation gives rise to segmental instability and causes collapse of both the implant and the disc space. The end-plate preservation does not allow sufficiently viable bone contact with HA for bone ingrowth. Thus, interbody implantation of porotic HA is presumed to require a better contact with cancellous bone, e.g. like shown in the study of Suetsuna et al. (1999), to be impeded into the vertebral spongiosa, and adjunctive internal fixation is obligatory.

7. CONCLUSIONS

Based on the aims of the present study, the results can be summarized in the following conclusions:

1. The use of a curved biodegradable polyglycolide (PGA) implant as particle containment showed less migration of HA granules compared to the conventional subperiosteal injection technique. The bone ingrowth into the HA layer, however, was better when conventional augmentation was used, but PGA-containment did not hinder the bone ingrowth. The PGA implant interfered with tissue nutrition giving rise to infections in 35 % of cases. Infections caused dehiscence and particle loss.

2. When particulate HA was pre-loaded inside the curved implant made of non-coloured PGA, the rate of infections was lower compared to the preliminary study, in 19 % of augmentations. The connective tissue response inside the HA graft varied from 75 % to 80 % and was higher when compared to conventional HA augmentation and even higher comparing pure HA inside the separate PGA containment. There was, however, only negligible bone ingrowth seen inside the HA graft. Foreign-body reactions were seen at the interface of the host bone as a sign of interference of the PGA/PLA copolymer and hindrance to bone ingrowth.

3. PGA and PDLLA fibres caused some local foreign-body and fibrous type tissue reactions, when fibre reinforced coralline HA blocks were implanted either in cancellous or cortical bone defects in the rabbit tibiae. These reactions did not disturb bone ingrowth into the HA blocks. Maximal bone ingrowth was seen at six weeks, and thereafter the amount of bone remained fairly constant up to the end of the 24-week follow-up. In terms of bone ingrowth there was no difference between the PGA- and PDLLA-reinforced implants. The mean percentual new bone ingrowth was 12,9 % in cancellous and 17,1 % in cortical implantation. The bone response inside the porous implant was shown to follow Wollf's law being dependent on the mechanical load pattern transmitted through the implant.

4. In minipig lumbar interbody implantation the plain radiographs showed some ossification in 12 out of 15 HA implanted disc spaces but in CT only in two out of 15 disc spaces, both implanted with coralline HA. The plain radiographs exaggerated the ossification due to the peripheral ossifications, e.g. ligamentous calcification and anterior and posterior osteophytes In MRI, universal finding was an increased signal intensity of the operated vertebrae. Histologically, the bone ingrowth in the reinforced coralline implants was promising and was seen in eight out of ten implants. In-

stead, no new bone was seen inside the synthetic porous PDLLA reinforced implants. In terms of disc space ossification, the histology and plain radiographs showed no correlation, whereas CT correlated better in terms of histologic ossification.

5. After discectomy and end-plate roughening the lumbar interbody implantation with PDLLA-reinforced coralline HA implants in growing pigs resulted in a significant loss in the disc spaces and it was similar to the loss of the emptied disc spaces. The loss of height was due to the fragmentation and resorption of the implants leading to instability and secondary changes, such as anterior osteophytes and local kyphosis. The measured local mean kyphosis was 18,0° at 16 weeks. The connective tissue reaction inside the implant increased until the end of the study and was mean 79,4 % at 16 weeks. The percent amount of HA decreased from 33,7 % at three weeks to 19,2 % at 16 weeks. According to the microradiographs, the implants fractured and lost their inner structure from six weeks on and they were dispersed. The bone ingrowth was minimal in every follow-up period, but rates of only 0,7–1,7 % could be measured.

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