Acta of Bioengineering and Biomechanics
Vol. 9, No. 2, 2007

A polyethylene-high proportion hydroxyapatite implant and its investigation in vivo

F. SARSILMAZ[b]*, N. ORHAN[b], E. UNSALDI[a], A.S. DURMUS[a], N. COLAKOGLU[c]

[a] Department of Surgery, Faculty of Veterinary, Firat University, 23119 Elazig, Turkey
[b] Department of Metallurgy Education, Firat University, 23119 Elazig, Turkey
[c] Histology and Embryology Department, Medical Faculty, Firat University, 23119 Elazig, Turkey

An implant from hydroxyapatite and polyethylene (HA+PE) composite was investigated for the usability in large bone defects. With this aim, the implants were manufactured in blocks by hot compacting the mixture of 80% HA and 20% PE weight ratio. Powders were machined in a lathe in the dimensions of diaphysis of the radius of the mongrel dogs. Then a defect, 1.5 cm in length, was made in the diaphysis of the radius with an operation performed under general anaesthesia in 16 healthy mongrel dogs. The defects were filled with implant as a block.

The dogs were observed radiologically in 15-day intervals and examined clinically in certain intervals. The bone samples were taken out from four dogs for the histopatological examinations at the end of the 2nd, 4th, 6th and 12th months, respectively.

Clinical examinations indicated the occurrence of slight lameness in all cases at the first month of experiment, but lameness completely disappeared in a further examination.

Progressive resorption and new bone formation began in the implants from the first month, but complete resorption was not observed in any case at the end of 12-month period.

SEM and optical microscope examinations revealed fibroblast cell with its clear cytoplasmic extensions and osteoblast cells in endosteum in the inner region. Bone formation increasing and extending to the pores of implant in time and blood vessels with lamellar structure and Haversian system were observed.

As a result, it was indicated that HA+PE composite implants could be applied with confidence and are useful in treatment of large bone defects in long bone of dogs.

Key words: *hydroxyapatite, polyethylene, implant, bone defect*

1. Introduction

In the last decade of the twentieth century, there were many developments in orthopaedic surgery. The most important are these in implantology. The materials used in implantology are subjected to very hard conditions in vivo [1]. Properties of the material used, design of implant and fixation method determine the performance of the implant [2]. Strength, fatigue, surface corrosion, allergenic reactions and biocompatibility of material are the common issues being presented in the research literature. Many implants endure up to 20 years inside the organism, which seems still considerably short compared to the human life [3]. Therefore, it is vital to develop implants exhibiting high strength and biocompatibility [2], [4]–[6]. Composites which comprise a bioactive filler and ductile polymer matrix are desirable as implant materials since their both biological and mechanical properties can be tailored for a given application [2], [7].

Hydroxyapatite (HA – $Ca_{10}(PO_4)_6(OH)_2$), a synthetic calcium phosphate ceramic resembling bone mineral, was used in various biomedical fields such as dental material, bone substitute and hard tissue paste [3]. HA can accelerate the formation of bone-like apatite on the surface of implant [4], [12]. Recently, it has been reported that HA can be osteoconducive and osteoinducive because they can induce bone formation when implanted in dogs [13]. On the other hand,

* Corresponding author: Department of Metallurgy Education, Firat University, 23119 Elazig, Turkey. E-mail: fsarsilmaz@firat.edu.tr
Received: May 29, 2007
Accepted for publication: June 30, 2007

stress distribution across the implant/bone interface during stress application is also very important and depends on the respective Young's and shear moduli of the implant and tissue [4]. Thus, an implant material with an elastic modulus similar to that of bone is required.

The elastic modulus of high-density polyethylene (PE), commonly used as an implant material, takes a value of approximately 1 GPa, and was thus selected as the matrix for a HA filler (Young's modulus of approximately 85 GPa) of the bone analogue material. HAPEX™ is one of these materials [2], [7], [8]. It comprises a 40% volume of HA in PE, and its Young's modulus is equal to 4.4 ± 0.7 GPa, which approaches the lower end of the modulus value for cortical bone (Young's modulus of 14–20 GPa) [2], [7]. At this volume fraction the fracture mode is still ductile, rather than brittle, so that the composite can be said to be fractured tough [9], [10]. HAPEX™ offers the potential for a stable implant–tissue interface during physiological loading and has established clinical uses for middle ear and orbital floor implants [10]. The development of biological and mechanical characteristics is important for the future advancement of HAPEX™ as a material which could act as a dense scaffold, encouraging the growth of bone directly onto the implant in load bearing situations [10], [11].

Material surface topography is known to be important in the cell–material interaction, for cell orientation and migration. Different cell types respond to topography in different ways. Osteoblastic phenotype and degree of bone contact have been shown to be responsive to topography, with bone formation preferentially observed within grooves and crevices [3].

Therefore this study focused on producing a PE and HA composite with a considerably high HA content using a relatively simple and cheap production method.

2. Materials and methods

In the study, a composite was produced from polyethylene and hydroxyapatite powders with an average particle diameter of 60 μm. With this aim, HA and PE powders were mixed in the proportions of 80 and 20% in weight, respectively, in a mixer for one hour to obtain a homogeneous mixture [7], [14]. Then this mixture was cold pressed in a metal die (see figure 1) under a pressure of 45 MPa. The die was heated and held at 175 °C for a time required PE to surround and bind HA powder.

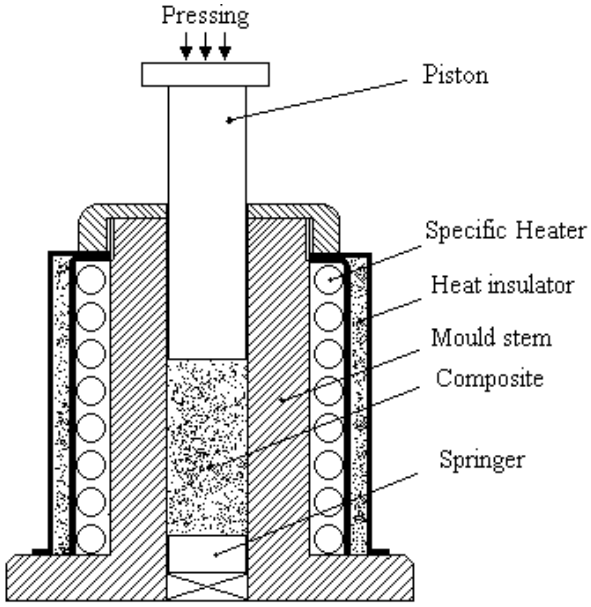


Fig. 1. A metal die for HA+PE composite producing by HIPing

After composite was produced, the composite bars were machined in a lathe to the diameter of the radius where they were to be implanted (outer diameter D = 10 mm, inner diameter d = 5.5 mm and d/D = 0.55 for maximum strength) (see figure 2). In order to help cell adhesion, the outer surface of the implant was finished by a rough cutting tool.

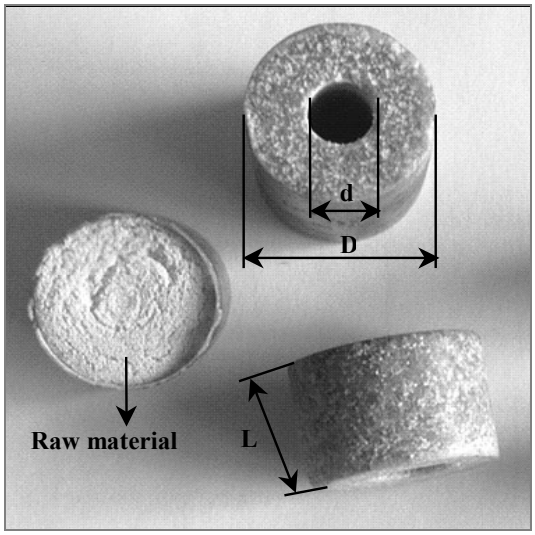


Fig. 2. Final illustration of HA+PE composite with dimension symbols

The mechanical properties of the implant manufactured, such as tensile and compression strengths, Poisson ratio and Young's modulus, were determined with a Hounsfield tensile test machine.

Microstructure of the composite was examined under optical microscope and SEM. Compositions of HA

and PE were determined by EDS (figure 4). Interface between HA (reinforcement) and PE was examined.

16 dogs were used in order to examine the implants in vivo.

Anaesthesia was maintained by 1.5 cm^3/10 kg Rompun (xylazine hydrochloride, 23.32 mg/cm^3, Bayer) given ten minutes after the injection of 15 mg/kg Ketalar (ketamine hydrochloride, 50 mg/cm^3, Parke–Davis), intramuscularly.

Skin was incised along the craniolateral edge of the radius. Diaphysis of the radius was exposured by the conventional incision of subcutaneous fascia and muscles [15]. A 1.5 cm defect was created by Gigli's wire saw in the diaphysis of the radius. The block was implanted into the defect area and fixed by a metal plate.

Operation wound was closed by the conventional operational techniques. All cases were examined radiographically immediately after the operations. Antibiotics were parenterally administered for five days in order to prevent possible postoperative infections.

The dogs were radiographically investigated and clinically checked in 15-day intervals. Bone samples were taken 2, 4, 6 and 12 months after the implantation.

Bone samples were fixed in 4% paraformaldehyde/0.1 M phosphate buffer (pH 7.4) for 72 h at 4 °C, decalcified in EDTA, dehydrated and embedded in wax. 5-mm thick sections were cut by microtom and stained with haematoxylin. The sections were viewed and photographed using an Olympus BH2 microscope.

For SEM examination fixed tissue samples were dehydrated using first ethanol series and then the series composed of amilacid and ethanol mixture. At the last stage, tissues were placed in pure amilacid. The samples were dried in critical point drier under 70 bars for 2 hours and finally they were covered with silver.

3. Results

3.1. Material properties

From optical and SEM micrographs it is seen that hydroxyapatite particles are binded by the melted polyethylene and then shrinked after solidification during hot isostatic pressing. Some pores are also seen in the structure. As shown by SEM in figures 3 and 4, the hydroxyapatite particles are distributed almost homogeneously and interconnected in the structure.

Mechanical properties of the implant produced are given in the table [2], [8]. As can be seen from the table, maximum compressive strength (see figure 5) and Young's modulus of the implant are lower than these of human cortical bone, and Poisson ratio approaches unity due to the polyethylene included.

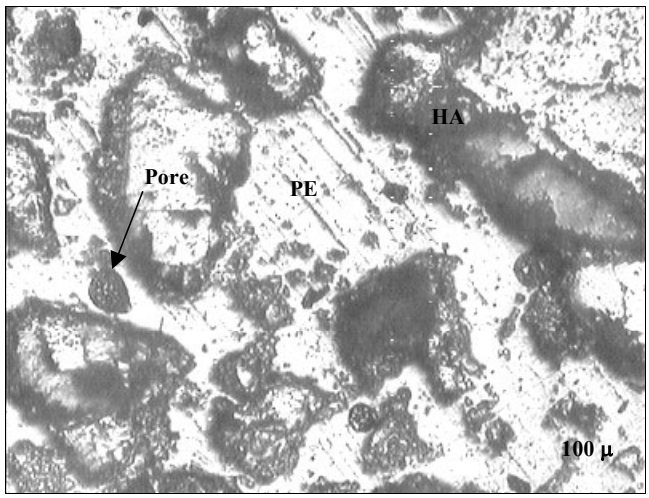


Fig. 3. Optical observation of HA+PE composite after its production (100×)

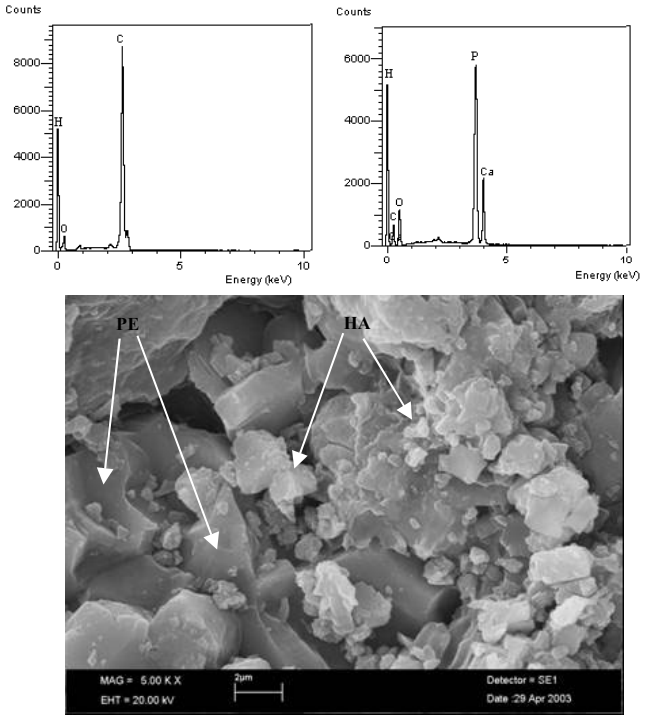


Fig. 4. SEM and EDS observations of HA+PE composite after its production (500×)

3.2. Surgical and radiological results

In the clinical examinations, slight lameness was observed in all the cases in the first month after the operation. At the end of the first month lameness dis-

Table. Mechanical properties of different human tissues, ceramics and 80% w/w HA+PE composite

Material	Ultimate compressive strength (MPa)	Young's modulus (GPa)	Modulus of resiliency (MPa)	Hardness (Vickers, kg/mm)	Poisson ratio	References
Human bone (cortical)	88–230	3–30	–	–	0.22–0.63	[18]
Human bone (dentine)	300–380	15–20	62,7	–	–	
Human tooth (enamel)	250–550	10–90	–	340	–	18]
Poly(ethylene)	35	0.88	–	–	–	[2]
Hydroxyapatite	300–900	80–110	6–13	600	0.28	[2], [18]
Zirconia	1700–2000	195–210	30–500	1100–1200	0.27	[2], [18]
Polyethylene–hydroxyapatite composite	75–80	4.4	–	–	0.95	

appeared in all the cases after PVC supported bandage was removed.

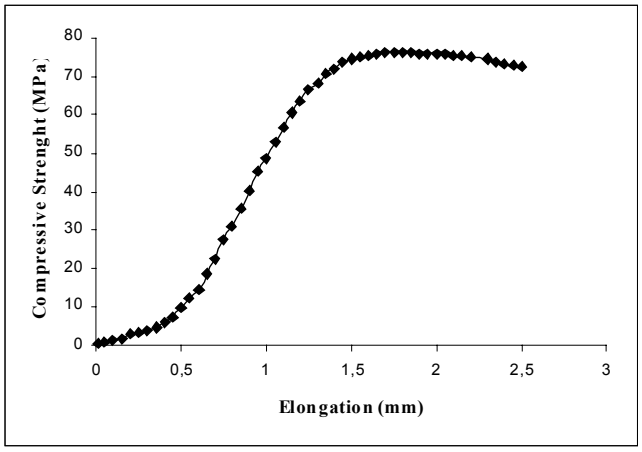


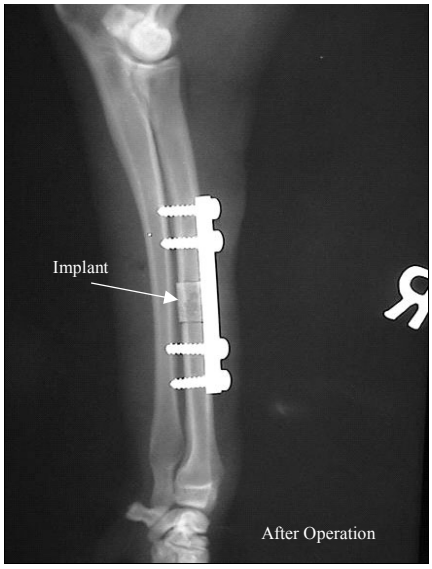
Fig. 5. Compressive strength curve of HA+PE composite

In the radiographical examinations, the bonding of the bone–implant did not change significantly in the second month. Even if bonding started, radiodensity of the implant decreased gradually and changed into decreasing radiolucent contrast after the third postoperative month.

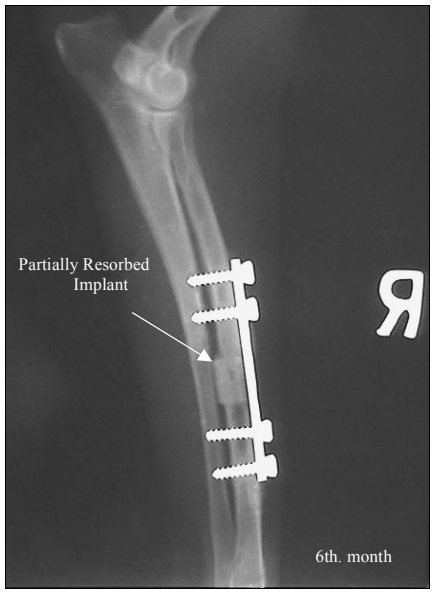
Exact resorption was detected in no cases. But partial resorption can be seen in the following 6-month control group. The best resorption has been observed in 12-month control group. Radiograms from various durations are given in figures 6a, b and 7a, b.

3.3. Histopathological results

In histopathological examinations, a response in the implantation region due to regeneration and a dense fibrous callus formation in the periostal region

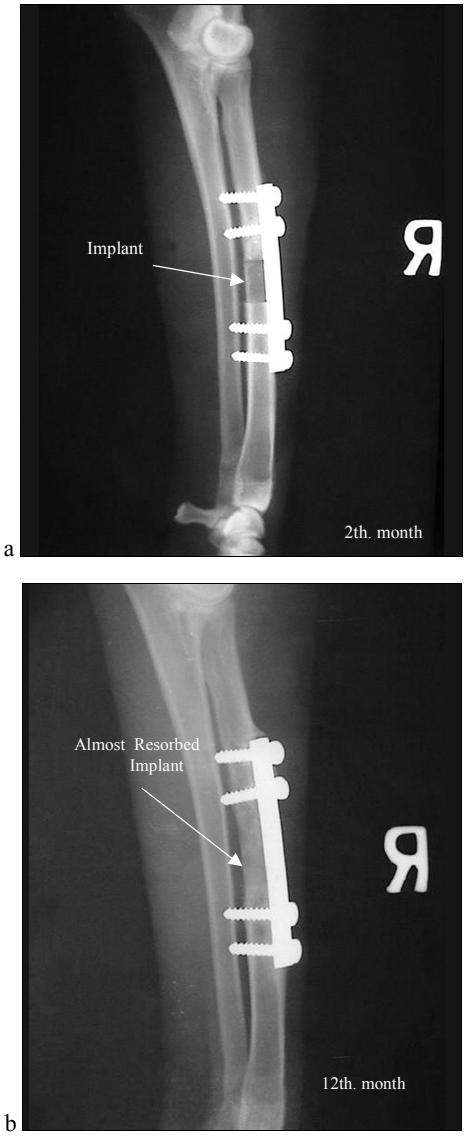


a



b

Fig. 6. Radiogram of the case after surgery (a) and in the postoperative 6th month (b)



a

b

Fig. 7. Radiogram of the case in the 2nd month (a) and in the postoperative 12th month (b)

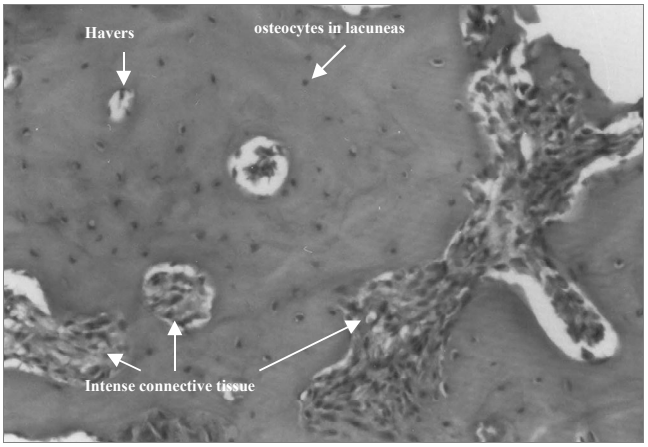


Fig. 8. A cross-section in the 6th month, abundant connective tissue, havers and lacunae can easily be seen. Haematoxylin

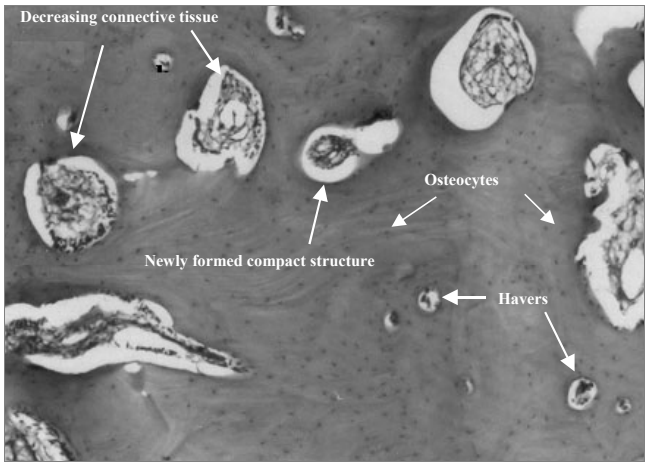


Fig. 9. A cross-section in the 12th month, connective tissue changing to compact structure; havers and osteocytes can easily be seen. Haematoxylin

Fig. 10. A cross-section in the 12th month, havers and chondrocytes in active endochondral bone region can easily be seen. Haematoxylin

was observed at the end of the second postoperative month. Although a slight inflammatory response to biomaterial being rich in neutrophils was provoked, the regeneration of new bone tissue was much more prominent. Local young cancellous bone tissues and biomaterial pieces trapped in the callus were conspicuous. In some areas, osteoid tissue penetrated into the implant and osteoblast activity increased.

Histopathological examinations after implanting revealed a response due to bone regeneration and an immense fibrous callus formation in periostal region. Although an inflammation, manifesting itself as the production of aboundant light neutrophils, neobone formation was more prominent. Biomaterial pieces locally trapped inside young collagen bone tissue and callus were seen. Osteoid tissue penetrated into biomaterial and osteoblast activity increased in some regions.

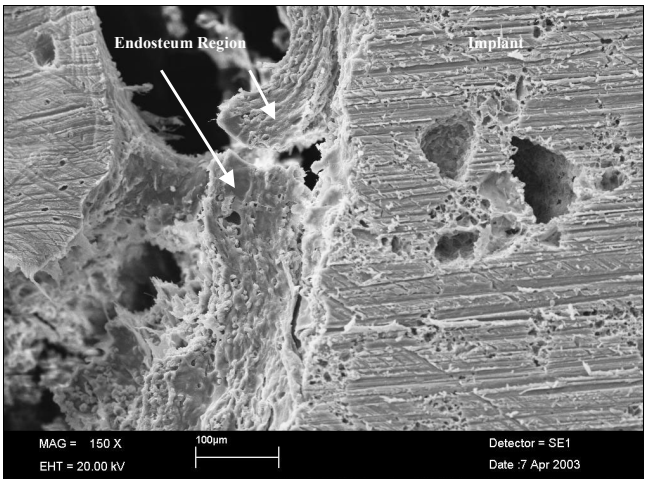


Fig. 11. SEM observation in the 6th month, intersection between endosteum region and implant can be seen

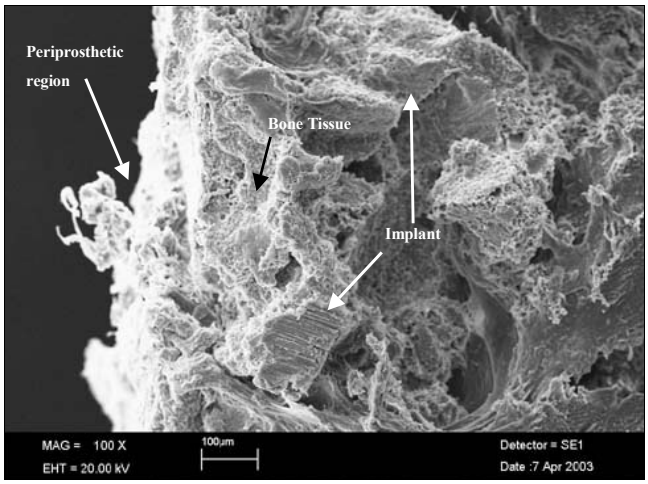


Fig. 13. SEM observation in the 12th month, periprosthetic region, bone tissue and implant region can be seen

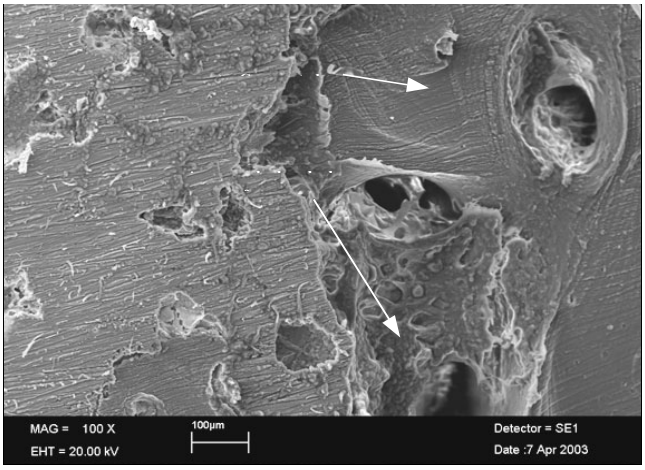


Fig. 12. SEM observation in the 6th month, intersection between active endochondral bone region and compact structure and implant can easily be seen

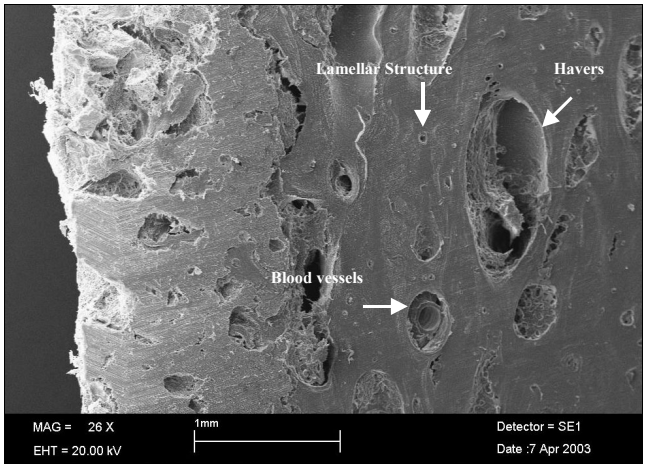


Fig. 14. A longitudinal section in the 12th month, lamellar structure, havers and blood vessels can easily be seen in SEM

Optical microscope examination showed that compact structure had not formed completely and there was abundant connective tissue in bone cavities at the end of the sixth postoperative month (see figure 8).

In the 12-month group a compact structure was more regular, connective tissue decreased considerably and in some areas active endochondral bone formation occurred (figures 9 and 10).

SEM examinations revealed bone tissue formation, blood vessels that extended to the pores and the channels formed as a result of the resorption of HA in the implant in the right proportion with time when the samples of the 6th and 12th months were considered (figures 11 and 12). It was seen that periosteum and bone tissue were formed on the outer surface of the implant in the samples from the 12-month group (figure 13). In this group, it is also seen from SEM photos that blood vessels were formed besides Havers channels and lamellar structure (figure 14).

4. Discussion and conclusion

In the study, the HAPE used was produced combining a bioinert material (PE) with an active material (HA) with a mechanical binding force. The surface of the implant was kept as reactive as possible [16], or interactive with the neighbouring tissue by adding the greatest possible amount of HA. Since it is well known that cell profileration and adhesion increase in the case of a rough surface [17], the outer surface of the implant was machined by a rough cutter, thus obtaining a rough finish.

Mechanical properties of the implant produced were close to these of the human cortical bone except the Poisson ratio [8]. The Poisson ratio of approximately 1 could be thought a problem regarding mechanical strength, but it was preestimation of the researchers in material side of the study that this could be eliminated by the penetration of the osteoblasts into the composite via pores and resorbed HA. This estimation was proved by the radiographical and histopathological results.

Histopathological examinations after the second postoperative month revealed the following: although regenerative reaction in the implant region, more immense fibrous callus formation in periosteal region, and a light reaction rich in neutrophils were determined, prominence in neobone formation, diffusion of osteoid tissue into the implant in some regions and the increase in osteoblast activity showed that the material used exhibits the behaviour of a typical bioinert–bioactive composite. Penetration of osteoid tissue into the implant is due to a higher amount of HA in the implant material. Since penetration of osteiod tissue into implant is a typical characteristic of bioabsorbable materials [9], it can be said that the biomaterial used is partially bioabsorbable.

In addition, a light inflammation infers that the material did not show any foreign body reaction and longer time is required for full absorption of the bioactive portion of the implant material by the surrounding tissue. Bone is a very regenerative tissue. The formation of hard tissue in normal fractures takes more than two months [18], [19]. When tissue adaptation of the implant material is also concerned, full absorption for this material is naturally expected to take longer time.

The response of the tissue surrounding the implant and the bone cells to the implant is the most important factor showing whether or not the implant will be encapsulated by fibrous tissue or bone regeneration will begin. It is seen that implant materials were replaced by the tissue death in toxic materials, fibrous tissue formation in bioinert materials, connective tissue formation at the interface between bioactive materials and surrounding tissue in bioabsorbable materials [16], [17].

Based on the light microscope examinations of the implants 6 months after the implantation we can infer that this time is not enough for an exact compact structure and fat tissue formation in the cavities of the bone. A duration of 12 months is much more effective in formation of compact structure, decrease in fat tissue and active endochondral bone formation in some areas. In the photos of 12-month group, formation of blood vessels and permanent bone tissue together with Haversian channels can be seen clearly. Since extension of the osteoid tissue to biomaterials is a bioabsorbable material property [3], [15], [16], we can define this material as a partially bioabsorbable.

As a result, it can be concluded that the material produced in this study (hydroxyapatite–polyethylene composite) is a cheap, easily producible and formable material which is proper for orthaepedics surgery. Moreover, due to very high hydroxyapatite content (80%) it is suitable for bone regeneration and does not cause any biocompatibility problem in vivo.

Acknowledgements

The authors acknowledge the Scientific Research Foundation, Firat University (FUBAP-Project No.705), for their financial support.

References

[1] MURUGAN R., RAMAKRISHNA S., *Bioresorbable composite bone paste using polysaccharide based nano-hydroxyapatite*, Biomaterials, 2004, 25, 3829–35.

[2] MURUGAN R., RAMAKRISHNA S., *Development of nanocomposites for bone grafting*, Composites Science and Technology, 2005, 65, 2385–2406.

[3] ANSELME K., *Osteoblast adhesion on biomaterials*, Biomaterials, 2000, 21, 667–681.

[4] MURALITHRAN G., RAMESH S., *The effects of sintering temperature on the properties of hydroxyapatite*, Advanced Materials Research Centre (AMREC), Ceramics International, 2000, 26, 221–230.

[5] DURMUS A.S., UNSALDI E., *Comparison of coral and cancellous autograft applications in experimental femoral fractures with large bone defect in dogs*, Firat University Journal of Health Sciences, 2001, 15(1), 101–112.

[6] UNSALDI E., BULUT S., OZERCAN I., DURMUS A.S., *Comparison of the usage of coral and cancellous autograft as a fusion stimulator in experimentally performed stiffle joint arthrodesis in dogs*, Turkish Journal of Veterinary Surgery, 2001, 7(1–2), 28–37.

[7] ABU BAKAR M.S., CHEANG P., KHOR K.A., *Tensile properties and microstructural analysis of spheroidized hydroxyapatite–poly(etheretherketone) biocomposites*, Materials Science and Engineering, 2003, A345, 55–63.

[8] JUHASZ J.A, BEST S.M, BROOKS R., KAWASHITA M., MIYATA N., KOKUBO T., NAKAMURA T., BONFIELD W., *Mechanical properties of glass-ceramic A–W-polyethylene composites: effect of filler content and particle size*, Biomaterials, 2004, 25, 949–955.

[9] CHANDRA R., RUSTGI R., *Biodegradable polymers*, Progress in Polymer Science, 1998, 23, 1273–1335.

[10] TENHUISEN K.S., MARTIN R.I., KLIMKIEWICZ M., BROWN P.W., *Formation and properties of a synthetic bone composite: hydroxyapatite–collagen*, J. Biomed. Mater. Res., 1995, 29, 803–10.

[11] GREEN D., WALSH D., MANN S., OREFFO R.O.C., *The potentials of biomimesis in bone tissue engineering: lessons from*

the design and synthesis of invertebrate skeletons, Bone, 2002, 30, 810–5.

[12] KURTZ S.M., MURATOGLU O.K., EVANS M., EDIDIN A.A., *Advances in the processing, sterilization, and crosslinking of ultra-high molecular weight polyethylene for total joint arthroplasty*, Biomaterials, 1999, 20, 1659–1688.

[13] SANTIS R.D., AMBROSIO L., NICOLAIS L., *Polymer-based composite hip prostheses*, Journal of Inorganic Biochemistry, 2000, 79, 97–102.

[14] SILVIO L.D., DALBY M.J., BONFIELD W., *Osteoblast behaviour on HA/PE composite surfaces with different HA volumes*, Biomaterials, 2002, 23, 101–107.

[15] PIERMATTEI D.L., GREELEY R.G., *An Atlas of Surgical Approaches to the Bones of the Dog and Cat*, second ed., W.B. Saunders Comp., Philadelphia, 1979, XII + 202.

[16] BONFIELD W., *Design of bioactive ceramic–polymer composites*, Biol. Biomech. Performance Biomaterials, 1986, 299.

[17] LAING P.G., *World standarts for surgical implants: An American perspective*, Biomaterials, 1998, 15, 405.

[18] THOMSON R.G., *General Veterinary Pathology*, second ed., W.B. Sounders, London, 1984.

[19] ANDERSON J.R., *Muir's Textbook of Pathology*, twelfth ed., English Language Book Society, London, 1985.