JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **11** (2000) 711–717

Bone healing in porous implants: a histological and histometrical comparative study on sheep

Y. L. LIU*, J. SCHOENAERS
Department of Oral and Maxillofacial Surgery, Catholic University of Leuven, Capucijnenvoer 7, 3000 Leuven, Belgium
K. DE GROOT, J. R. DE WIJN
Biomaterials Research Group, Faculty of Medicine, University of Leiden, Prof. Bronkhorstlaan 10, 3723 MB Bilthoven, The Netherlands
E. SCHEPERS
Department of Prosthetic Dentistry, Catholic University of Leuven, Capucijnenvoer 7, 3000 Leuven, Belgium

Tissue integration in four types of porous implant materials (Interpore 200® or Corallin hydroxyapatite, hydroxyapatite blocks, hydroxyapatite granules and polymethylmethacrylate) was evaluated *in vivo*. Porous blocks measuring 20 mm × 10 mm × 8 mm were implanted in mandibles and iliac crests of sheep. Bone healing in porous blocks was studied at 2 and 6 months after implantation. The behavior of the material itself was also analyzed. Histological and histomorphometrical analysis revealed bone healing depending upon healing time and material. On the basis of analysis of variance, differences in amounts of bone ingrowth at 2 and 6 months were statistically significant ($p = 0.0039$ in mandible; $p = 0.0351$ in iliac crest). The longer the time span, the more mineralized tissues were observed in the specimen. Our data confirmed that hydroxyapatite has osteoconductive capacities. Porous PMMA was found to be biocompatible, but it showed less bonegrowth within the pores. Interpore 200®, which had the highest surface to volume ratio was found to display the highest level of osseointegration and biodegradation.

1. Introduction

Autogeneous bone is the best substitute for replacing defect bone at this moment. However, autogeneous bone has a limited availability for grafting and is associated with donor site morbidity. Suitable and biocompatible substitutes for bone grafts are therefore required. As a consequence, various bone substitutes have been developed [1–5]. If they have osteogenic (osteoconductive) capacity, certain alloplastic materials can be used successfully for the filling of bone cavities and the replacement of bone lost after tumor removal or trauma [6, 7].

Bone replacing materials, especially bioceramics made of calcium phosphate salts are available in porous and dense forms [6, 8, 9]. The pores of these calcium phosphate materials resemble the porous structures of cancellous bone and appear to allow for better bone repair. The minimum pore size required for an effective bone ingrowth into the porous structures is approximately 100 microns [10, 11]. Moreover, macroporous and microporous implant materials can display extremely high surface to volume ratio. The relatively large surface area of these porous forms, up to 32–50 m^2/g, facilitates contact osteogenesis, thus preventing the intervention of connective tissues which hampers the long-term stability of the implant [12, 13]. Volumes of granules mimic a three-dimensional porous structure, which allows for tissue ingrowth and subsequent mineralization.

The most well known Ca/P material is hydroxyapatite (HA), since it resembles the mineral phase of bone. Hydroxyapatite ceramics are produced in many shapes, dimensions and compositions. Porous or granular hydroxyapatite has similarity to the mineral phase of bone and shows a good biocompatibility [2, 11]. However, biodegradation of HA materials is possible and results in two phenomena. First, physico-chemical dissolution reduces the size of an implant. Secondly, tiny particles of materials which are dissolved and disintegrated by the first phenomenon are ingested and presumably digested intracellularly by phagocytic cells [2, 14, 15].

The purpose of this study was to analyze histologically and histomorphometrically the hard and soft tissue ingrowth into 3 forms of porous hydroxyapatite

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*Author to whom all correspondence should be addressed. Current address: Biomaterials Research Group, Prof. Bronkhorstlaan 10, 3723 MB Bilthoven, The Netherlands.

materials, and to compare their influence on the healing process. We have taken the non-ceramic material polymethylmethacrylate (PMMA) as a comparison.

2. Materials and methods

2.1. Implant materials

Four types of implant materials which are in clinical use were chosen in this study (Table I and Figs 1–4). Three of these four materials, Interpore 200® or Corallin hydroxyapatite (IP200®), hydroxyapatite blocks (HAB) and hydroxyapatite granules (HAG) belong to the group of the CaP bioceramics, and one is composed of porous resin PMMA. These four materials with the size of 20 mm × 10 mm × 8 mm have similar pore volumes of 40–50%.

2.2. Experimental design and surgical procedure

The experiment was carried out in eight adult female sheep. Each of these sheep received four implants. In the angular regions of the mandible, they received Interpore 200®, and an equal volume of porous sintered HA blocks. In the iliac crests, each sheep received a porous PMMA block and an equal volume of dense sintered HA granules, these blocks having the same size as those in the mandible.

The sheep were operated under general anaesthesia which was administered in a semi-closed ventilation system (Halothane: 1% to 1.5%; oxygen 33.0%; NO_2 66.0%), the lateral periosteum was reflected and a bony cavity measuring 20 mm × 10 mm × 8 mm was prepared, using an electric driven dental hand-pressed drill. The bony cavities of iliac crests were made in the same way. Daily inspection and wound care were given. Antibiotics were continued for one week. The eight sheep were divided into two groups, the first group of four sheep was sacrificed at two months. The second group of four sheep was sacrificed at six months.

2.3. Qualitative and quantitative analysis

Each implant was further divided into three parts (A, B, C) representing the anterior, the middle and the posterior of the implant. These specimens were fixed immediately in a solution of neutralized formaldehyde and ethanol, dehydrated in a series of graded ethanol, soaked in purified methylmethacrylate monomer and polymerized.

Serial sections of approximately 80 microns were made on a sawing microtome (Leitz®, Wetzlar,

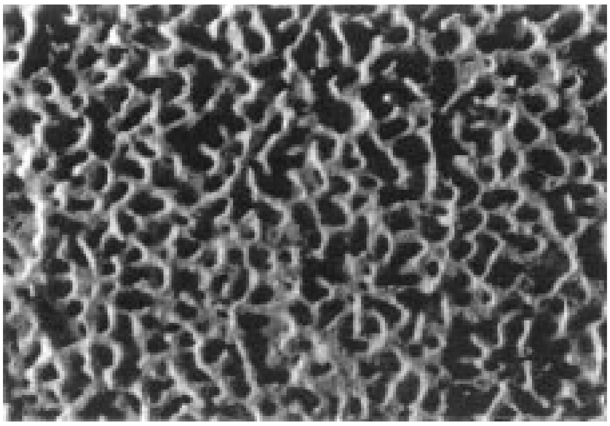

Figure 1 Interpore 200® (Corallin hydroxyapatite).

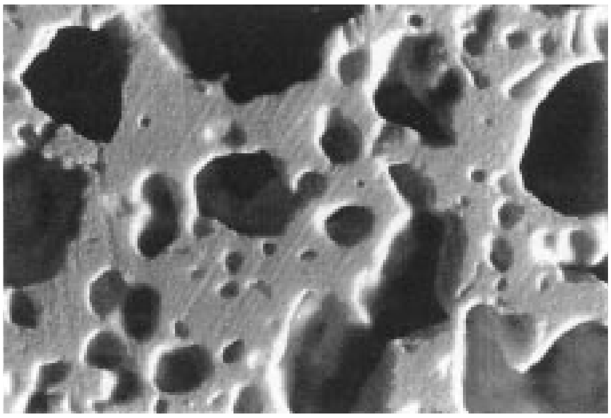

Figure 2 Hydroxyapatite blocks.

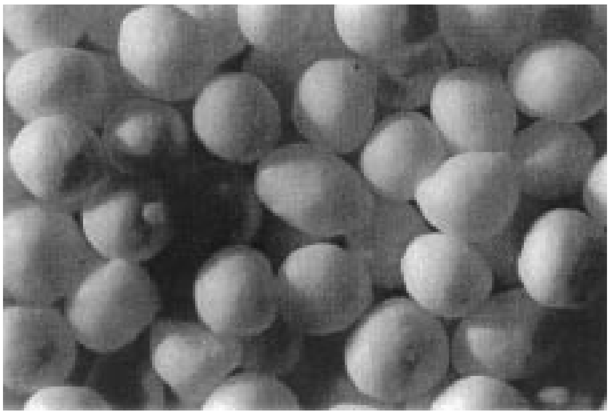

Figure 3 Hydroxyapatite granules.

TABLE I The characteristics of four types of implant material

Name of material	Porosity characteristics	Manufacturer
IP200® (Interpore 200® or Corallin hydroxyapatite)	Pores are homogeneous and interconnected, pore diameter is 180–230 microns	Johnson & Johnson, USA
HAB (hydroxyapatite blocks)	Pores are irregular and mostly not interconnected, diameter is 100–400 microns	Free University, Amsterdam, The Netherlands
HAG (hydroxyapatite granules)	Pores are homogenous and interconnected. The diameter is 300–400 microns	Free University, Amsterdam, The Netherlands
PMMA (Polymethylmethacrylate)	Pores are homogenous and interconnected, the diameter is 100–400 microns	Catholic University, Nijmegen, The Netherlands

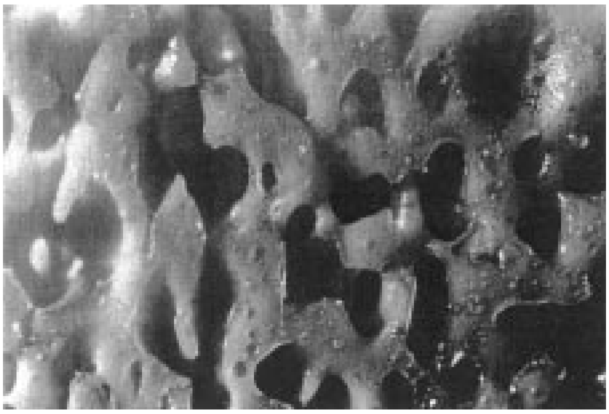

Figure 4 Polymethylmethacrylate.

Germany). The final thickness of the sections at 50–60 microns were obtained by grinding and polishing in a semi-automatic way (Minimet®, Buehler Ltd, Illinois, USA) with SiC papers (Carbimet® Paper Discs, Buehler Ltd, Illinois, USA) up to 600 grid using superfluous water cooling. The sections for light-microscopic examination were stained with Stevenel's blue and Von Gieson's picro-fusion. Histological examination and photomicrography of the sections were performed on a light microscope (Leitz Labonlux S, Wetzlar, Germany) with a camera (Heerbrugg, Switzerland) and a monitor (JVC TM–1500PS, Japan) at magnifications of 9 × , 20 × , 40 × and 100 × . A Leitz CBA 8000 system (Leitz®, Wetzlar, Germany) was used to examine all sections qualitatively and quantitatively.

Three sections of each implant specimen were measured. Five optical fields per section were scanned, at a magnification of 32 times, which stands for 51.7% of the whole sectional area. After obtaining the surface percentages of bone and implant materials, the ''third compartment'' was calculated as follows:

$$\begin{aligned}&100\% - \text{bone}\% - \text{implant}\% \\ &= \text{``third compartment (Fibrous)'' percentage}\end{aligned}$$

The bone content of the pores was calculated as:

$$\text{Bone}\%/(100 - \text{implant}\%)$$

Histometrical results were statistically compared in a mixed model with the approximate F-test at propinquity level 0.05 to analyze differences of the sections. The total amounts of mineralized tissues (bone) and the percentages of bone-fillings in the porous spaces as well as the total amounts of the implants were analyzed at two and six months.

3. Results

3.1. Microscopical observation

3.1.1. Mandible

Healing in IP 200® after two and six months. After two months of implantation in the mandible, in some areas bone growth over long distances inside the interconnected pores of the material was observed. Osteoblast activity is seen on the surface of the bone. The presence of large bands of osteoid tissue and active osteoblasts confirms considerable bone growth activity. The implant is lined with mineralized tissues which shows a smooth surface. The bone growth on the surface of the material suggests an osteoconductive nature of the coralline hydroxyapatite, that is, no fibrous tissue layer separates the bone from the implant material. In some places where the implant is surrounded with soft tissues, a significant number of multinuclear giant cells are eroding the surface of the IP 200® implant (Fig. 5). The histological observation indicates that, after six months of implantation, the bone tissue has almost completely filled the pores of the implant at the surface. Also, a considerable amount of mineralized bone tissue can be detected in the interior part of the implant (Fig. 6). The presence of osteoid tissues and a band of active osteoblast indicate that bone tissue apposition is still active. The surface of the material in contact with fibrous tissue is at times lined up with giant cells. The roughness of the pellet surface suggests disintegration and resorption of the material.

Healing in HAB after two and six months. Microscopical analysis of HAB after two months reveals the formation of a thin layer of bone on the surface of the material. Bone tissue is most noticeable at the peripheral region close to the bony wall of the defect. Bone growth in the implant is limited. At the center of this material absence of bone or soft tissues is observed. Also after six months of implantation the entrance area of this material is

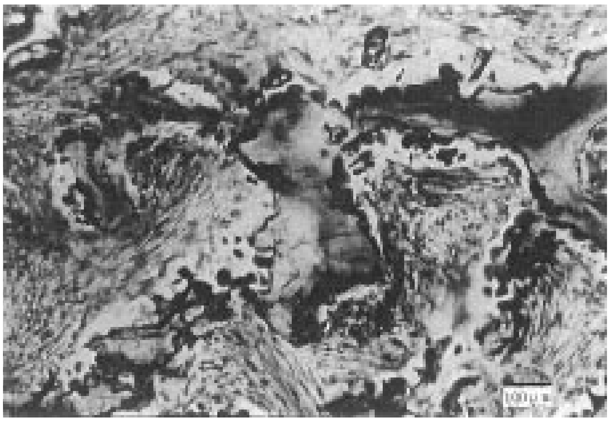

Figure 5 After two months of implantation in the sheep's mandible, there are giant cells (arrows) at the surface of the Interpore 200®.

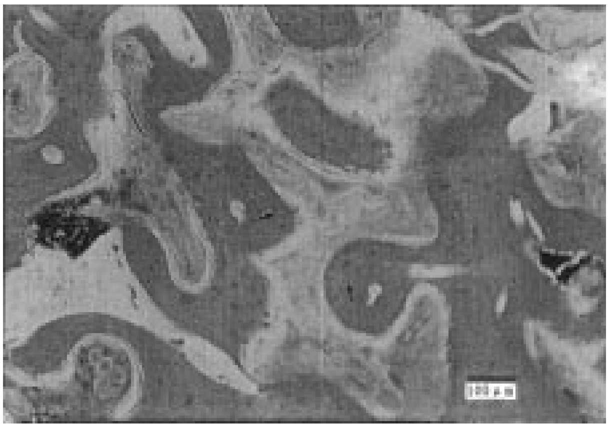

Figure 6 After six months of implantation in the sheep's mandible, mineralized bone (arrows) fill the pores of Interpore 200® and it is also visible in the center of the implant.

occupied by some bone trabeculae. Most of them do not show an intimate contact with the surface of the block. Bone tissue has penetrated to some extent into the porous HAB, starting from the walls of the defect towards the center. The pore surface in the materials show a limited osteoconductive nature. In the center of the material, the cavities are mostly covered by fibrous tissues.

3.1.2. Iliac crests

Healing in HAG after two and six months. After two months of implantation, bone growth can be seen between the granules in contact with the surface. Bone regeneration is starting from surrounding bone. Multinucleated cells are seen at the implant surface where they are surrounded by soft tissue (Fig. 7). The new bone is mainly of a woven, not of a lamellar structure. There is no bone tissue formation observed in the center of the granular implant. Foreign body giant cells can be detected in these areas. They are disintegrating the material into small pieces and the material is being chipped off and migrating away from the original implant surface into the void. After six months of implantation, the histologic situation around the HA granules material is basically the same as that after two months, though the total amount of bone tissue has increased. Now, mature lamellar bone covers most of the implant surfaces. The outside of the defects are almost entirely enclosed by bone tissue. However, the bone growth in the center of the defect areas is limited, and these bony areas are only occasionally in contact with the granules. The rest of the granules are surrounded with fibrous tissue. The erosion of the implant surfaces, after six months of implantation has a somewhat more ragged, eroded appearance, indicating some degree of biodegradation. Foreign body giant cells, involved in the degradation of the hydroxyapatite granules are also observed.

Healing in PMMA after two and six months. After two months of healing, bone tissue is observed in the peripheral pores of the porous PMMA. It is progressing from the surrounding bone. A few islands of mineralized tissues are in contact with the implant surfaces. In the central area of the porous PMMA implant, soft tissues fill the pore spaces. To a great extent the porous surfaces are covered by fatty cells, which are surrounded by vascular structures. No multinucleated giant cells are noticed. There is no homogeneity with regard to the distribution of porous spaces and the shape of the porous in the PMMA material. After six months some bone growth is noticed at the exterior of the porous PMMA block. Bone growth appears over a short distance near the block's surface which is contacted with pre-existed bone (Fig. 8). In the center of the material, bone is not observed in the porous spaces, most of the pores are filled with soft tissues and fat cells. No osteoclast activity is observed. Foreign body giant cells and other phagocytosing cells are not detected.

3.2. Histomorphometrical comparative results

Histomorphometrical data in four different materials and at two different times are presented in Table II.

3.2.1. Mandible

IP200® and HAB at two and six months (Figs 9 and 10, Table II). After two months of implantation, the percentages of bone-filled pores in IP200® and in HAB are not significantly different ($p = 0.1133$). After six months the bone-filled pores in IP200® material is greater than in HAB material; statistical analysis indicates the significance of the observed differences in bone-filling ($p = 0.0001$). At six months the bone growth in IP200® material is clearly more extensive than after two months ($p = 0.0001$). The remaining amount of implant material itself is less after six months than after two months ($p = 0.0001$). The analysis of the HAB implant reveals a better bone-fill response after the six month period than after the two month period ($p = 0.0139$). The amount of HAB material itself shows a higher percentage after six months than after two months ($p = 0.0288$).

3.2.2. Iliac crests

HAG and PMMA at two and six months (Figs 9 and 11, Table II). Comparing the tissue ingrowth in the granular

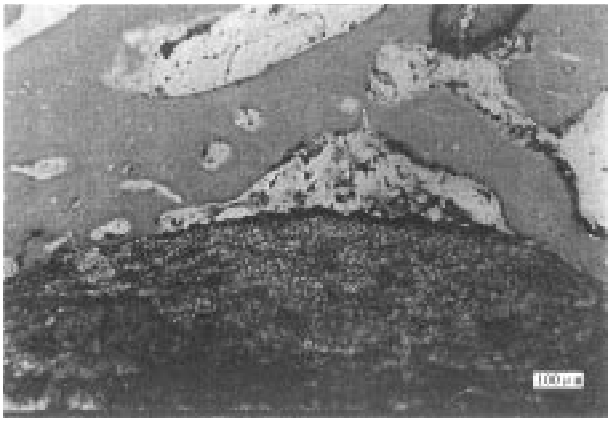

Figure 7 Giant cells (arrows) line-up at the surface of hydroxyapatite granules.

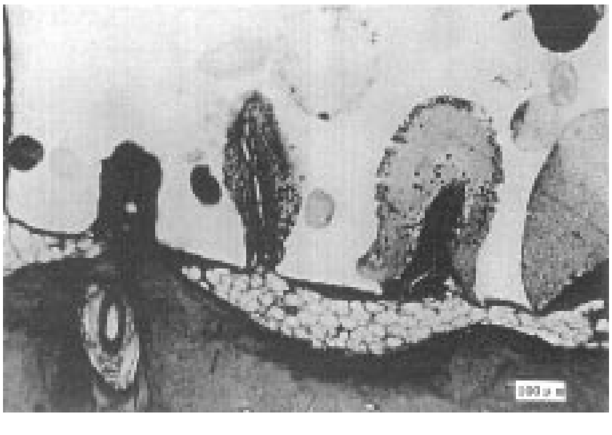

Figure 8 After six months of implantation in the sheep's iliac crest, bone growth in the porous PMMA is only scarcely noticed.

TABLE II Means and standard deviation revealed at two and six months of tissue ingrowth in four different materials, bone content of the pores and the behavior of material itself

Mandible Time	Material	A Bone growth (%)	SD	B Implant (%)	SD	100 –(A + B) Fibrous (%)	SD	A/100 – B Pore fill (%)	SD
2 months	IP200®	7.24	(6.27)	58.33	(5.69)	34.39	(9.02)	17.98	(15.44)
	HAB	5.34	(4.94)	55.07	(8.72)	39.57	(7.69)	11.36	(9.80)
6 months	IP200®	32.11	(11.92)	31.75	(5.52)	36.14	(13.21)	47.05	(16.25)
	HAB	11.41	(6.36)	61.07	(6.35)	27.52	(9.38)	29.31	(18.52)

Iliac Crest Time	Material	A Bone growth (%)	SD	B Implant (%)	SD	100 – (A + B) Fibrous (%)	SD	A/100 – B Pore fill (%)	SD
2 months	HAG	7.39	(6.16)	57.54	(5.22)	35.08	(5.22)	16.75	(12.63)
	PMMA	1.37	(1.51)	46.56	(6.50)	52.07	(5.75)	2.41	(2.55)
6 months	HAG	13.08	(6.15)	57.45	(5.61)	29.47	(7.66)	31.02	(14.90)
	PMMA	4.51	(3.49)	52.54	(7.60)	42.94	(7.83)	9.62	(7.21)

Values of A and B are measured as surface percentages in the sections.

HAG material with the porous PMMA, more bone is detected in HAG material after two months than in PMMA ($p = 0.0001$). At six months, the amount of bone growth in HA granules is also significantly larger than that in PMMA ($p = 0.0001$). These results confirm that the amount of mineralized tissues in the spaces of granules at the six month healing period is significantly higher than at the two month healing period ($p = 0.0351$). The histometrical comparison of the tissue integration in PMMA shows that the absolute amount of mineralized tissue observed is very low at two months. A significant increase occurred in the six month interval ($p = 0.0351$), although the amount is still very low.

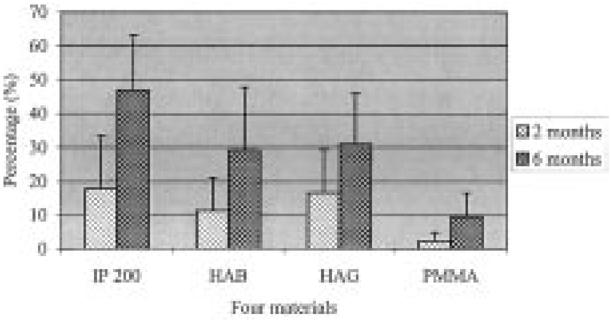

Figure 9 Bone-fill percentages in pores of four materials after two and six months (SD).

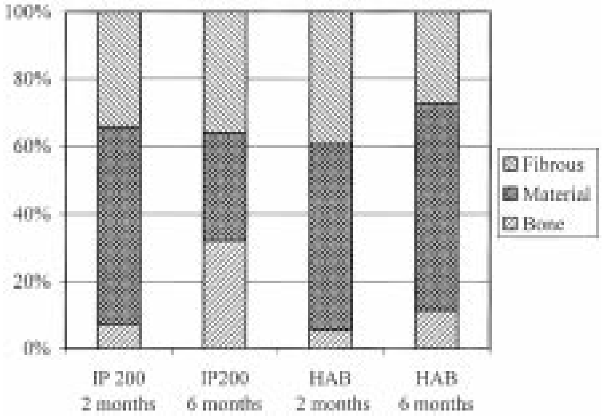

Figure 10 Comparison of the percentages in IP200® and HAB in the mandible after two and six months.

4. Discussion

Many investigations in the field of experimental biomaterials research in animals have been carried out, sometimes using one implant material in one species. Most studies have been undertaken, using several bone substitutes in the same animal [14, 16, 17]. In our study we have evaluated tissue integration in four different materials, in two different sites in the sheep.

Sheep were chosen as experimental animals because they have morphological and physiological characteristics in their bone structure which are comparable to those of human bone. The sheep's mandible has ample experimental surface area. Sheep are docile, and they need only simple care. Sheep also do well under general anaesthesia. There exists an acceptance of projecting the outcome of bone healing studies to human tissue repair [18].

A bony defect size of $20 \times 10 \times 8\,\mathrm{mm}^3$ was chosen because in a previous canine healing experiment, bone regeneration in coralline hydroxyapatite and in auto-

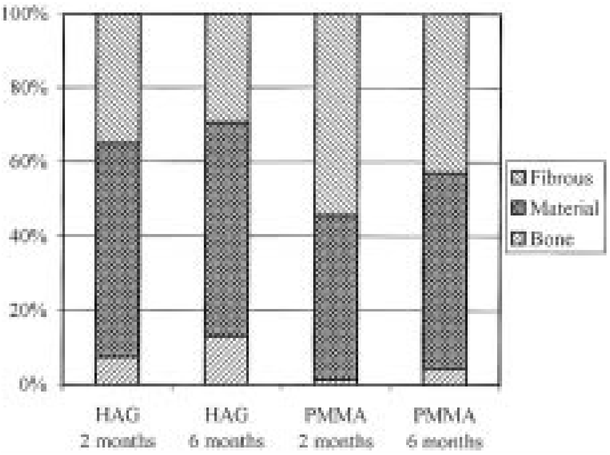

Figure 11 Comparison of the percentages in HAG and PMMA in the iliac crest after two and six months.

genous bone grafts had been studied in defects of the same dimensions [17].

Bone healing in the porous materials is confirmed by the results of this study: the osteoconductive nature of porous hydroxyapatite is noticed, giant cells and osteoclast cells line-up on the surface of HA implants, which indicates the degradation of the implant. Similar findings have been reported by several authors [7, 14, 19]. However, the different calcium phosphate materials in this study (IP200®, HAB and HAG) did not behave in the same way.

In the mandible, the bone content of the pores of IP200® is more pronounced than HAB. The IP200® material is reduced substantially ($p = 0.0001$) in contrast to HAB which seemingly increased somewhat in volume. Of course this can be explained in respectively increase and decrease of the pore volume. Also in another study IP200® was found to become surrounded by phagocytosing cells and tend to be fragmented and dissolved [14]. The findings here may be explained by the following reasoning: the pore size of IP200® (180–230 microns) is approximately similar to the pore size in bone (200 microns), which is ideal for bone growth. The pores are interconnected and display an extremely high surface to volume ratio [12]. The pore size and type of HAB are not the same as in IP200®: the pores are irregular, and there are less interconnections between pores. The biocompatibility of the material and its surface characteristics are essential for the material to be accepted or integrated in the host [14]. Biointegration as well as biodegradation processes occur at the surface between implant and bone. For each of the phenomena, blood supply, sustaining cell vitality, as well as biochemical and physical reactions, are of utmost importance. Whether blood supply is adequate or not depends on the permeability of the implant. This occurs when pores are linked. The quality and the quantity of the connections between porous cavities are of major importance for biointegration or biodegradation of porous implants [20]. Thus, the percentage of porosity, the shape and the dimensions of the pores and the interconnection of the porous spaces, all contribute to the healing or biodegradation process.

In the iliac crest, the amounts of bone healing in HAG are much different compared to bone healing in PMMA. They can be explained by two reasons: first, the pores of HAG and PMMA are interconnected as in IP200®, bone healing in these materials can be explained in the same way as in the mandible. Second, the physical-chemical properties are different between PMMA and HAG implant materials.

In 1979 Holmes determined bone ingrowth in IP200® [21], using a computerized scanning electron microscope image analysis. 11% and 88% of the coralline hydroxyapatite void were filled with bone in dog mandibles at two and six months. In our study, bone-filled in IP200® is 17.98% and 47.05% at two and six months in the mandible of sheep. Bone growth rates in HAB, HAG and PMMA are lower than that in IP200®. The three porous ceramics are osteoconductive (i.e. they provide a lattice on which local cells can form new bone). Bone healing in to the porous structure starts from the pre-existing bone and moves towards the center part. Our histomorphometrical data clearly showed that the bone healing increases from two months to six months after implantation, no matter if they are in mandible or in iliac crest. However, the amounts of bone in the pores vary in different materials, even in the same time period. This may be due to the difference of the density, the size, and the interconnectivity of pores in the implant materials. Also the chemical composition of the materials may be considered to be important [11]. In this study we have seen large differences in osteoconductivity between the hydroxyapatite implants and porous polymethylmethacrylate, the advantage of calciumphosphate chemistry over the organic polymeric compound being obvious. Minor differences in the chemical composition and crystallinity of hydroxyapatite may also be of importance – although not considered in this study – especially where the rate of degradation is concerned. The combination of pore geometry and chemical composition constitute the important factors for the successful selection of a porous material that would permit bone rather than connective tissue growth.

5. Conclusions

This animal experimental method is valuable for a simultaneous study of our four porous materials. The histomorphometrical technique applied on non-demineralized sections, is a reliable method to quantify bone healing in the porous implants. Interpore 200® with high surface to volume ratio, displays most signs of osseointegration and biodegradation. Compared with the other three materials, Interpore 200® has the highest osteoconductive nature. The bone ingrowth and bone volume increased with time so that after six months the bone tissue appeared much more than after two months. This study confirmed that hydroxyapatite material has capacities of an osteoconductive nature. PMMA is a biocompatible material but possesses less osteogenic potential than hydroxyapatite.

Acknowledgments

The authors would like to thank the F. Verbiest Foundation, Catholic University Leuven, Belgium, especially Fr. Jeroom Heyndrickx for their financial support and encouragement. Profs M. Brussuyt and E. Verbeken, Dr H. Bras and Dr L. Barbier are gratefully acknowledged.

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Received 15 December 1998
and accepted 8 June 1999