JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **16** (2005) 789–793

Histological study on sinus lift grafting by Fisiograft and Bio-Oss

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The work aims to provide a histological investigation of Fisiograft®, a PLA/PGA copolymer, used as filler for bone defects in humans. The study was performed on biopsies of sinus lifts where Bio-Oss® and Fisiograft® gel were applied as graft material. Bone regeneration was satisfactory in all sinus lifts, even when Fisiograft® was applied alone. Due to remarkable osteoclast activity, Bio-Oss® granules were cleared from the majority of biopsy cores. At histology, Fisiograft® gel appeared as globes enveloped by fibroblasts, displaying an epithelial-like cell appearance. Due to its solubility in solvents, undegraded Fisiograft® (recorded for 7 months or more) did not stain whereas degraded Fisiograft® stained positive. The loose connective tissue, that surrounded Fisiograft® and bone contained isolated mastocytes. Bone grew inside the loose connective and often reached the surface of Fisiograft® by intervening cells. The results seem to indicate that Fisiograft® may be considered both a polymer useful for fastening bone substitutes inside a defect and in addition a material capable of prompting bone regeneration, with or without the use of a bone substitute. In addition to space-former and space-maintainer functions, Fisiograft® shows potential bone stimulation function, which may be labelled as osteopromotive capability.

1. Introduction

Bioresorbable polymers have been widely used as osteosyntesis appliances given their advantages over metallic materials [1]. Appliances made of these polymers can fix skeletal segments during the early phases of fracture healing and afterwards degrade spontaneously, avoiding the need for additional surgery.

After the initial enthusiasm for polydioxanone and polyglactin [1], particular attention was has been focused on glycolic acid [2] and lactic acid [3] polymers (PGA & PLA) and their copolymers [4]. The possibility of manufacturing biodegradable devices for osteosynthesis, well tolerated by biological tissues [5], has promoted the diffuse employment of these materials in dentistry, maxillofacial and orthopedic surgery. PLA, PGA and copolymers have been installed as membranes to perform guided tissue regeneration (GTR) [6] or guided bone regeneration (GBR) [7] in intraoral applications. Sponge or spheres of PLA/PGA have been employed as a carrier of rHBMP-2 to stimulate bone osteogenesis [8, 9]. The capability of tissue regeneration by these polymers has been further studied using amorphous forms in intraoral districts [10].

Fisiograft® is a low-molecular-weight PLA/PGA copolymer designed for use in bone grafts as a space filler and maintainer for GTR and GBR treatments. After the first studies on biocompatibility and bone formation in experimental animals, subsequent reports published in 1999 [11] have employed Fisiograft® in humans with satisfactory results [12, 13].

The aim of this work, where Fisiograft® was used as bone graft to achieve augmentation of the maxillary sinus floor (sinus lift) in humans, was to perform histological studies on cellular activities during bone formation in PLA/PGA copolymers, a topic receiving little attention to date. The study was run on undecalcified PMMA embedded biopsies using suitable staining to highlight cellular and tissue events.

2. Materials and methods

Sixteen patients, males and females ranging from 48 to 64 years of age, underwent monolateral lifting of

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the floor of the maxillary bone (sinus lift). All 16 patients gave informed consent to the procedure. After cutting and reflection of the soft tissues, sinus access was achieved through a window 10–15 mm wide and 10–12 mm high cut by a round diamond bur under a continuous jet of sterile saline. After bone total abrasion, the mucous membrane (Schneider membrane) of the floor of the maxillary sinus was gently freed up to the palatal wall. In fifteen patients, the cavity was filled with a 50:50 mixture of Bio-Oss®, deproteinized bovine bone (Geistlich Söhne AG, Wolhusen, Switzerland), and Fisiograft® gel, a PLA/PGA (50:50) co-polymer (Ghimas SpA, Bologna, Italy), to reach a fixed height. In one patient, the only who gave consent to the procedure, the cavity was filled with only Fisiograft® gel. In all patients, the window was covered with a non-resorbable membrane (Gore-Tex®-W.L. Gore & Associates, Inc., Flagstaff, AZ, USA), the flap then repositioned and carefully sutured.

At patient follow-up (19–35 weeks after surgery), a core sample (Ø 3 × 5 mm) was obtained by means of a hollow mill at 2,000 RPM under saline jet from the zone where the graft mixture filled the cavity below the sinus membrane.

The cylindrical bone biopsies were fixed in 4% paraformaldehyde (all reagents Fluka Chemie AG, Switzerland) in 0.1 M phosphate buffer, pH 7.2 for 4 h at room temperature. Specimens were dehydrated through an ethanol series, and embedded in methylmethacrylate without decalcification.

The methacrylate blocks were serially sectioned using a diamond saw microtome (1600 Leica, Wetzlar, Germany) along the longitudinal axis of the biopsy up to its center. Five-micron-thick sections were obtained using a bone microtome (Autocut1150, Reichert-Jung GmbH, Nußloc, Germany) starting from that level. They were stained with Toluidine Blue, Gomori trichrome or Solochrome cyanine/Congo red methods. A thick section (200 μm) was then obtained from the center of each biopsy. The thick sections, reduced by grinding to 100 μm and perfectly polished with emery paper and alumina, were X-ray microradiographed (Italstructures, Riva del Garda TN, Italy) at 8 kV and 12.5 mA on Kodak SO 343 film. The microradiographs and the sections were analyzed and photographed under transmitted ordinary light using a photomicroscope (Axiophot, Carl Zeiss, Oberkochen, Germany). The Trabecular Bone Volume (TBV) of the biopsies (index of the amount of bone tissue [14]) was calculated

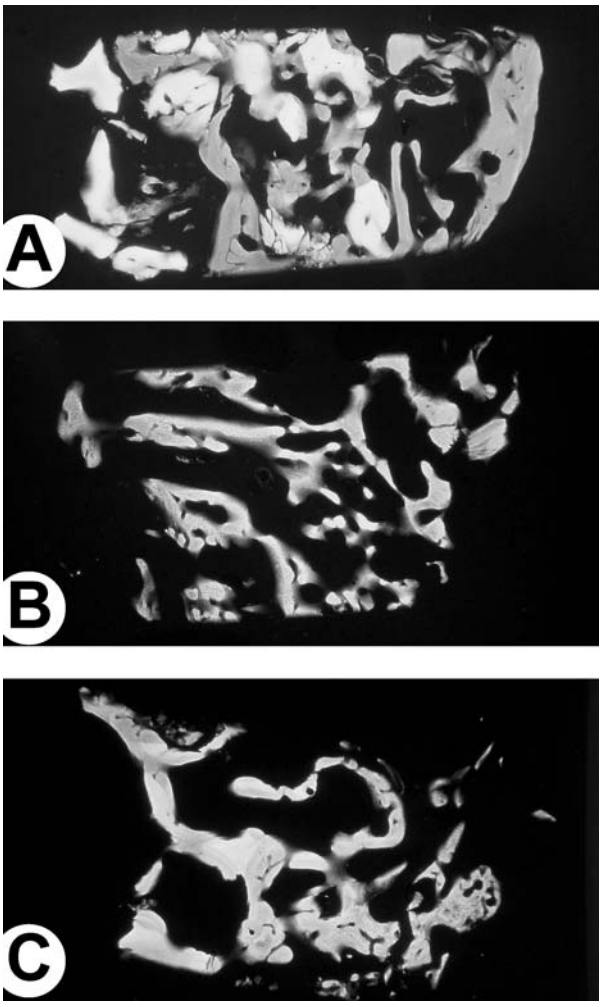

Figure 1 Microradiographs of thick sections of biopsies where Bio-Oss® and Fisiograft®, (A) and (B), or only Fisiograft®, (C), were used as sinus lift graft. Biopsies taken 224 (A), 208 (B) and 135 (C) days after surgery. Note the great amount of mineralized materials of (A), TBV = 48.4%, and the similar amounts of (B), TBV = 26.1%, and (C), TBV = 30.2%. Note also the Bio-Oss® granules (white in (A)) that disappear in (B) and the disordered arrangement of bone in (C). Field width (A) = (B) = (C) = 5500 μm.

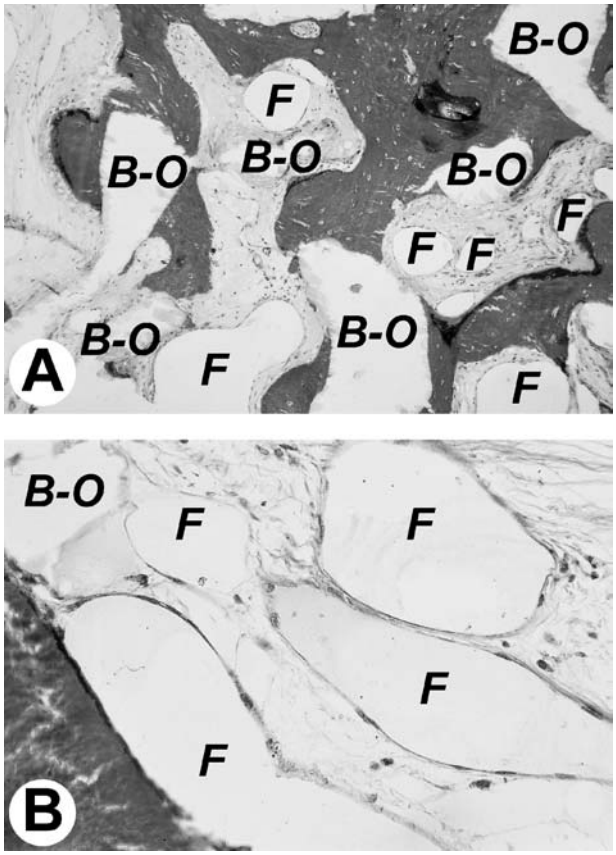

Figure 2 Images of sections of 224-day-old (A) and 217-day-old (B) biopsies containing Bio-Oss® (BO) and Fisiograft® (F). Note the new bone (dark gray) formed in apposition to some BO granules and the soft tissue surrounding F granules in (A). Note in (B) how F granules are enveloped by fibroblastic cells, showing an epithelial-like appearance, and loose connective tissue. Trichrome Gomori stain. Field width (A) = 1440 μm; (B) = 360 μm.

on microradiographs using an image analyzer (VIDAS, Carl Zeiss, Germany) and suitable software to evaluate the volume of Bio-Oss® and newly formed bone.

3. Results

At radiographic analyses, all sinus lift procedures produced an adequate amount of new bone. In one patient microradiographs of the biopsies revealed persistence of an appreciable amount of Bio-Oss® and a very high TBV value (about 50%) (Fig. 1(A)). Indipendent of the time elapsed from surgery, the remaining fourteen patients displayed scantiness or absence of Bio-Oss® granules in the biopsies (Fig. 1(B)) and a TBV ranging between 25 and 35%. The one patient treated with only Fisiograft® gel as graft material had a bone content, arranged in a very disordered architectural array way (Fig. 1(C)), with a TBV value of around 30%.

Histology indicated the absence of bacterial aggression and inflammatory reaction in all biopsies. No granulocytes, lymphocytes, histiocytes and plasmocytes were found inside the soft tissue. Some isolated and random mastocytes were observed, especially near the Fisiograft® gel or Bio-Oss® granules. No evidence of giant cell foreign body reaction was found in any biopsy; on the contrary, some osteoclasts were observed. Soft tissue was made up of loose connective tissue rich in fibroblasts, collagen fibers and amorphous ground substance (Fig. 2). Sometimes the connective tissue had become more fibrous, particularly around the Bio-Oss® granule (Fig. 3(A)).

In a few patients, Bio-Oss® granules formed a structured network with the newly formed bone (Fig. 2(A)). In the remaining patients, Bio-Oss® granules were completely absent or surrounded by only fibrous tissue (Fig. 3(A)). New bone frequently originated in between the granules instead of coming into contact with the Bio-Oss® granules (Fig. 3(A)). Sometimes, a few residual Bio-Oss® granules, small in size, were completely surrounded by fibrous tissue and located at a distance from the new bone. On the contrary, in 4–8 month-old biopsies of sinus lift with Bio-Oss® granules used as graft material, several osteoclasts were observed around the Bio-Oss® granules. Osteoclasts were often present on Bio-Oss® granules in contact with new bone (Fig. 3(B)), whereas they were generally absent on granules completely surrounded by fibrous tissue (Fig. 3(A)). Contrary to the typical picture, osteoclasts preferred to resorb the more highly mineralized substrate (Bio-Oss®) than the more readily erodible (comparatively lower mineralized) newly formed bone (Fig. 3(B)).

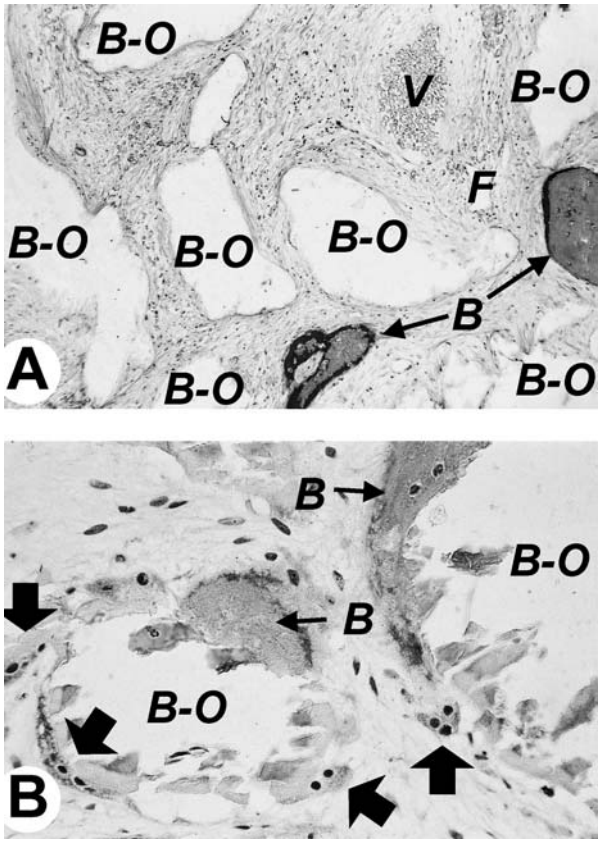

Figure 3 Images of sections of 165-day-old (A) and 217-day-old (B) biopsies containing Bio-Oss® (BO) and Fisiograft® (F). Note in (A) how BO granules are enveloped by loose connective tissue and that new bone (B—dark gray) is formed inside the tissue and not in apposition to BO. The arrows in (B) point to multinuclear osteoclasts which resorb BO granules instead of the more easily erodible bone (B—dark gray). V = Vessel containing red cells. (A) = Trichrome Gomori stain; (B) = Solochrome cyanine/Congo red stain. Field width (A) = 1440 μm; (B) = 360 μm.

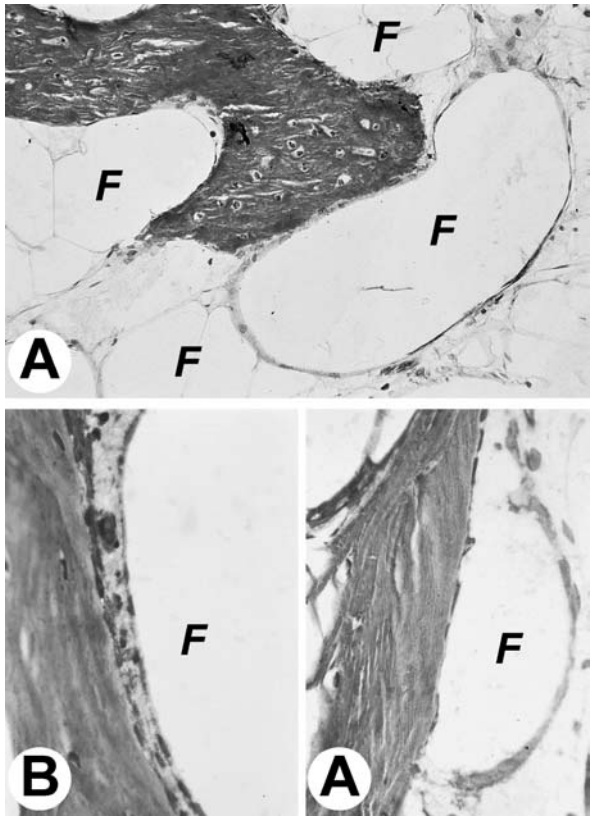

Figure 4 Images of sections of 154-day-old (A) and 213-day-old (B) biopsies containing both Bio-Oss® and Fisiograft® (F). Note in (A) how the newly formed bone (dark gray) grows between F granules, reaching their surface, and propagates superseding the fibrous tissue; two layers of cells in (B): on the left on bone (dark gray) and on the right enveloping the F granule. In (C) a discontinuous layer of cells (lining bone cells or fibroblasts?) intervenes between bone (dark gray) and F granule. (A) = Trichrome Gomori stain; (B) and (C) = Solochrome cyanine/Congo red stain. Field width (A) = 563 μm; (B) = (C) = 225 μm.

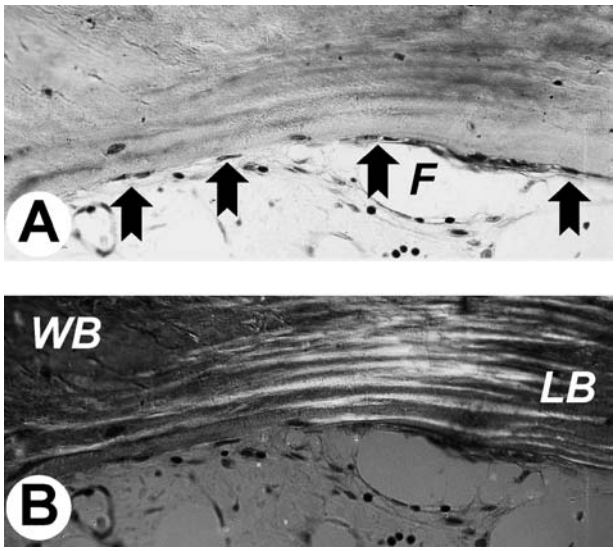

Figure 5 Images of a section of 165-day-old (B) biopsy containing Bio-Oss® and Fisiograft® (F). The arrows in (A) point to the discontinuous layer of lining cells. Note in (B) that the latest formed bone has a lamellar structure. WB = woven bone; LB = lamellar bone. Toluidine blue stain under ordinary (A) and polarized (B) light. Field width (A) = (B) = 563 μm.

Fisiograft® gel formed globe-like formations enveloped by loose connective tissue (Fig. 2(B)). Fibroblastic cells, with an epithelial-like appearance, surrounded the globules (Fig. 2(B)). The loose connective tissue showed several cells and collagen fibers, sometimes faintly condensed. Fisiograft® globules were transparent to transmitted light in stained sections due to their high solubility in MMA-embedding-treatment fluids. Staining was positive only when globules were partially degraded, probably due to protein or glycoprotein perfusion by biological fluids.

The bone newly formed with Fisiograft®, with or without Bio-Oss® granules, generally had a woven structure (Fig. 4(A)). Moreover, a good amount of parallel fibered or, in particular, lamellar bone was displayed inside 7–8 month-old biopsies (Fig. 5). The bone grew inside the connective tissue filling the interlaying spaces (Fig. 4(A)). Bone apposition advanced up to a residual layer of connective tissue behind the osteoblasts (Fig. 4(B)). Bone apposition ceased and osteoblasts transformed into lining bone cells when the bone reached the Fisiograft® implant (Fig. 4(C)).

4. Discussion and conclusion

PGA, PLA and PLA/PGA copolymers are designed to form degradable appliances to be used in various skeletal districts. Several works have been performed in the last quarter of century to find the optimal trade-off between degradability and mechanical properties. Notwithstanding, PGA/PLA devices sometimes fall short of achieving satisfactory results in bone fixation [15]. These results should not necessarily be taken as a negative factor, since they are related to degradability of the tested materials. PLA/PGA copolymers are not suitable for devices where a high tensile resistance is needed: to obtain this property a fiber reinforcement can be used [16]. The mechanical quality of a device made of PLA/PGA copolymers finds applications in appliances to be used in oral (resorbable membranes [17]) or maxillofacial districts [18, 19].

Some authors have seen in the degradability properties of PLA/PGA copolymer a chance for tissue neogenesis [20, 21]. Our results seems to confirm these opportunities. The results reveal osteoclast activity against Bio-Oss® granules in the presence of Fisiograft® gel. They highlight good bone formation in strict relation to Fisiograft® globules. Bone grows in between the Fisiograft® globules and continues to grow even when it reaches the Fisiograft®, changing growing direction inside the fibrous tissue. Similar results are not possible when an inert material, such as alumina or zirconia, having a size similar to Fisiograft® globules is used. The results do not elucidate if osteoblasts (which are transforming into lining bone cells) or fibroblastic cells are the cell type intervening between the two materials when bone reaches the Fisiograft® surface. Sometimes it seems that no cells intervene between the two materials, so that a question rises: is it possible that bone fluids freely communicate with the marrow or does the Fisiograft® globule acts as a canaliculus plug? If the last supposition was true, what happens when the globules degrade? Only studies on epon-embedded samples using TEM will be able to tackle these questions.

Particular importance must be conferred to the implant site. Maxillary bone, and in particular the maxillary floor lift, represents an unloading of bone with a very low trophism, a site where bone growth with inert materials is very unlikely. This fact may support our speculation on the bone stimulation properties of Fisiograft®, in agreement with the findings of Hollinger [20]. Fisiograft® has been presented as a material capable of forming and maintaining space inside a bone defect during healing. Our results suggest a further role of Fisiograft®: its degradation may have an interaction on bone processes. Fisiograft® might act as an osteoinductive or osteoconductive material, and so may be considered to have osteostimulative material. More in depth studies on experimental animals and cultured cells, using more enhanced methods of analysis (e.g. TEM, etc.) will be needed to define the osteostimulative properties of Fisiograft®. At present, in consideration of the space former and maintainer capabilities of Fisiograft®, with a purported activity on osteogenetic processes, we suggest that it may be considered an osteopromotive substance [22, 23], a term not in vogue in bone graft substitution. This term identifies a material capable of spatially and structurally promoting all processes involved in bone formation. It appears that this term may be the most well suited descriptor for Fisiograft®, in particular until studies defines its role in osteogenetic processes.

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Received 19 March
and accepted 29 April 2004