LETTER TO THE EDITOR

POLY-L-LACTIC ACID ß-TRICALCIUM PHOSPHATE SCREWS: A PRELIMINARY *IN VIVO* BIOCOMPATIBILITY STUDY

N. MAGARELLI, M.A. SAVASTANO, D. PALMIERI[1], R. ZAPPACOSTA[2], G. LATTANZIO[2], V. SALINI[1], C.A. ORSO[1], G. GUGLIELMI[3] and C. COLOSIMO

Istituto di Radiologia, Università "G. d'Annunzio" di Chieti, [1]Clinica Ortopedica e Traumatologia, Università "G. d'Annunzio" di Chieti, [2]Sezione di Anatomia Patologica, Dipartimento di Oncologia e Neuroscienze, Università "G. d'Annunzio" di Chieti, [3]Dipartimento di Scienze Radiologiche, Ospedale IRCCS Casa della Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy

The aim of this study is to assess the biocompatibility of two types of Poly-L-lactic acid (PLLA) screws (with either hydroxiapatite (HA) or β-tricalcium phosphate (β-TCP)) implanted in the left femur of four sheep euthanized at 42, 50, 57 and 84 days after surgery. Titanium screws were also implanted for comparison purposes. No signs of inflammation were seen in the 240 specimens. A rating of "+/-"for macrophages and "-"for neutrophils was assigned to all specimens. All specimens were assigned a rating which ranged from "+/-" to "+++" for fibroblasts and osteoblasts. The presence of macrophages, neutrophils and fibroblasts/osteoblasts was not statistically different for the four implantation periods. PLLA implants with β-TCP have a biocompatibility comparable to PLLA implants with HA.

In recent years, the number of biodegradable implants has notably increased, mostly due to the intrinsic properties of the materials used to construct these implants and the reduced risk of complications.

The use of metallic implants (for example, titanium) presents two major practical problems and several minor problems. They protect the bone from mechanical solicitations which result in a delaying-effect on healing. They are also subject to corrosion which releases metallic ions triggering immunological reactions due to foreign antibodies. The minor problems include a subsequent surgical procedure for removal, and problems caused by these implants when Magnetic Resonance Images are required, even for medical questions that do not concern the area of the implants (1-3).

Bio-degradable implants are less rigid and do not protect the bone from mechanical solicitations, thereby favoring bone cicatrization. Due to their molecular composition, these implants are metabolized by the normal physiological processes of surrounding cells and they can stimulate the formation of new bone. Ideally, they should not stimulate inflammatory processes or immunological responses. The inflammatory processes and immunilogical response can be either macrophage or neutrophil mediated. They are intentionally constructed to be re-absorbed at a specific rate and do not therefore require a second procedure for removal. CAT and MRA can be acquired even in proximity of the implants without distortions typically seen with metallic implants.

The basic concept of re-absorbable implants is to initially provide a solid anchorage, followed by a slow biodegradation with substitution by host tissue. Since their introduction over 40 years ago, various polymers have been studied in animals: poly-DL-

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lactic acid and poly-glycolic acid (PDLLA-co-PGA), poly-L-lactic acid and poly-DL-lactic acid (PLLA-co-PDLLA), poly-DL-lactic acid (PDLLA), poly-glycolic acid and trumethylin carbonate (PGA-co-TMC) and poly-L-lactic acid (PLLA) (3-5). The results of various studies indicate that the most suitable polimer for bone implants is PLLA. This material requires a longer period of time for re-absorption and, therefore, corresponds with the time required for bone repair, while still presenting the physical resistance comparable to that of titanium implants. Presently, the majority of implants used are made of PLLA with hydroxyapatite (HA).

The aim of this study is to assess the biocompatibility of two types of PLLA screws (with either HA o β-tricalcium phosphate (β-TCP)) implanted in the left femur of four sheep euthanized at 42, 50, 57 and 84 days after surgery. Titanium screws were also implanted for comparison purposes.

MATERIALS AND METHODS

This study was approved by the local ethics committee. Four genetically-related lambs were used as subjects. They were given a pre-anaesthetic (bezodiazepine) and a local anaesthetic (lidocaine) followed by general anaesthesia (intramuscular injection of Ketamine). The pre-surgical procedures and the intra-surgical monitoring were performed by an experienced veterinarian. Two different cannulated biodegradable screws were implanted in the left femur. The biodegradable screws were composed of PLLA (70%) and either HA (dimension: 7 mm x 20 mm; Smith & Nephew, U.S.A.) or β-TCP (dimension: 7 mm x 23 mm; Mitek, U.S.A.) for the remaining 30%.

The same surgical procedure was performed for each subject by an experienced orthopaedic surgical team: tricotomy of the external covracondylar region of the left femur with a longitudinal incision with bone removal and preparation of the parallel 7 mm holes corresponding to the tangent of the sovracondylus. The position of the two screws was alternated randomly. Postoperatively, a compressive bandage was applied for two days and intramuscular injections of penicillin and analgesics were administered for seven days.

The subjects were randomly euthanized at 42, 50, 57 and 84 days after surgery by endovenous injection of a massive dose of pentobarbital. The left coxo-femoral joint was disarticulated and each limb containing the four screws was placed in formaldehyde for 30 days for fixation.

Each bone segment was fixed for 12 hours in a 4% solution of paraformaldehyde in a 0.1 M phosphate buffer, pH 7.2 (Fluka Chemie AG, Buchs, Switzerland). The samples were than rinsed with the buffer solution, reduced in size (in order to favour the infiltration and polymerization), dehydrated with increasing concentrations of alcohol, treated with methyl metacrilate monomer (NMA, B.D.H.) and placed in an infiltration solution (100 g MMA. 15G of Synperonic* NP10 and 3 g of benzoil peroxide – Fluka) for periods that raged from 24-48 hours, as indicated by the manufacturer. The scalable polyethylene cylinder (Kartell, Italy), each containing a sample, were filled with a polymerizing solution (100 ml of infiltrating solution + 15 g of polymetylmetacrilate (PMMA) in pearls – Fluka) for 72 hours.

The PMMA enclosed samples were then sliced in 200 μm slices using a circular diamond-blade microtome (Mod. 1600, Leica, Germany). The orientation of the slices were determined using radiographs in order to obtain longitudinal sections of the screws. Each slice was atached to a 180 μm polyester adhesive sheet to aid in their preservation. Random samples were then mounted on glass slides using cianoacrylic glue. They were then thined to 100 μm with a LS2 cleaner (Remet, Bologna, Italy), using abrasive paper with an increasing grit (from 400 to 1200) and water. The slides were colored using solid nuclear red or blue.

Two pathologists, who were blinded for the type of implant, independently evaluated all slides using a light microscope. They were asked to assess the presence of inflammation, the infiltration on immunological cells (macrophages and neutrophils) and the presence of fibroblasts and osteoblasts as either absent, present or abundant (scale ranged from "-" to "+++"). The presence of statistically significant differences in the results, reported by the two readers was assessed using chi-squared test. Intra-lamb (time differences were assessed using chi-squared test. Intra-screw differences were assessed using McNemar test.

RESULTS

All four lambs survived surgery. During the time period prior to euthanization, they did not preset clinical signs of rejection. At euthanization, signs or symptoms of inflammation were not manifested. The pathologists viewed 30 slides for each implant. Intra-reader results were not statistically different (chi-squared test), therefore the results for the two readers were averaged. No histological signs of inflammation were seen in the 240 specimens. The ratings of "+/-" for macrophages and "-" for neutrophils were assigned to all specimens. All specimens were assigned a rating which ranged from
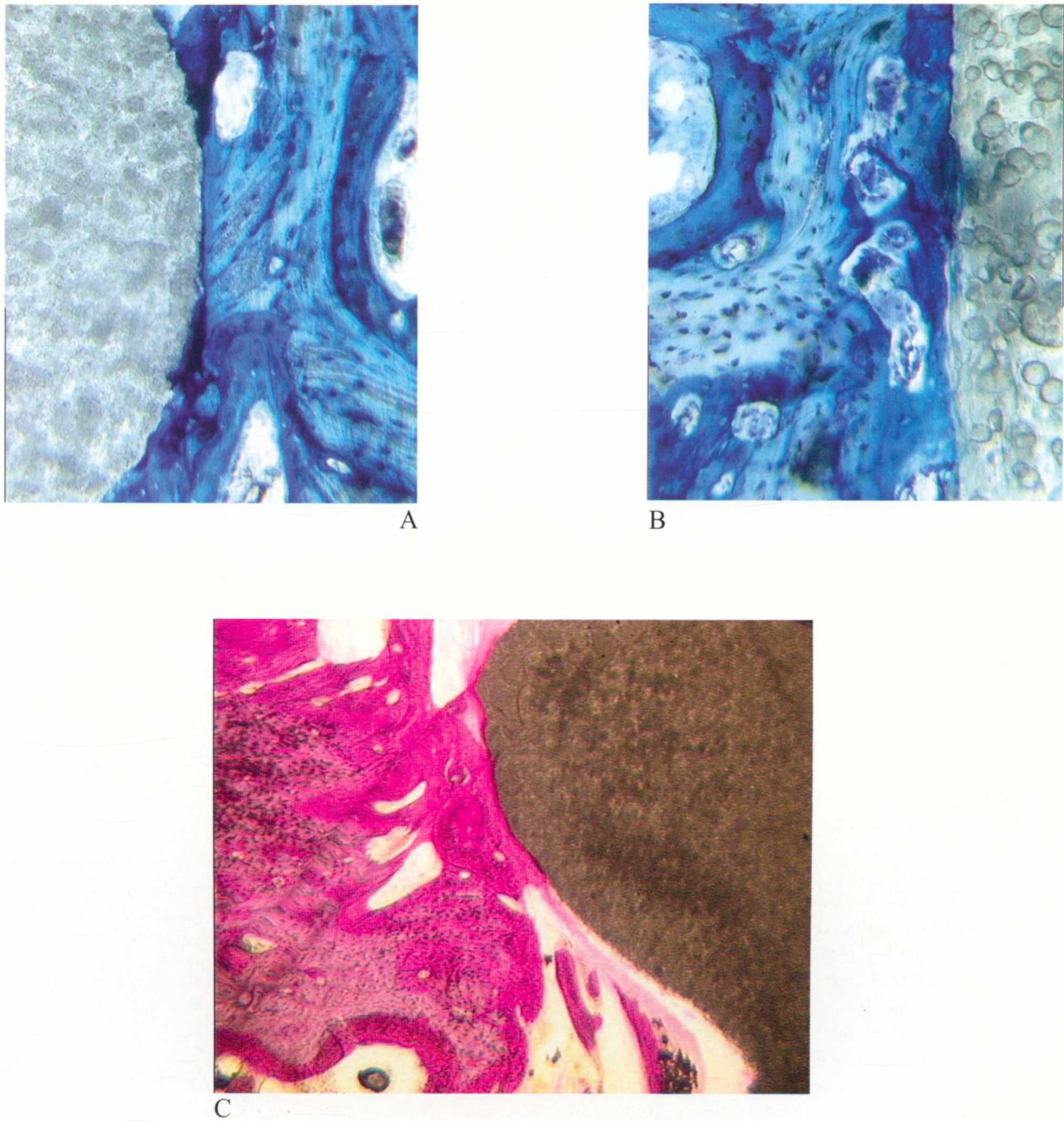
Fig. 1. *Samples obtained 84 days after implantation with a PLLA- β-TCP implant (A. Toluidine blue, 400x) or a PLLA-HA implant (B. Toluidine blue ,400x and C. acid fuchsin, 400x). Newly-formed bone was found in contact with the implant surface. Bone trabeculae were in close contact with the implant surface. In some areas, newly-formed blood vessels were observed. Osteoblast were actively secreting matrix directly on the implant surface, while, in other areas, a line of cuboidal-shaped osteoblasts could be observed directly on the implant surface. The bone-implant contact percentage was approximately 80 to 85%. No gaps, fibrous tissue or inflammatory infiltrates were present at the interfaces. In particular, no neutrophils, macrohages, lymphoctes or plasma cells were obsrved near the implant surface or within the bone. The differences for the two implants were not statistically significant.*

"+/-"to "+++" for fibroblasts and osteoblasts. The presence of macrophages, neutrophils and fibroblasts/osteoblasts was not statistically different for the four implantation periods (chi-squared test) or, overall, for the three screw types (McNemar test).

DISCUSSION

All possible mechanisms that could lead to failures must be studied prior to the implementation of an implant made of a new polymer. Foremost among these failure mechanisms is an immunological reaction by the host against the implant as a foreign body (7). However, an immunological reaction is necessary for the absorption of the biodegradable implants. Therefore, an equilibrium between these two extremes represents the ideal compromise. The use of HA produces particles at the prosthetic interface that activate phagocytic cells inducing a cascade reaction leading to bone re-absorption and aseptic loosening (8). HA also stimulates the production of the proinflammatory cytokine IL-18 by monocytes which have been observed in the interface tissue (8). This cytokine is an antagonist of IL-6, which has a stimulatory effect on osteoclastogenesis (9).

The presence of an elevated number of ED-2-positive macrophages has been seen as early as three days following implantation (10). The acute inflammatory phase was generally resolved after a varying period of time, while reabsorption of the implant begins after approximately one year. Time periods for this study were deliberately chosen so that this initial phase of acute inflammation should have been resolved but the formation of new bone should not have initiated. No absorption of biodegradable material as observed. This finding is concordant with prior studies which indicate that reabsorption begins approximately one year after implantation.

The results concerning the lack of inflammation are concordant with a similar study which used PLLA and stainless steal screws implanted in the tibia of rabbits with osteotomitus where the histological examination at 16 week did not reveal signs of inflammation (6). In conclusion, PLLA implants with β-TCP have a biocompatibility comparable to PLLA implants with HA. No signs of inflammatory or neutrophils, but an adequate number of macrophages and fibroblasts were seen in all four implantation periods for all implants. Further studies are required to compare bioabsorption.

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