Key Engineering Materials Vols. 396-398 (2009) pp 7-10
online at http://www.scientific.net
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Biological Response to Wollastonite Doped α-Tricalcium Phosphate Implants in Hard and Soft Tissues in Rats

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Keywords: Calcium phosphate, wollastonite, *in vivo* test, biocompatibility.

Abstract. The biological response following subcutaneous and bone implantation of β-wollastonite(β-W)-doped α-tricalcium phosphate bioceramics in rats was evaluated. Tested materials were: tricalcium phosphate (TCP), consisting of a mixture of α- and β-polymorphs; TCP doped with 5 wt. % of β-W (TCP5W), composed of α-TCP as only crystalline phase; and TCP doped with 15 wt. % of β-W (TCP15), containing crystalline α-TCP and β-W. Cylinders of 2x1 mm were implanted in tibiae and backs of adult male *Rattus norvegicus, Holtzman* rats. After 7, 30 and 120 days, animals were sacrificed and the tissue blocks containing the implants were excised, fixed and processed for histological examination. TCP, TCP5W and TCP15W implants were biocompatible but neither bioactive nor biodegradable in rat subcutaneous tissue. They were not osteoinductive in connective tissue either. However, in rat bone tissue β-W-doped α-TCP implants (TCP5W and TCP15W) were bioactive, biodegradable and osteoconductive. The rates of biodegradation and new bone formation observed for TCP5W and TCP15W implants in rat bone tissue were greater than for non-doped TCP.

Introduction

New bioceramics composed of α-$Ca_3(PO_4)_2$ (α-TCP) doped with β-$CaSiO_3$ (β-W) have been recently developed and evaluated *in vitro*. They are stronger and more reactive than pure α-TCP, and release ionic Si and Ca species when immersed in SBF [1], which are well recognized promoters of bioactivity and osteoinduction [2]. The new bioceramics are not cytotoxic against a culture of fibroblastic human cells. According to the results of *in vitro* studies they are promising bone repairing materials [1].

Therefore, the aim of this study was to evaluate the biological response following subcutaneous and bone implantation of β-W-doped α-TCP bioceramics in rats.

Materials and Methods

Tested materials were: TCP, consisting of a mixture of α- and β-polymorphs; TCP doped with 5 wt. % of β-W (TCP5W), composed of α-TCP as only crystalline phase; and TCP doped with 15 wt. % of β-W (TCP15), containing crystalline α-TCP and β-W. Cylinders of 2x1 mm were prepared by isostatic pressing of the respective powders and sintering as elsewhere [1].

Thirty six healthy male rats (*Rattus norvegicus, Holtzman,* ≈200g) were used. Groups of 12 animals were employed for each follow-up period of time (7, 30 and 120 days). Six animals of each group received TCP5W implants in the left side of the back and in the left tibia. Opposite right sites

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received TCP15W implants. TCP implants were placed in the left tibiae and backs of the other six animals. On the opposite right side, surgical defect were created and left empty to be used as control.

The animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (1 mL/kg, Agener União) and xylazine (0,1 mL/kg, Rompum-Bayer). The dorsal and anterior leg regions were shaved and externally disinfected. Two parallel longitudinal surgical incisions of 15 mm length were made at dorsal and subcutaneous cavities were created by mean of surgical tweezers. A longitudinal incision was carried out in the anterior region of each leg to expose the tibiae subperiosteally. At the mid-diaphyseal region, a drill hole was made transcortically using a No. 6 spherical dental burr (Broca Carbide, KG Sorensen) under saline irrigation. After implantation all wounds were sutured with Mononylon 5-0 (Ethicon, Johnson & Johnson) and an analgesic single dose of acetyl salicylic acid (120-300 mg/kg, Eurofarma) was orally administered after surgery. The animals were kept in isolated cages, fed on a standard diet and received water *ad libitum* according to the recommendations of the Canadian Council on Animal Care.

The animals were sacrificed by injection of thiopental (1.6 mL/kg, Cristália) after 7, 30 and 120 days. The dorsal and tibia segments containing the implant were sectioned with a scalpel or diamond saw, as required. The tissue blocks containing the implants were fixed for 72 h in Bouin solution. Afterwards, blocks were washed under tap water and tibiae specimens were decalcified in Morse solution and washed in phosphate buffer (pH 7.0). All samples were routinely dehydrated and included in paraffin wax. Semi-serial cuts (6 µm in thickness) were dyed with hematoxylin and eosin and examined and photographed by the transmitted light microscope (BX51 coupled to Camedia C-5060-5.1 MPix., Olympus). Tissue reaction to implant was classified as “intense”, “moderate”, “mild”, “discrete” and “absent”.

Results and Discussions

The results of the evaluation of the biological response of connective and bone tissue to each implant material are resumed in Table 1.

Table 1. Qualitative assessment of the magnitude of the tissue reaction for each implant material and period of implantation.

Material	Magnitude of tissue reaction					
	7 Days		30 Days		120 Days	
	Bone	Connective	Bone	Connective	Bone	Connective
TCP	Absent	Mild	Absent	Discrete	Absent	Absent
TCP5W	Absent	Moderate	Absent	Discrete	Absent	Absent
TCP15W	Absent	Mild	Absent	Discrete	Absent	Absent

Qualification scale: absent; discrete; mild; moderate; intense

Subcutaneous implantation. None of the three implant materials, TCP, TCP5W and TCP15W, were reabsorbed during any of the implantation periods. Thus, they had to be manually removed from the fixed blocks previously to paraffin inclusion and serial cutting.

7 days: The three materials induced the formation of a thin perimplant fibrous capsule. In Fig. 1a the fibrous capsule surrounding the TCP5W implant is identified as **c**. Signs of inflammation: mononuclear macrophagues and lymphocytes, giant cells (signaled by arrows in Fig. 1a for TCP5W) were also observed for the three materials. Blood vessels presenting clear signs of vasodilation (**v** in Fig. 1b for TCP15W) were also observed for the three ceramics. The intensity of inflammation was described as “moderate” for TCP5W (Fig. 1a) and mild for TCP and TCP15W (Fig.1d).

30 days: All implant sites were surrounded by the fibrous capsule and the adjacent connective tissue was normal with active fibroblasts (short arrows in Figs. 1b and e) and only a few

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inflammatory cells (long arrows in Figs. 1b and e). Reaction was classified as “discrete” for the three materials.

120 days: Thin and stable fibrous capsule and normal fibroblasts (thin arrows in Figs. 1c and f) and organized connective tissue surrounded all implant sites. Scarce and isolated giant cells were still observed (bold arrows in Fig. 1c and f). Tissue reaction was considered “absent” for all materials. Evidences for ectopic osteogenesis were not observed in any case.

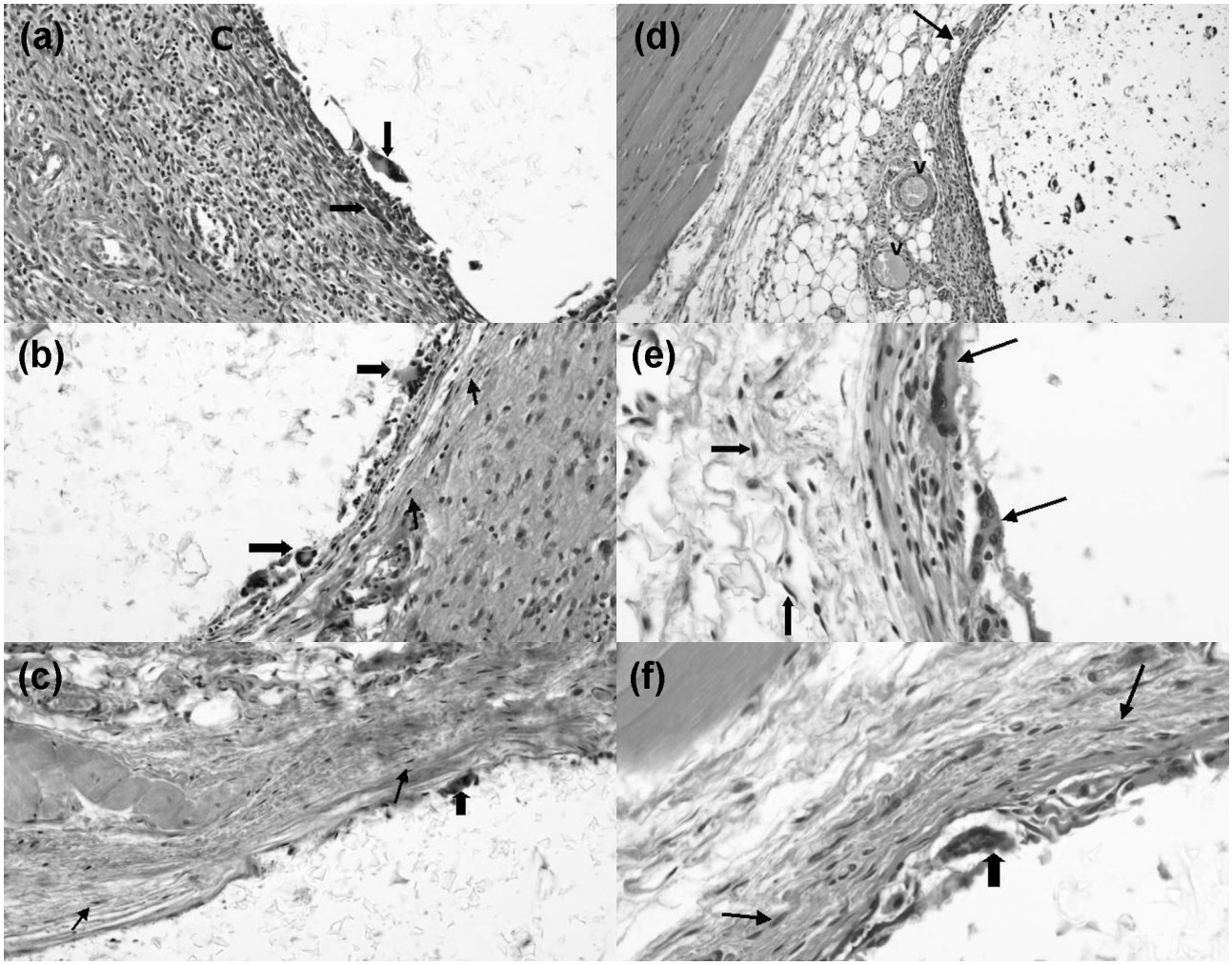

Fig. 1. Micrographs of thin serial sections of connective tissue implant sites at 7 (a and d), 30 (b and e) and 120 days (c and f) for TCP5W (a, b and c) and TCP15W (d, e and f).

Tibiae implantation

7 days: The implant sites were covered by periosteum and the inflammatory reaction was “absent” for the three implant materials. The implants degraded partially. Intense angiogenesis (**v** in Fig. 2a) and osteocyte activity (arrows in Fig. 2a) in the neighborhood of all implants was observed.

30 days: New bone replaced almost totally TCP5W and TCP15W implants. A lot of vessels and osteocytes were visible in the new bone tissue (Figs. 2b and d). TCP implant site was only partially replaced by new bone.

120 days: Mature bone completely filled the implant cavities of TCP5W and TCP15W, no rests of the implants remain (Figs. 2c and 2f). Cavity filling for TCP was not complete and portions of the implants remain.

Conclusions

TCP, TCP5W and TCP15W implants were biocompatible but neither bioactive nor biodegradable in rat subcutaneous tissue. They were not osteoinductive in connective tissue. However, β-W-doped α-TCP implants (TCP5W and TCP15W) in rat tibiae were biodegradable, bioactive and

osteoconductive. They presented faster biodegradation and new bone formation than non-doped TCP implants placed in rat bone tissue.

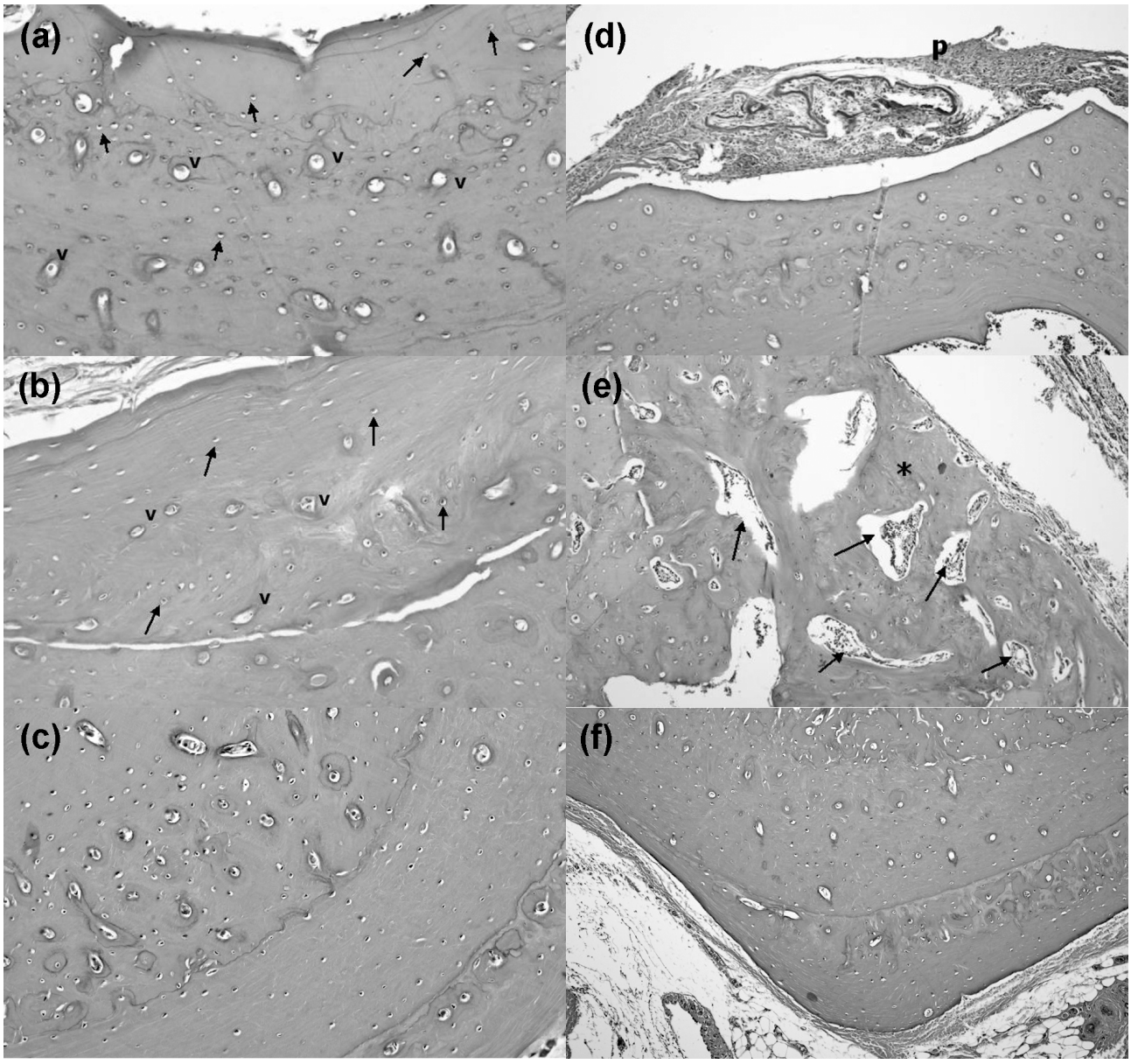

Fig. 2. Micrographs of thin serial sections of tibia implant sites at 7 (a and d), 30 (b and e) and 120 days (c and f) for TCP5W (a, b and c) and TCP15W (d, e and f).

Acknowledgements

The study was partially supported by the Project CICYT MAT2006-12749-C02-01-02 SONBEDE of the Ministry of Science and Education of Spain.

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