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ORIGINAL ARTICLE

Osteoinductive potential of 4 commonly employed bone grafts

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Abstract

Objectives Guided bone regeneration (GBR) aims to predictably restore missing bone that has been lost due to trauma, periodontal disease or a variety of systemic conditions. Critical to this procedure is the ability of a bone grafting material to predictably serve as a 3-dimensional scaffold capable of inducing cell and bone tissue in-growth at the material surface. Although all bone grafts are osteoconductive to bone-forming osteoblasts, only a small number of commercially available bone grafts with FDA approval are osteoinductive including demineralized freeze-dried bone allographs (DFDBA) and scaffolds containing bone morphogenetic proteins (BMPs). Recently, a class of synthetic bone grafts fabricated from biphasic calcium phosphate (BCP) sintered at a low temperature have been shown to form ectopic bone formation in non-skeletal sites without the use of growth factors. Therefore, the present study aimed to compare the osteoinductive potential of this group of synthetic BCP alloplasts with autografts, allografts and xenografts.

Materials and methods In the present study, 4 types of bone grafting materials including autogenous bone harvested with a bone mill, DFDBA (LifeNet, USA), a xenograft derived from bovine bone mineral (NBM, BioOss, Geistlich, Switzerland) and a novel synthetic biphasic calcium phosphate (BCP, Straumman, Switzerland) were implanted into intramuscular pouches of 24 rats and analysed histologically for their ability to form ectopic bone formation around grafting particles. A semi-quantitative osteoinductive score was used to quantify the osteoinductive ability of each bone graft.

Results The results from the present study reveal that (1) autogenous bone resorbed rapidly in vivo, (2) the xenograft showed no potential to form ectopic bone formation and (3) both DFDBA and BCP were able to stimulate ectopic bone formation.

Conclusion These studies demonstrate that these newly developed synthetic bone grafts have potential for inducing ectopic bone formation similar to DFDBA. Future clinical testing is necessary to reveal their bone-inducing properties in clinical scenarios including GBR procedures and in combination with implant dentistry.

Clinical relevance Novel BCP scaffolds are able to induce ectopic bone formation without the use of osteoinductive growth factors such as BMP2 and thus demonstrate a large clinical possibility to further enhance bone formation for a variety of clinical procedures.

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Introduction

Despite the increasing number of new bone grafting substitutes that have emerged in recent years, to date, there exists no single ideal replacement grafting material [1]. Autogenous bone has been considered the golden standard for bone replacement procedures in dentistry due to its release of osteogenic growth factors including bone morphogenetic proteins (BMPs) able to promote the proliferation and differentiation of progenitor cells [2, 3]. Furthermore, they carry no risk of immunologic reaction or disease transmission and their use provides an optimal environment for new blood vessel formation [2, 3]. In contrast, many bone grafting substitutes fabricated from a variety of synthetic materials such as hydroxyapatite, β-tricalcium phosphate and bioactive glasses may provide an osteoconductive matrix but without osteoinductive potential [4].

The field of osteoinductive bone grafting materials has been the cornerstone of much research over the past few decades [5]. Historically, osteoinduction refers to the process by which one tissue, or product derived from it, causes a second undifferentiated tissue to differentiate into bone [5]. In 2 classical studies by Marschall Urist in 1965 and 1967, osteoinduction was defined as "the mechanism of cellular differentiation towards bone of one tissue due to physicochemical effect or contact with another tissue" [6]. In that study, the ability of an implanted demineralized bone matrix to induce in-growth ectopic bone formation in connective tissue of rabbits, dogs and rats was investigated [7]. Later, a group of low molecular weight proteins extracted from demineralized bone matrix (DBM), termed bone morphogenetic proteins (BMPs), were isolated and characterized showing more osteoinduction than DBM alone [8]. Despite these early advancements in the field of osteoinductive biomaterials, to date, only DFDBA and bone biomaterials containing BMPs are approved by the FDA as truly osteoinductive materials [5].

Recently, Yuan et al. demonstrated that synthetically fabricated bone grafts sintered at low temperatures possess the capability of forming ectopic bone formation in large animal models [9]. Bone defects loaded with these synthetic bone grafts were also shown to heal as rapidly as autogenous bone [9]. In light of these recent findings, our group aimed to investigate the use of this novel synthetic scaffold fabricated from biphasic calcium phosphate (BCP). BCP bone grafting particles were compared to 3 commonly employed bone grafting materials including autogenous bone chips derived from a bone mill, demineralised freeze-dried bone allografts (DFDBA) and a commonly employed xenograft (a natural bone mineral, NBM). The osteoinductive potential of each bone graft was compared for their ability to induce ectopic bone formation in an animal model.

Materials and methods

Bone graft selection

Autogenous bone

Autogenous bone was harvested from Wistar rats using cortico-cancellous block grafts harvested with a trephine and ground to particulated bone chips using a bone mill. The selection of bone mill as the selection for autogenous bone harvesting is because it stimulated the highest osteogenic response and ability to release growth factors to the surrounding environment [10, 11].

Allograft bone

Demineralized freeze-dried bone allograft (DFDBA) from LifeNet (USA) was utilized as the DFDBA of choice due to previous handling and its ability to form ectopic bone formation in vivo [12, 13].

Xenograft bone

A natural bone mineral (NBM) of bovine origin (BioOss, Geistlich, Switzerland) was used as the xenograft of choice due to its widespread use in dentistry and our laboratories' previous use in its handling [14–16].

Alloplast bone ceramic

In the present study, a biphasic calcium phosphate (BCP) was tested. The BCP graft was utilized using a 90:10 ration of beta-tricalcium phosphate and hydroxyapatite (Vivoss, Straumann AG, Basel, Switzerland) as previously described [17].

Scanning electron microscopy

Bone grafting materials were fixed in 1 % glutaraldehyde and 1 % formaldehyde for 2 days for SEM. Following serial dehydration with ethanol, samples were critically point dried (Type M.9202 Critical Point Dryer, Roth & Co. Hatfield, PA, USA) followed by overnight drying. The following day, samples were sputter coated using a Balzers Union Sputtering Device (DCM-010, Balzers, Liechtenstein) with 10 nm of gold and analyzed microscopically using a Philips XL30 FEG scanning electron microscope to determine surface variations between bone grafts.

Animal experiments

Twenty-four male Wistar rats (mean body weight 200 g) were used with all handling and surgical procedures in accordance with the policies of the Ethics Committee for Animal Research at the University of Wuhan, China. Animals were given food and water ad libitum with constant temperature at 20–25 °C.

All operations were conducted under strictly sterile conditions. For surgery, the rats were generally anesthetized with intraperitoneal injection of chloral hydrate (10 %, 4 ml/kg body weight) as previously described [18]. After skin preparation and disinfection, bilateral muscle pouches were made in the gastrocnemius muscle of each animal as previously described [13]. Subsequently, an equal mass of autogenous bone (20 mg per pouch), DFDBA particles (20 mg per pouch), NBM (20 mg per pouch) and BCP (20 mg per pouch) were implanted intramuscularly and incisions were sutured in two layers. Postoperatively, penicillin (400,000 IU/ml, 0.1 ml/kg) was injected for 3 days. After 3 and 6 weeks postimplantation, rats were sacrificed and samples were removed and prepared for histological analysis.

Histological analysis

The samples were decalcified in 10 % EDTA replaced twice weekly for 3 weeks at room temperature. Samples were then dehydrated in a series of graded concentrations of ethanol from 30 to 100 % followed by embedding in paraffin as previously described [19]. Serial sections of 5 μm were cut and mounted on polylysine-coated microscope slides and stained with H&E (Sigma #S2255; Sigma-Aldrich, St. Louis, USA) in accordance with the manufacturer's protocol and also as previously described to visualize ectopic bone formation. All samples were observed for evidence of ectopic bone formation and remaining bone grafting particles. Furthermore, the ability of the samples to induce new ectopic bone formation was semi-qualitatively evaluated by two independent observers (QZ, YZ) blinded to treatment and rated according to a previously published scheme [20–24]. Three consecutive sections (3 to 4 μm each) were obtained at 3 different levels throughout the block along the longitudinal axis for evaluation. The ability of the samples to induce new bone was qualitatively evaluated by the 2 independent observers and rated accordingly as described below. In total, 4 separate fields in

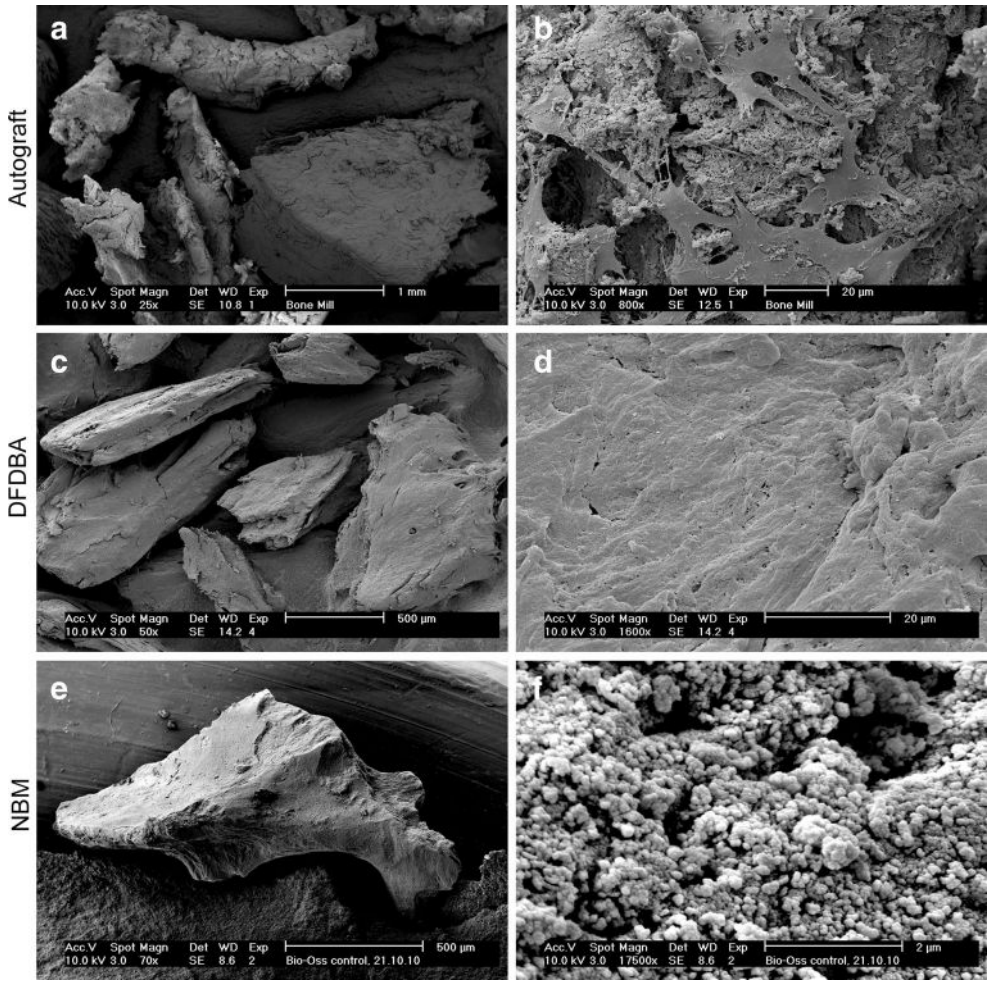

Fig. 1 SEM images of autogenous bone harvested via bone milling **a**, **b**, an allograft (DFDBA) **c**, **d** and **a** xenograft (NBM) **e**, **f** at low **a**, **c**, **e** and high **b**, **d**, **f** magnification

each of the 3 different sections were evaluated for ectopic bone formation according to the following score: A score of 1 indicated the presence of particles without any bone; 2 indicated the production of a new bone in one site within the section and covering less than 40 % of the surface area examined; 3 indicated the production of a new bone in more than one site, covering more than 40 % but less than 70 %, of the surface area examined; and 4 indicated the production of a new bone in more than one site, covering more than 70 % of the surface area examined. The overall grade for each implant was obtained by averaging the scores from all specimens in the group.

Results

Scanning electron microscopy

SEM was utilized to visualize bone grafting particles both at low and high magnification (Figs. 1 and 2). Autogenous bone chips derived from a bone mill showed various shapes and sizes from 0.1 mm to slightly greater than 1 mm (Fig. 1a). The high-resolution magnification demonstrated a number of proteins remaining on the particle surface with a number of cells still present on its surface following sample harvesting (Fig. 1b). On the other hand, DFDBA particles were slightly smaller in size with particle surface devoid of any visible proteins and with a very smooth surface (Fig. 1c, d). Xenograft particles were also very similar in size to DFDBA ranging from 0.3 to approximately 1 mm (Fig. 1e). Interestingly, the material surface had quite a roughened surface when compared to either autogenous bone or the alloplast (Fig. 1f). The material surface was completely devoid of all proteins (Fig. 1f).

Following analysis of 3 commonly used bone grafting materials, BCP grafts were visualized (Fig. 2). As can be depicted from the SEM images at a low magnification, the new synthetic bone grafts present with many macro-topographies (Fig. 2a). Furthermore, the high-resolution SEM images demonstrate extremely roughened surfaces (Fig. 2c).

Ectopic bone formation

Following SEM analysis, ectopic bone formation was investigated for all bone grafting particles at 3 and 6 weeks postimplantation (Fig. 3). Interestingly, autogenous bone demonstrated some signs of ectopic bone formation at 3 weeks (Fig. 3a, arrow). By 6 weeks, the bone graft was entirely resorbed and muscle was found surrounding the original defect site (Fig. 3b). NBM derived from bovine origin demonstrated no signs of ectopic bone formation either at 3 or 6 weeks (Fig. 3c). Following 6 weeks, the xenograft material was still present in muscle pouches with a large number of infiltration inflammatory monocytes found within connective tissues surrounding the bone particles (Fig. 3c). The implantation of DFDBA to the muscle pouches demonstrated signs of ectopic bone formation at both time points (Fig. 3e, f). Results varied between the 6 animals tested, largely dependant

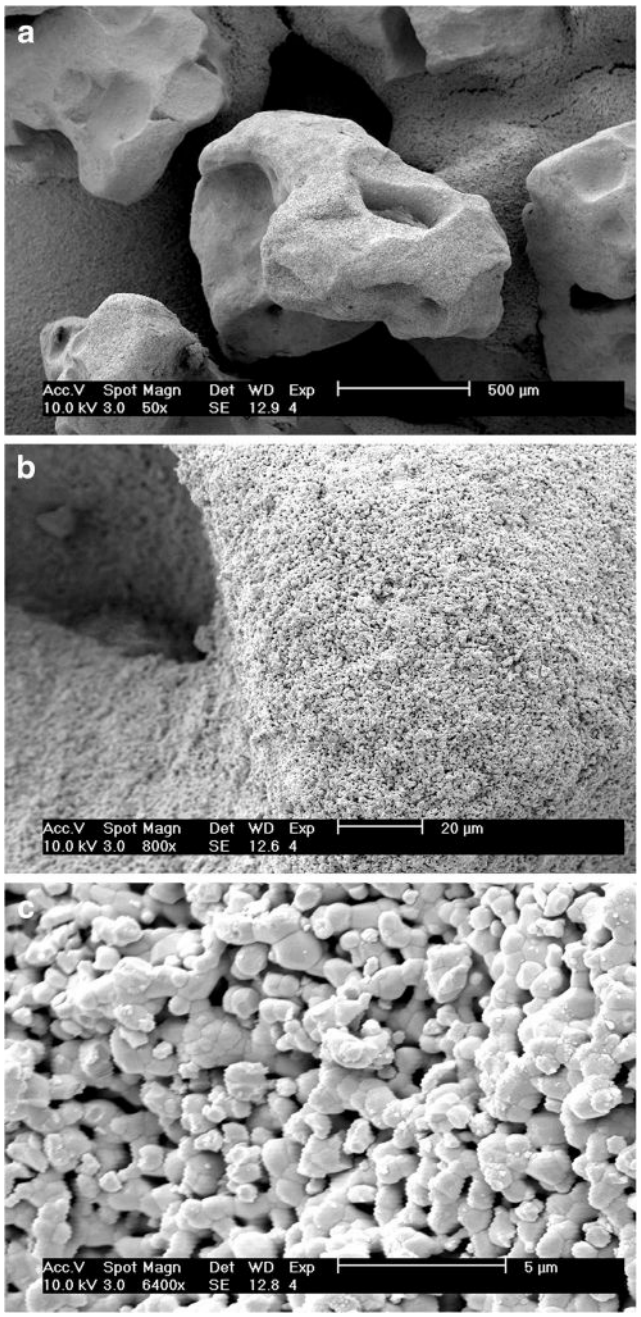

Fig. 2 SEM images of novel BCP bone grafting material demonstrating many macro- and nano-topographies at low **a**, moderate **b** and high **c** magnification

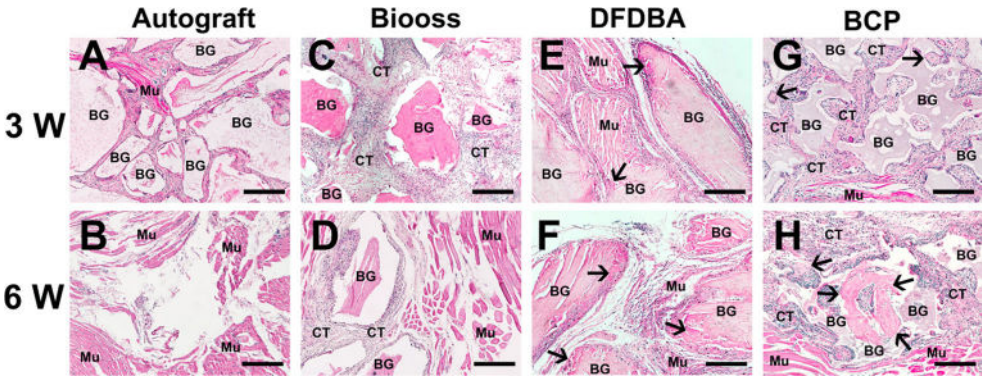

Fig. 3 H&E staining of bone grafts (BG) tested for ectopic bone formation in the gastrocnemius muscle of rats at 3 and 6 weeks postimplantation. (*Bar* = 500 μm; *BG* = bone graft, *CT* = connective tissue, *Mu* = muscle; *arrows* indicate new ectopic bone formation)

on the batch of DFDBA chosen; however, most of the new bone formation was found adjacent to the scaffold surface (Fig. 3e, f, arrows). Interestingly, the BCP group with a high macro- and microtopographies (Fig. 2) was able to form ectopic bone formation as depicted in Fig. 3g, h. Ectopic bone formation was found in all samples adjacent to the bone grafting particle surface at 3 weeks with an additional bone being formed by 6 weeks (Fig. 3h). All samples were quantified using a semi-quantitative osteoinduction score (Fig. 4). By 6 weeks, all autografts were resorbed with some signs of osteoinduction visible at 3 weeks. The NBM had no ability to form ectopic bone formation and remained present at 6 weeks (Fig. 4). Both DFDBA and BCP demonstrated the ability to form ectopic bone formation. More variability was found for DFDBA samples (Fig. 4).

Discussion

The aim of the present study was to test the osteoinductive potential of 4 bone grafting materials commonly utilized in the field of implant dentistry. There exists a large variability between the bone-forming capabilities of various bone grafts, and the osteoinductive potential remains one of the key features to improve the integration of implanted bone grafts [5]. While the field of implant dentistry has made a number of significant advances in recent years, the use of an autogenous bone has remained the gold standard for bone grafting procedures over the past several decades. Despite this, numerable attempts have been made to substitute autogenous bone grafts with other replacement options due to their drawbacks that include increased patient morbidity, limited availability and extra surgical time/costs.

Therefore, the present study investigated a newly developed synthetic bone graft fabricated from a biphasic calcium phosphate fabricated from 10:90 ratio of hydroxyapatite and β-tricalcium phosphate (Fig. 2). These novel grafts show promising features as a bone grafting material by demonstrating extremely high macro- and nanoporosities, ideal characteristics for bone growth [25–27] capable of forming ectopic bone formation (Fig. 3). Previously, Dahlin et al. compared these same BCP bone grafts with a 2nd BCP-II grafting material composed of 60 % hydroxyapatite/40 % β-TCP, as well as a NBM in surgically created defects in the mandible of minipigs [28]. It was found that BCP-I showed significant higher amounts of newly formed bone when compared to the other 2 groups including the NBM utilized in our study [28]. Therefore, consistent with those results, the osteoinductive model utilized in the present study demonstrated similar in vivo characteristics on new bone formation as presented previously by Dahlin et al. [28].

Not surprisingly, the natural bone mineral derived from bovine origin was unable to show signs of ectopic bone formation. These bone grafts have been used extensively in bone grafting procedures over the years due to their ability to remain present with little to no evidence of bone resorption even years after implantation [29–31]. While these grafts demonstrated numerous clinical advantages, there drawbacks include no osteoinductive potential limiting their ability to rapidly stimulate new bone formation. Previously, Donos et al. further

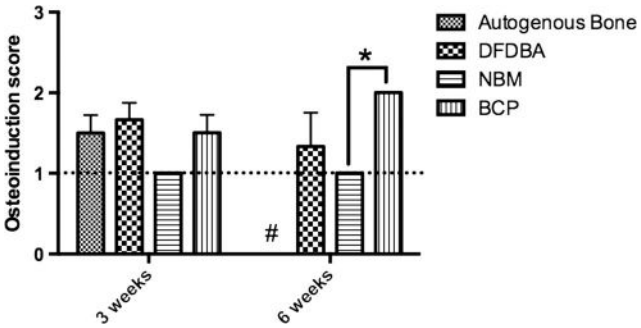

Fig. 4 The effect of different bone grafts on osteoinductivity using a qualitative scoring system: *0* = no evidence of any bone graft, *1* = only original bone graft present, *2* = one site with visible ectopic bone formation, *3* = two or more sites of ectopic bone formation, *4* = >70 % of field at ×10 covered by a new bone (*$p < 0.05$, #$p < 0.05$; group lower than all other groups)

showed that these xenografts were also incapable of forming ectopic bone formation [32], consistent with the results found in our study.

It was also noted in the present study that the allografts demonstrated selective ability to form ectopic bone formation with variable rates of bone around the implanted grafts. It has previously been reported that DFDBA has variable rates of bone formation largely dependent on donor age, medical condition as well as sterilization procedures [13, 33]. Thus, while the ability for DFDBA to form ectopic bone formation was confirmed in the present study, one of the advantages of using a synthetic material could more predictably produce new bone formation when compared to DFDBA from various donor batches.

One of the key remaining questions presently investigated by our group is to determine the mechanism by which these novel BCP bone grafting materials are able to direct and induce ectopic bone formation. Recent studies have begun to implicate immune cells as the possible governing cells responsible for dictating new bone formation around certain classes of bone biomaterials [34–36]. A special subset of macrophages (osteal macrophages, also referred to as OsteoMacs) is currently being investigated as possible key players responsible for guiding new bone formation around these bone biomaterials [37]. While limited available data still exists in the literature, future strategies to further characterize these cells within osteal tissues as well as their plausible roles in bone induction of biomaterials remains necessary.

In conclusion, the results from the present study demonstrate that these novel BCP scaffolds possess an osteoinductive potential by demonstrating ectopic bone formation in skeletal sites in a rat muscle. While autogenous bone is still considered the gold standard of bone grafting materials due to its excellent combination of osteogenesis, osteoinduction and osteoconduction, novel synthetic materials are slowly paving a path which may demonstrate equivalent bone-forming abilities in the near future.

Compliance with ethical standards

Conflict of interest BCP grafts were kindly provided by Straumann AG, Switzerland. Benjamin Pippenger and Michel Dard are both employees of Straumann AG who contributed to the experimental design and provided the bone grafting materials. All other authors declare no conflict of interest.

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Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the University of Wuhan, Department of Oral Implantology, China.

Informed consent No informed patient consent was necessary.

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