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Bone ingrowth in macroporous Bonelike® for orthopaedic applications

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Abstract

The aim of this study was to evaluate the biological behaviour of porous scaffold structures of Bonelike® which is suitable for either direct clinical use or tissue engineering applications. Porous cylindrical specimens 8×10 mm were implanted in the lateral aspect of the tibia of 13 patients (mean age 54 years), during osteotomy surgery for the treatment of medial compartment osteoarthritis of the knee. Implanted cylinders were retrieved at the same time as the removal of the blade plates at 3, 6, 9 and 12 months. Scanning electron microscopy and histological evaluations were performed to observe the biological responses of human bone tissue to porous Bonelike®. The penetration depth was determined for all implantation periods, and after 6 months it was already possible to see new bone in the centre of the implanted cylinders, which gives 100% of penetration depth for all implantations periods except for 3 months when bone could only be seen in the peripherical region. Regarding the percentage of the area covered by new bone calculated from two-dimensional histological sections, values of 53 ± 15, 76 ± 12 and $88 \pm 9\%$ were achieved for 6, 9 and 12 months, respectively. Due to its structural features porous Bonelike® permitted effective vascularization and bone ingrowth, and therefore was fully osteointegrated as shown in the histological surveys. A slow biomaterial degradation with implantation time is envisaged since the material has displayed surface degradation. Bonelike® scaffolds show potential for complete ingrowth of osseous tissue and restoration of vascularization throughout the defected site.

Keywords: Bonelike®; Porous materials; Bone graft; Orthopaedic

1. Introduction

In the treatment of monocompartmental osteoarthritis of varus knees, high tibial osteotomy (HTO) is considered as to be an effective procedure to reduce pain and control the progression of the disease, delaying more aggressive surgeries as total knee arthroplasties. This effect is based in the reduction of the stress and weight over the affected compartment that is produced when the mechanical axis is transferred from the medial compartment to the junction of the lateral third with the two medial thirds. This corresponds to an alignment of 9–10° valgus. Some authors have demonstrated that one year after a HTO, it is possible to detect radiologically a reduction in the bone density in the medial compartment, thus reflecting a slower progression of the ostheoarthritis [1]. Others have described some degree of histological cartilage regeneration after the joint surface has been unloaded [2].

The closing wedge osteotomy that was used in this study is the most common technique, but recently open wedge procedures have been described with autografts [3], allografts, cement [4] and hydroxyapatite [5]. This second

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technique provides a more accurate correction of varus deformity when compared with the conventional lateral closing-wedge HTO, but in a time of great developments in knee arthroplasties, autologous chondrocyte implantation and growth factors, there is still a place for both approaches.

A careful selection of the candidates is essential not only for the success of the surgery but also for the success of a clinical trial aiming to assess a new bone graft substitute. Because bone is a porous tissue material, there is a physiological rationale for the use of porous materials in its replacement. Moreover, porous bone grafts are advantageous for the early incorporation of the graft into or apposed to the bone tissue surrounding it [6–8]. Several key aspects should be considered when designing porous materials for bone grafting [6,9,10]: (i) the size of pores at the surface of the graft, to actually allow for bone to "flow into" the graft and fill all of its structure; (ii) the size of porous network connecting the surface pores to allow areas of bony ingrowth to "meet up" within the porous graft; (iii) the extent (percentage) of the porosity; (iv) the ability for blood vessels and canaliculi to form within the porosities.

Regardless of the use of porous materials, bone engineering is an area of considerable ongoing scientific exploration, and it seems that the ultimate porous ceramic has yet to be designed. A variety of fabrication methods have been proposed [6,9–19] to produce porous scaffolds with interconnected pore networks, such as the polymeric sponge method or foaming processes, but a general problem is how to control the processing and the ultimate material properties. There is also a new family of forming techniques, known as direct consolidation techniques, which allow generation of complex shapes, but require a good knowledge of the rheological behaviour of concentrated biomaterial suspensions [20–23].

The balance between porosity and initial mechanical strength must be precisely controlled in order to allow the optimal scaffold for each surgical application to be engineered while maintaining the structural parameters necessary for host bone ingrowth. Some controversial issues have arisen regarding the importance of specific structural parameters, such as pore morphology, percentage of porosity, pore connectivity and the strut microstructure, to the biocompatibility of the bone graft [24–31]. For instance, a number of works have suggested [32–35] that the degree of interconnectivity is more critical than the pore size since the vascular network needed for new bone formation or repair is strongly influenced by the degree of structural interconnectivity among pores, whereas other works seem to give the same importance to both parameters.

Despite the fact that nature can be hardly ever reproduced, it is well established that improved mechanical properties of hydroxyapatite (HA) and better chemical similarity between HA grafts and bone can be obtained through liquid phase sintering route using $CaO–P_2O_5$ glasses as a sintering aid; this has been confirmed by extensive published data regarding the development of Bonelike® novel bone graft [36–41]. During the sintering process $CaO–P_2O_5$ glass reacts with HA, forming β-tricalcium phosphate (β-TCP), which then can transform into α-TCP at higher temperatures. The relative proportions of the β- and α-TCP phases in the final microstructure depend upon several experimental factors, including the glass content and composition. Bonelike® has been reported to be osteoconductive and bioactive, supporting the formation of mechanically and chemically bonded bone directly on its surface [42–47]. The bioactivity of Bonelike® is determined by an optimal balance of the least soluble phase of HA and most soluble phase of TCP.

This study aimed at evaluating the biological responses of human bone tissue to porous Bonelike® by implanting cylindrical specimens in the lateral aspect of the tibia of patients with medial compartment osteoarthritis of the knee during osteotomy surgeries.

2. Materials and methods

2.1. Material preparation

Commercial, synthetic, medical-grade HA powder (Plasma Biotal Limited, UK) underwent calcining and milling procedures to yield an average particle size of about 1.5 μm. Well-dispersed aqueous suspensions were prepared with solid loadings as high as 60 vol.% following a deagglomeration procedure, in the presence of a ammonium polycarbonate, as previously described [20,21]. These suspensions were then used to consolidate macroporous cylinders by suitably combining the microstructural capabilities of the foaming method with the shaping capabilities of starch consolidation method, as reported elsewhere [21,22]. Briefly, starch was added to the stock suspension prior the incorporation of the foaming agents, in a proportion of 10 vol.% relative to HA. The amount of foaming agents added was 4 wt.%, relative to the mass of water present in the suspension, and the mass proportion between foam-bath concentrate and sodium lauryl sulphate was 80/20. The final mixtures were poured into a closed mould and consolidated at 80 °C for 1 h to obtain cylindrical samples of 8 × 10 mm. The samples were demoulded, dried and pre-sintered at 1100 °C for 1 h. These pre-sintered ceramic bodies were impregnated with a glass solution, in optimized conditions as previously described [23], in order to incorporate about 4 wt.% of glass (glass composition used: $65P_2O_5–15CaO–10CaF_2–10Na_2O$ in mol.%), and then sintered at 1300 °C at 4 °C min^{-1} for 1 h.

2.2. Material characterization

The apparent density was evaluated from the weight and dimensions of the samples. Scanning electron microscopy analysis (S-4100, Hitachi, Tokyo) performed on fracture surfaces of the samples was used to characterize the microstructure and qualitatively assess pore size and pore morphology.

X-ray diffraction (XRD) analyses (Rigaku, Tokyo) were carried out in the sintered samples to evaluate the final crystalline phases present in the structure of the materials.

2.3. Clinical trial applications

After approval by the Ethics Commission of Hospital de São João (Porto, Portugal), 13 patients with medial compartment osteoarthritis of the knee were selected and gave informed consent to participate in the clinical trial.

The study took place between June 2003 and September 2004 and included ten women and three men, with a mean age of 54 years (range 48–66 years). The left side (knee) was most frequently affected. The inclusion criteria were active patients with medial compartment osteoarthritis of the knee, aged not more than 70 years, varus deformity of less than 15° and no associated inflammatory disease. Mild-to-moderate patello-femoral osteoarthrosis was not considered as a contraindication for HTO. Respecting what had been previously agreed with the patients, the blade plates were removed at 6, 9 and 12 months. Four patients were involved for each time period. In one patient a deficient osteosynthesis technique forced an earlier plate removal after 3 months of implantation and allowed the retrieval of a sample with a very short implantation time.

Pre-operatively, all patients were clinically evaluated according to the standard protocol of the Orthopaedic Department of Hospital de São João (Porto, Portugal), which is the International Knee Score (IKS). The radiological protocol included: anteroposterior knee view standing on each leg; lateral view with 30° flexion; skyline views and hip–knee–ankle radiographs, with the patient standing on both legs, which allowed the global alignment of the limb to be determined and operative planning in order to obtain a slight hypercorrection of 2° or 3° valgus.

With general or loco-regional anaesthesia, all patients were submitted to a prior arthroscopy of the affected knee. Although it has not been demonstrated that this procedure influences the long-term clinical outcome, it allowed the treatment of associated lesions of the meniscus or removal of loose bodies. A 12 cm curved incision was usually made, beginning midway between the patella and the fibular head and extending distally to the crest of the tibia. The fascia was then opened and a subperiosteal dissection was made to expose the lateral tibia and tibiofibular ligaments.

The division of superior tibiofibular ligaments was performed in 12 patients. One patient required a large correction, so, prior to the tibial approach, a fibular shaft osteotomy was performed in the region between its middle and distal thirds.

A Coventry [48–50] type closing wedge HTO was performed. The use of special instrumentation helped to improve the precision of the bone cuts and to apply the Giebel blade plate that was used to internally fixate the bone. Special care was given to keep the medial cortex intact. The correction control was achieved with intraoperative fluoroscopy.

To evaluate the biological behaviour of the Bonelike® at regenerating bone-defected areas, an 8 mm diameter hole was created in the lateral aspect of the tibia, 3 cm distal to the entry point of the screws. A porous cylinder of exactly the same size was press-fitted there in order to fill the cavity as shown in Figs. 1 and 2. A suction drain was placed (and left for 48 h) and the fascia, subcutaneous tissue and skin were closed. The second operation, which allowed samples to be collected at predetermined dates for the subsequent histological study, was performed using the same approach, with a 12 mm trephine to cut the cortical bone around the specimens.

2.4. Evaluation of retrieved samples

For histological examination, retrieved samples were immediately placed in a neutral formaldehyde fixative solution (6%) for seven days, then dehydrated in a series of alcohol solutions (70, 80, 90 and 100%) and embedded in a methylmethacrylate resin. After polymerization, specimens were sectioned with a diamond saw and polished down to the thickness of 40 ± 10 μm with a diamond disc to prepare histological slices. These sections were stained with haematoxylin/eosin and Solo-Chrome R and finally

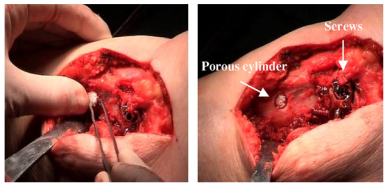

Fig. 1. Macroporous cylinders were press-fitted into a 8 mm hole, 3 cm distal to the entry point of the screws.

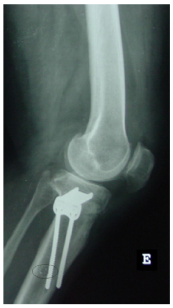

Fig. 2. Post-operative X-ray after implantation of cylindrical samples; the position of the implanted sample relative to the Giebel blade plate can be seen.

examined using an Olympus BH-2 transmitted light microscope. The interface between biomaterial and new bone was examined by scanning electron microscopy (SEM, JEOL JSM 630IF).

Image analysis software (PAQI, developed by CEMUP-UP) was used on both optical microscope and back-scattered SEM images to assess the bone ingrowth within the pore structures by quantifying the bone penetration depth and relative area filled with new bone. For statistical analysis, Student's *t*-test was performed using a 5% significance level.

3. Results

Fig. 3 shows the typical microstructure of a porous sample after sintering at 1300 °C for 1 h. The estimated value of total porosity based on the determined apparent density was around 90%, and it can be observed that over 90% of the total porosity consists of open and interconnected pores.

The XRD pattern of the porous specimens sintered at 1300 °C revealed the presence of HA, α-TCP and β-TCP as previously published (data not shown) [11,12].

The post-operative radiological evaluation started 48 h after surgery with a standard A-P and lateral view of the knee in order to confirm the proper positioning of the implant. The X-rays were then repeated at 6, 12 and 24 weeks. At 6 weeks, it was possible to see in all patients signs of good integration without any lucent line surrounding the cylinders. In later observations, the radiographic appearance became progressively similar to the host bone, showing that Bonelike® was completely osteointegrated by new bone.

The histological analysis revealed no collapse of the Bonelike® cylinders by microfracture, and direct apposition of bone on the Bonelike® surface was found in all tissue sections with no fibrous tissue interface. The pores located in the periphery were the first to be filled with bone as envisaged from the histological surveys after 3 months of implantation (Fig. 4a). A centripetal bone colonization from the periphery to the middle of the cylinder was observed, but this was continuous with the surrounding host bone. The percentage of new bone coverage on the porous Bonelike® internal surface increased with implantation time. This newly formed bone consists of osteoid tissue as observed on stained histological sections.

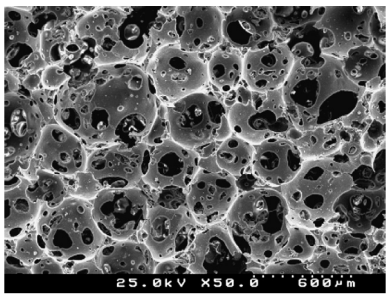

Fig. 3. Microstructure of the sample sintered at 1300 °C.

As shown in Fig. 5 there was evidence of the direct apposition of bone on internal pore surfaces, with osteocytes in close proximity to the implant material after 6, 9 and 12 months of Bonelike® implantation. Furthermore, an extensive vascular network was evident within the porous structure after 6 months.

From the results obtained at short implantation periods it was noted that bone ingrowth tended to be situated on the Bonelike® pore surfaces rather than free standing within the pores. However, the pore structure was not entirely covered by bone, with portions of the implant exposed directly to the bone marrow at early implantation periods although new bone formation penetrated to the middle of the samples. Histomorphometry has been used to characterize the degree of bone integration, and the bone penetration depth and the relative cylinder area filled with new bone were quantified using this technique.

The penetration depth was determined from a radial line measuring the distance of the intersection of the line with newly formed inner bone tissue and the external surface of the implant. However, after 6 months it was already possible to see new bone in the centre of the implanted cylinders, which gives 100% of penetration depth for all implantations periods except for 3 months when bone could only be seen in the peripheral region. Regarding the percentage of the area covered by new bone calculated from two-dimensional histological sections, a value of $53 \pm 15\%$ was achieved for 6 months and no statistical differences were found between the values obtained for 9 and 12 months of implantation since bone was found to penetrate the channels and fill up most of the channel spaces (76 ± 12 and $88 \pm 9\%$, respectively).

Fig. 5 gives an example of mature bone with regular osteon structure surrounded by areas where the new bone tissue was still at the early stages of maturation. After 9 and 12 months, numerous intralacunar osteocytes were embedded within the newly mineralized bone matrix formed in the porous structure of Bonelike®. The results confirmed the highly osteoconductive behaviour of porous Bonelike® structures and the ability of the porous network to promote tissue ingrowth. Significant degradation of the Bonelike® was not detected in the short implantation periods under study although clear surface dissolution was visible after 9 months of implantation (Fig. 6).

4. Discussion

In tissue repair applications an interconnected three-dimensional structural network is required to promote extensive cell attachment and organization of cells; this

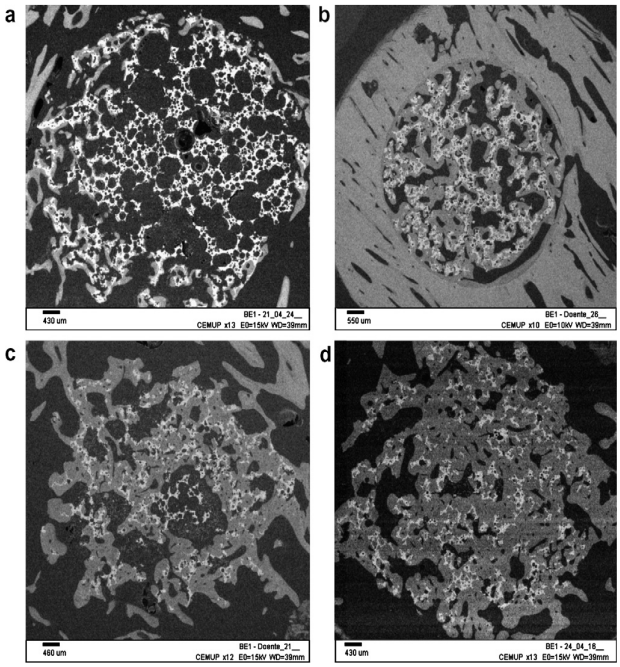

Fig. 4. Back-scattered SEM images of implanted Bonelike® after (a) 3 months, (b) 6 months, (c) 9 months and (d) 12 months of implantation, showing bone regeneration inside the implants.

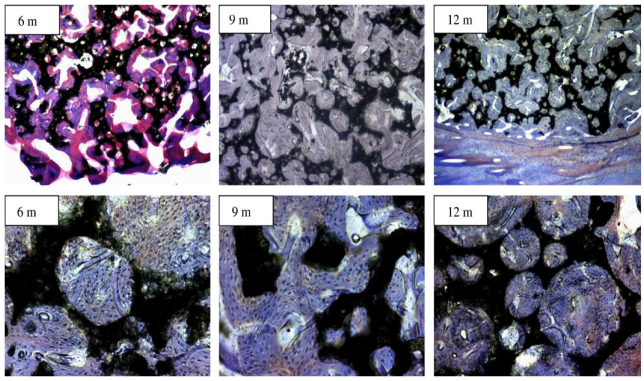

Fig. 5. Histological slices of a Bonelike® porous sample retrieved after 6, 9 and 12 months of implantation (top images 25×; bottom images 100×).

leads to tissue ingrowth with vascularization, and good implantation integration. The tissue ingrowth rates depend upon the pore morphology, the degree of pore connectivity and pore volume. It has been reported that pores larger than 100 μm are necessary to allow blood and nutrients access for bone mineralization within a graft [25–28].

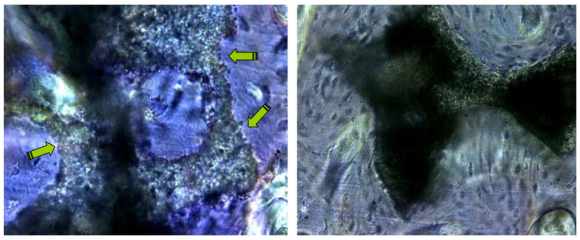

Fig. 6. The surface degradation of a Bonelike® porous sample can be clearly seen, as indicated by arrows, after 9 months of implantation.

The microstructure of the developed porous samples showed that the fraction of closed pores is relatively modest if one considers that foaming methods tend to form structures of essentially closed porosity. The results suggest that the pores left by the burning out of starch granules enhance pore interconnectivity, and therefore it is possible to combine the foaming and the starch consolidation methods to achieve the three-dimensional architectures that fulfil the pore size requirements for bone ingrowth [6–10]. Previous works [32–35] demonstrated that pore interconnectivity seem to play the most important role in glass impregnation in comparison with total porosity.

Synthetic bone grafts provide an alternative to the limited resources of autographs, and the problems associated with the use of allogenic and xenogenic grafts. Bonelike® is a glass-reinforced HA and is believed to be a promising alternative to natural bone grafts, especially in a porous form where the three-dimensional architecture of the material allows cellular ingrowth and vascularization of the graft, thus promoting a greater degree of osteointegration.

During the preparation of the porous Bonelike®, during the drying of the pre-sintered and glass solution soaked samples, the liquid phase has tended to migrate to the evaporating surfaces where the soluble glassy phase started precipitating preferentially after reaching a supersaturated concentration. During sintering, the glassy phase enhances the densification in the above-mentioned parts, eventually closing the microcracks that exist in the struts, and therefore exerting an overall reinforcing effect on the porous structure. Therefore, the innovative approach used to prepare Bonelike® porous structures has the advantage of reinforcing the porous structure especially in the target sites where microfractures may occur.

The use of Bonelike® with standardized and well-defined physicochemical and biological properties in its dense/granular forms led to predictable results when using it in prefashioned porous structures as done in this study. Previous studies, performed *in vitro* and using animal models, have already shown the positive effect on the biological response to the microstructure and chemistry of Bonelike®. Thus, the high level of affinity shown by the host bone to the surface of Bonelike® observed in this study should be a result of a combination of its chemistry and its porous structure.

Extensive penetration of bone ingrowth was noted within the Bonelike® porous structure at time points as early as 6 months, which suggests a rapid fixation of the bone graft. The porosity of the Bonelike® structure allowed osteoprogenitor cell penetration, cell migration and attachment, enabling new bone ingrowth and thus forming a strong bond with the host bone, which provides stability and osteointegration. After the initial period, the bone growth and fixation occurring on the surface of Bonelike® appeared to induce the deposition of more ordered bone on its internal surfaces and within its pores, thereby acting as a support for new bone and encouraging bone growth on its surfaces. The weak mechanical properties of a porous structure should be eliminated once incorporation and bone ingrowth into the pores has been achieved.

The complete penetration of bone into the centre of Bonelike® cylinders must be a consequence of the good interconnectivity among the pores within the structure of Bonelike®, which allowed for nutrient and oxygen availability, leading to faster bone apposition and/or angiogenesis. The literature describes that in the short to medium term, bone apposition is influenced in the first instance by the rate of cellular invasion, which is determined by the pore morphology, and in the medium to long term, by the rate of vascularization, which is determined by the connectivity network [25–28].

HA has been characterized as being much more stable compared to α- and β-TCP phases. Therefore porous Bonelike® has the ability to provide the scaffold for new bone growth due to the presence of the HA, as well as the ability to foster osteogenesis due to the presence of the degradable TCP phases and by the release of specific ions present in its composition. A series of dissolution–precipitation processes *in vivo* and the release of ions, stimulated by the high surface area, will have a beneficial effect on cell biology activity, as has also been demonstrated in several *in vitro* cellular studies and in vivo experiments [42–47].

5. Conclusions

This study demonstrates that porous Bonelike® is highly biocompatible, since direct apposition of bone to the Bonelike® surface is the desired clinical outcome with full

osteointegration and vascularization. Porous structures of Bonelike® can be applied in human adults for bone repair; new bone ingrows into the pores with time of implantation. The porous structure of Bonelike® offer the potential to use this material in tissue engineering.

Acknowledgments

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