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ORIGINAL ARTICLE

Osteogenic gene array of osteoblasts cultured on a novel osteoinductive biphasic calcium phosphate bone grafting material

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Abstract
Objectives Recently, novel biphasic calcium phosphate (BCP) scaffolds have emerged as a new class of bone grafts with osteoinductive potential demonstrating the ability to form ectopic bone in extra-skeletal sites. The aim of the present study was to perform an osteogenic gene array to target possible genes responsible for eliciting the changes in cell expression responsible for inducing osteoblast differentiation.
Materials and methods Human MG63 osteoblast-like cells were seeded for 24 h on tissue culture plastic or osteoinductive BCP particles and analyzed for upregulated genes using an osteogenesis super-array. Osteoblast-related genes including those transcribed during bone mineralization, bone metabolism, cell growth and differentiation, as well as gene products representing extracellular matrix molecules, transcription factors, and cell adhesion molecules were investigated.
Results An upregulation of genes transcribing biglycan (1.7-fold), bone morphogenetic proteins 1, 2, 4, 6, and 7 (1.5–2.1-fold), various collagen isoforms including 1a1, 1a2, 2a1, and 5a1 (1.73–2.72-fold), colony stimulating factor 2 (2.59-fold), fibroblast growth factor receptor 2 (1.79-fold), fibronectin (2.56-fold), integrin alpha 1, 2, and 3 (1.82–2.24-fold), SOX9 (2.75-fold), transforming growth factor beta receptor 2 (1.72-fold), vitamin D (1.89-fold), and vascular endothelial growth factor A and B (2.00, 1.75-fold) were all significantly ($p < 0.05$) increased on BCP particles when compared to control tissue culture plastic.
Conclusion In summary, a number of activated genes were involved in bone formation following osteoblast attachment to BCP particles. The involvement of key chondrogenic genes hints that bone grafts capable of spontaneously inducing ectopic bone formation may implicate endochondral ossification. Further investigations in the triggered pathways involved in the process of ectopic bone formation are necessary to understand the key inductive properties of these novel osteoinductive BCP particles.
Clinical relevance Novel osteoinductive BCP particles demonstrate a wide range of significant increases over several key molecules implicated in osteogenesis that may be implicated in their ability to form ectopic bone formation.

Keywords Osteoinduction · Osteoinductive potential · Bone graft · Ectopic bone formation · Guided bone regeneration

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Introduction

Over five decades have now passed since the discovery of osteoinductive factors largely pioneered by Marschall Urist's work on bone grafts [1, 2]. By studying demineralised freeze-dried bone allografts (DFDBA), it was discovered that they

possess the ability to induce ectopic bone when implanted in extra-skeletal locations in rabbits, dogs, and rats [1, 2]. Later, a class of growth factor proteins, later termed bone morphogenetic proteins (BMPs), were isolated and characterized for their ability to induce ectopic bone formation [3]. Since then, the use of osteoinductive growth factors such as BMPs as well as bone grafting materials with osteoinductive potential (DFDBA) has commonly been utilized in the fields of orthopedics and dental medicine [4–8].

To date, bone grafting materials have commonly been characterized based on their regenerative potential in three areas. The ideal grafting material should:

1) Be osteoconductive by providing a three-dimensional scaffold which allows vascular invasion and cellular infiltration.
2) Possess osteoinductive potential capable of attracting mesenchymal progenitor cells and inducing their differentiation into bone formation osteoblasts.
3) Contain living progenitor cells able to lay new bone matrix (osteogenesis).

Consequently, the gold standard of bone grafting is autogenous bone due to its excellent combination of osteoconduction, osteoinduction, and a living osteogenic cell population. Autogenous bone possesses numerous advantages over many synthetically fabricated bone grafts in that it induces osteoblast proliferation, differentiation, and release a multitude of osteopromotive growth factors to their surrounding tissues [9, 10]. Despite its many advantages, numerous attempts to pursue alternatives have been investigated due to its drawbacks which include limited supply/availability, additional surgical time and costs, and additional donor site morbidity. Although most of the bone grafting materials fabricated in the past two decades have been osteoconductive to bone-forming osteoblasts, only scaffolds carrying BMP2 or BMP7 as well as scaffolds which hold growth factors naturally within their matrix (DFDBA) have demonstrated any form of osteoinductive potential with FDA approval [11].

Interestingly, it was recently demonstrated that a new type of synthetically fabricated bone graft made from biphasic calcium phosphate (BCP) sintered at lower temperatures possesses osteoinductive potential by demonstrating the ability to form ectopic bone in extra-skeletal sites [12, 13]. Their ability to induce bone formation in sites where bone should otherwise not be formed without containing potent growth factors such as BMPs raises numerous hypotheses on their potential mechanisms. Some factors suggested to contribute to their ability to induce ectopic bone formation include varying sintering temperatures during scaffold preparation, material composition, compressive forces, surface topography, and dissolution rates of bone grafting materials [14–19]. Furthermore, our group has recently demonstrated that the bone grafting material used in the present study is able to promote early cell differentiation of mesenchymal progenitor cells into osteoblasts as well as induce ectopic bone formation in the calf muscle of beagle dogs and epithetical tissues of rats, thus confirming its osteoinductive potential [19, 20].

The aim of the present study was to perform an osteogenic super-array to investigate the response of osteoblasts seeded on these novel osteoinductive BCP particles and analyze cell behavior over a wide array of genes involved in osteogenesis including those transcribed during bone mineralization, ossification, bone metabolism, cell growth and differentiation, as well as gene products representing extracellular matrix molecules, transcription factors, and cell adhesion molecules.

Methods

Bone grafting material and cell source

BCP particles were kindly provided by Institut Straumann AG, Basel, Switzerland (Vivoss®). MG-63 human osteoblast-like cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA USA). For all in vitro experiments, 500 mg of BCP material was placed in 6-well culture dishes to cover the bottom. Osteoblasts were seeded at a density of 500,000 cells per well in 6-well dishes for super-array analysis after 24 h. Culture media was supplemented with 1 % antibiotics and 10 % fetal bovine serum (Invitrogen, Basel, Switzerland).

Scanning electron microscopy

BCP particles were fixed in 1 % glutaraldehyde and 1 % formaldehyde for 2 days for scanning electron microscopy (SEM). Following serial dehydration with ethanol, samples were critical point dried (Type M.9202 Critical Point Dryer, Roth & Co. Hatfield, PA, USA) followed by overnight drying. In 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 topographies of BCP particles.

Super-array of osteogenic potential

The initial expression of osteoblast-related genes was examined after culture of cells for 24 h. Total RNA was isolated using TRIZOL reagent and RNAeasy Mini kit (QIAGEN, Basel, Switzerland). A TaqMan® Human Osteogenesis 96-well plate super-array (4,414, 096, Applied Biosystems, Rotkreuz, Switzerland) was employed for the analysis. Osteoblast-related genes

include those transcribed during bone mineralization, ossification, bone metabolism, and cell growth and differentiation. The gene products represent extracellular matrix molecules, transcription factors, and cell adhesion molecules among others. Real-time RT-PCR was performed according to manufacturer's protocol using 20-μl final reaction volume of TaqMan®'s one step master mix kit (Applied Biosystems) as previously described [21]. RNA quantification was performed using a Nanodrop 2000c (Thermo Scientific, Waltham, MA, USA), and 100 ng of total RNA was used per sample well. Gene fold increases represent data from BCP versus control tissue culture plastic.

Statistical analysis

Gene array analysis was performed for both control (n = 4) and BCP groups (n = 4). Means and standard deviations (SE) were calculated, and the statistical significance of differences among each group were examined by Student t test between both the groups (*p values <0.05).

Results

Scanning electron images are visualized in Fig. 1 to demonstrate the topographical features of BCP particles. The low magnification images (Fig. 1a) demonstrate particles ranging in size between 200 and 800 μm with many micro- and macro-topographical features. At low magnification, the surfaces visibly demonstrate a roughened surface topography (Fig. 1a, b). The high magnification images demonstrate a surface composed of many nano-topographies ideal for osteoblast attachment and differentiation (Fig. 1c).

Following SEM, MG-63 cells seeded on BCP were analyzed for a wide range of genes included in osteoblast differentiation. The full set of genes investigated in the present study is listed in Table 1 in alphabetical order. As depicted, a number of genes involved in osteogenesis were upregulated with the vast majority between 1.5- and 2.5-fold when cells were cultured on BCP particles when compared to tissue culture plastic (Table 1). All genes underwent a standard t test with genes demonstrating a p value <0.05 were considered significant and highlighted in red in Table 1.

The addition of BCP to standard tissue culture plastic was able to significantly increase gene expression of biglycan (BGN, 1.7-fold), various bone morphogenetic proteins (BMPs) including 1, 2, 4, 6, and 7 (1.84-, 1.63-, 2.15-, 1.54-, and 1.63-fold, respectively), various collagen isoforms including 1a1, 1a2, 2a1, and 5a1 (1.99-, 1.73-, 2.17-, and 2.72-fold, respectively), colony stimulating factor 2 (CSF, 2.59-fold), fibroblast growth factor receptor 2 (FGFR2, 1.79-fold), fibronectin (FN, 2.56-fold), GLI family zinc finger 1 (GLI1, 2.45-fold), intercellular adhesion molecule 1 (ICAM1, 1.54-fold), insulin-like growth factor 1 receptor (IGF1R, 1.92-fold), integrin alpha 1, 2, and 3 (1.82-, 2.24-, and 1.85-fold, respectively), matrix metallopeptidase 2 and 9 (MMP2 and 9, 1.83- and 1.75-fold), nuclear factor of kappa light (NFKB1, 1.41-fold), SOX9 (2.75-fold), transforming growth factor beta receptor 2 (1.72-fold), vitamin D (1.89-fold), and vascular endothelial growth factor A and B (2.00- and 1.75-fold, respectively) when compared to control tissue culture plastic.

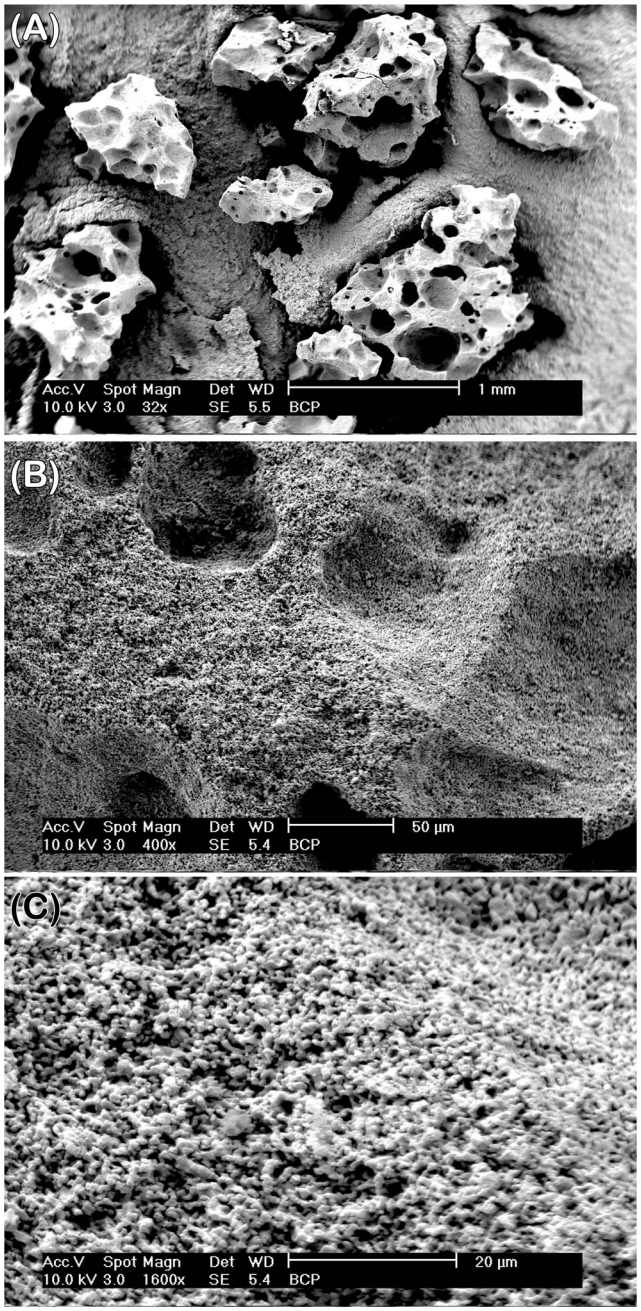

Fig. 1 SEM images of BCP bone grafts at **(a)** low (32×), **(b)** medium (400×), and **(c)** high (1600×) magnification

Table 1 Gene array samples of human MG-63 cells cultured on osteoinductive BCP bone grafting materials in comparison to cells cultured on standard tissue culture plastic following 24 h

Well	AVG ΔC_t (Ct(GOI) - Ave Ct (HKG))		$2^{-\Delta C_t}$		Fold change	*T* test	Fold up- or downregulation
	Test sample	Control sample	Test sample	Control sample	test sample / control sample	*p* value	test sample / control sample
ACVR1	6.69	7.22	9.7E−03	6.7E−03	1.44	0.074234	1.44
AHSG	12.80	13.81	1.4E−04	7.0E−05	2.01	0.080878	2.01
ALPL	12.77	13.55	1.4E−04	8.3E−05	1.72	0.142755	1.72
ANXA5	1.19	1.47	4.4E−01	3.6E−01	1.22	0.186405	1.22
BGLAP	7.34	7.55	6.2E−03	5.3E−03	1.16	0.249878	1.16
BGN	0.90	1.68	5.3E−01	3.1E−01	1.72	**0.023346**	1.72
BMP1	6.79	7.67	9.0E−03	4.9E−03	1.84	**0.007049**	1.84
BMP2	9.30	10.00	1.6E−03	9.7E−04	1.63	**0.016356**	1.63
BMP3	13.35	14.08	9.6E−05	5.8E−05	1.66	0.489320	1.66
BMP4	10.80	11.90	5.6E−04	2.6E−04	2.15	**0.039697**	2.15
BMP5	11.87	11.77	2.7E−04	2.9E−04	0.94	0.864329	−1.07
BMP6	12.40	13.02	1.9E−04	1.2E−04	1.54	**0.014018**	1.54
BMP7	8.72	9.43	2.4E−03	1.5E−03	1.63	**0.030869**	1.63
BMPR1A	6.81	7.01	8.9E−03	7.8E−03	1.14	0.327031	1.14
BMPR1B	12.75	13.12	1.5E−04	1.1E−04	1.29	0.199787	1.29
BMPR2	5.70	5.97	1.9E−02	1.6E−02	1.20	0.288379	1.20
CALCR	13.86	14.53	6.7E−05	4.2E−05	1.59	0.206337	1.59
CD36	12.40	12.76	1.8E−04	1.4E−04	1.28	0.290171	1.28
CDH11	7.28	7.60	6.4E−03	5.2E−03	1.24	0.072918	1.24
CHRD	12.50	12.97	1.7E−04	1.2E−04	1.39	0.271937	1.39
COL10A1	13.18	14.05	1.1E−04	5.9E−05	1.84	0.222193	1.84
COL14A1	10.81	11.36	5.6E−04	3.8E−04	1.47	0.256818	1.47
COL15A1	12.28	12.92	2.0E−04	1.3E−04	1.56	0.091677	1.56
COL1A1	2.09	3.08	2.3E−01	1.2E−01	1.99	**0.001376**	1.99
COL1A2	2.59	3.38	1.7E−01	9.6E−02	1.73	**0.002691**	1.73
COL2A1	12.68	13.80	1.5E−04	7.0E−05	2.17	**0.021557**	2.17
COL3A1	5.55	6.10	2.1E−02	1.5E−02	1.46	0.086962	1.46
COL5A1	4.85	6.29	3.5E−02	1.3E−02	2.72	**0.000041**	2.72
COMP	12.75	13.83	1.4E−04	6.9E−05	2.11	0.057861	2.11
CSF1	2.85	2.01	1.4E−01	2.5E−01	0.56	0.377666	−1.80
CSF2	7.20	8.57	6.8E−03	2.6E−03	2.59	**0.011749**	2.59
CSF3	10.63	11.00	6.3E−04	4.9E−04	1.29	0.216280	1.29
CTSK	8.52	8.94	2.7E−03	2.0E−03	1.34	**0.024137**	1.34
DLX5	18.22	18.66	3.3E−06	2.4E−06	1.35	0.364002	1.35
EGF	10.41	10.76	7.4E−04	5.7E−04	1.28	0.373179	1.28
EGFR	5.08	5.64	3.0E−02	2.0E−02	1.47	0.097823	1.47
FGF1	11.51	12.16	3.4E−04	2.2E−04	1.57	0.124878	1.57
FGF2	4.22	4.12	5.4E−02	5.8E−02	0.93	0.744603	−1.08
FGFR1	5.66	6.50	2.0E−02	1.1E−02	1.79	**0.014965**	1.79
FGFR2	12.41	12.78	1.8E−04	1.4E−04	1.29	0.190558	1.29
FLT1	12.49	12.88	1.7E−04	1.3E−04	1.32	0.334025	1.32
FN1	4.46	5.82	4.5E−02	1.8E−02	2.56	**0.003909**	2.56
GDF10	12.85	13.27	1.4E−04	1.0E−04	1.34	0.527424	1.34
GLI1	13.17	14.46	1.1E−04	4.4E−05	2.45	**0.009293**	2.45
ICAM1	5.03	5.65	3.1E−02	2.0E−02	1.54	**0.020859**	1.54

Table 1 (continued)

Well	AVG ΔC_t (Ct(GOI) - Ave Ct (HKG))		$2^{-\Delta C_t}$		Fold change	*T* test	Fold up- or downregulation
	Test sample	Control sample	Test sample	Control sample	test sample / control sample	*p* value	test sample / control sample
IGF1	13.24	14.01	1.0E−04	6.1E−05	1.70	0.124058	1.70
IGF1R	7.61	8.55	5.1E−03	2.7E−03	1.92	**0.014845**	1.92
IGF2	17.24	18.16	6.5E−06	3.4E−06	1.90	0.079166	1.90
IHH	18.75	19.95	2.3E−06	9.9E−07	2.30	0.174000	2.30
ITGA1	8.61	9.48	2.6E−03	1.4E−03	1.82	**0.042160**	1.82
ITGA2	6.07	7.23	1.5E−02	6.6E−03	2.24	**0.003792**	2.24
ITGA3	5.18	6.06	2.8E−02	1.5E−02	1.85	**0.008413**	1.85
ITGAM	12.73	13.67	1.5E−04	7.6E−05	1.92	0.064733	1.92
ITGB1	1.54	1.84	3.4E−01	2.8E−01	1.24	0.237455	1.24
MMP10	11.87	12.74	2.7E−04	1.5E−04	1.83	0.102947	1.83
MMP2	3.70	4.57	7.7E−02	4.2E−02	1.83	**0.012619**	1.83
MMP8	13.85	14.79	6.8E−05	3.5E−05	1.92	0.191511	1.92
MMP9	11.77	12.58	2.9E−04	1.6E−04	1.75	**0.039701**	1.75
NFKB1	6.83	7.33	8.8E−03	6.2E−03	1.41	**0.049263**	1.41
NOG	11.47	12.02	3.5E−04	2.4E−04	1.46	0.082593	1.46
PDGFA	8.33	8.74	3.1E−03	2.3E−03	1.33	0.139487	1.33
PHEX	8.83	9.16	2.2E−03	1.8E−03	1.25	0.107673	1.25
RUNX2	6.86	7.22	8.6E−03	6.7E−03	1.28	0.289751	1.28
SERPINH1	0.73	1.09	6.0E−01	4.7E−01	1.28	0.149506	1.28
SMAD1	7.19	7.38	6.8E−03	6.0E−03	1.14	0.493437	1.14
SMAD2	4.68	5.09	3.9E−02	2.9E−02	1.33	0.143975	1.33
SMAD3	7.34	7.76	6.2E−03	4.6E−03	1.34	0.207788	1.34
SMAD4	5.42	5.73	2.3E−02	1.9E−02	1.24	0.139882	1.24
SMAD5	5.01	5.33	3.1E−02	2.5E−02	1.25	0.304914	1.25
SOX9	6.95	8.42	8.1E−03	2.9E−03	2.75	**0.006158**	2.75
SP7	12.57	13.25	1.6E−04	1.0E−04	1.60	0.091864	1.60
SPP1	11.45	11.82	3.6E−04	2.8E−04	1.29	0.136702	1.29
TGFB1	2.63	3.21	1.6E−01	1.1E−01	1.49	0.505849	1.49
TGFB2	8.44	8.58	2.9E−03	2.6E−03	1.10	0.382456	1.10
TGFB3	10.60	10.88	6.4E−04	5.3E−04	1.21	0.251902	1.21
TGFBR1	7.52	7.72	5.5E−03	4.7E−03	1.15	0.293326	1.15
TGFBR2	6.04	6.82	1.5E−02	8.8E−03	1.72	**0.010130**	1.72
TNF	13.56	14.16	8.3E−05	5.5E−05	1.51	0.218802	1.51
TNFSF11	12.84	13.47	1.4E−04	8.8E−05	1.55	0.161509	1.55
TWIST1	7.72	7.76	4.7E−03	4.6E−03	1.03	0.756992	1.03
VCAM1	12.76	12.52	1.4E−04	1.7E−04	0.85	0.631422	−1.18
VDR	9.58	10.50	1.3E−03	6.9E−04	1.89	**0.005192**	1.89
VEGFA	2.04	3.04	2.4E−01	1.2E−01	2.00	**0.005841**	2.00
VEGFB	4.03	4.84	6.1E−02	3.5E−02	1.75	**0.019438**	1.75
ACTB	−1.28	−1.50	2.4E+00	2.8E+00	0.85	0.228635	−1.17
B2M	−0.32	−0.26	1.3E+00	1.2E+00	1.05	0.544996	1.05
GAPDH	−1.53	−1.35	2.9E+00	2.6E+00	1.13	0.180997	1.13
HPRT1	5.12	4.97	2.9E−02	3.2E−02	0.90	0.111606	−1.11
RPLP0	−1.99	−1.85	4.0E+00	3.6E+00	1.10	0.349142	1.10

Values in bold represent significant differences with $p < 0.05$

Discussion

The aim of the present study was to investigate the response of osteoblasts when seeded on novel osteoinductive BCP particles. The use of a gene array with a wide variety of genes transcribed during bone mineralization, ossification, bone metabolism, cell growth, extracellular matrix molecules, and transcription factors was utilized to discover the possible genes implicated in induced osteoblast differentiation. In recent years, much research has been dedicated to the development of alloplastic materials able to promote osteoblast proliferation and differentiation [18, 22–26].

In the present study, a wide number of genes implicated in osteogenesis were upregulated with multiple roles in bone physiology (Table 1). Interestingly, the gene that demonstrated the highest upregulation was SOX9, a transcription factor implicated in chondrocyte differentiation as well as cartilage formation [27, 28]. Furthermore, collagen type 2A1, the collagen required for cartilage formation [29, 30], was also elevated 2.17-fold, slightly higher than collagen type 1, the collagen required for bone formation [31, 32] (Table 1).

During tissue fetal development, bone is typically formed in one of two ways, by intramembranous or endochondral ossification [33]. Unlike intramembranous ossification which is formed directly from bone cells, endochondral ossification requires a cartilage matrix during the early phases which is later replaced by bone [34–36]. Endochondral ossification is essential for the formation of long bones and has its implications in natural bone fracture healing [34–36]. Although a significant deal of research has been addressed dealing with bone formation during tissue development and during bone healing, much less research has focused on the formation of bone around locally implanted biomaterials. The development of these novel biomaterials able to spontaneously induce bone formation in muscles and epithelial tissues has potent applications in the fields of orthopedics and dentistry. The use of these biomaterials suggests that once a cell from various cell origins adheres to the material surface with precise nano-topographies, rapid cell signaling is able to progressively recruit and differentiate cells into bone-forming osteoblasts. Therefore, it becomes critical to study the cellular mechanisms which are implicated in these novel biomaterials which are able to auto-induce cells either down the osteoblast/chondrocyte lineages.

Recently, there has been some suggestion that chondrocytes play a role in new bone formation around dental titanium implants [37]. Other identified genes associated with chondrocyte differentiation included pannexin3 (PANX3), asporin (ASPN), and proteoglycans (PGs) which were all found in elevated levels in numerous studies analyzing implant surface osseointegration into the bone [38–40]. Furthermore, in vitro studies using human MSCs cultured on implant discs similarly demonstrated early upregulation of chondrocyte genes including HAPLNs, CRTAP, and SOX9 which these authors have reported as unexpected [37, 41, 42]. Therefore, the transient expression of cartilage-related transcription factors and ECM molecules may be part of the early bone regeneration process around biomaterials whereby a cartilaginous extracellular matrix produced by chondrocytes serves as a mean for subsequent bone apposition to implanted biomaterials [37]. This hypothesis clearly needs further investigation.

In the present study, it was also observed that a number of genes expressing bone-related growth factors and their associated receptors where significantly upregulated when cells were seeded onto BCP particles (Table 1). It becomes difficult to assess what role these growth factors may be playing on osteogenesis versus chondrogenesis. For instance, it is well known that BMPs act by increasing osteoblast proliferation and differentiation; however, their role in chondroblast maturation has also been observed [43]. Interestingly, it has also been observed that certain BMPs, such as BMP6 (also significantly upregulated in the present study), is able to specifically differentiate progenitor cells down the chondroblast lineage [44]. Furthermore, FGF, IGF, TGFB, and VEGF all have positive roles in osteoblast and chondroblast maturation [45–52]. Thus, it becomes difficult to assess the exact nature of cells seeded on these scaffolds at present. Future studies designed at specifically blocking the osteogenic or chondrogenic pathways in response to cells seeded onto these osteoinductive scaffolds may give higher precision on the cellular pathways responsible for the osteoinductive phenomenon observed when cells are in contact with these novel biomaterials. Future study investigating these upregulated genes at the protein levels could provide further insights into the molecular mechanisms inducing ectopic bone formation by these BCP bone grafting particles.

Interestingly, one area of research having been suggested as one of the key reasons for the osteoinduction of these bone grafts are cells implicated in the immune system. Recent studies have show that part of the osteoinductive potential of certain classes of BCP bone grafts has to do with their response to immune cells including monocytes and macrophages [53–55]. There knock-down in certain animal models has been shown to completely eliminate the grafts ability to form ectopic bone formation. This special subset of macrophages termed osteal macrophages (or OsteoMacs) is currently being investigated as possible key player responsible for guiding new bone formation around these bone biomaterials [56]. While there is very limited available data to date, our research team is further investigating new strategies to fully characterize their implication in these bone-inducing osteoinductive BCP bone grafts. Furthermore, another area of research requiring future study could include a comparative study investigating these osteoinductive synthetic bone grafts in comparison to other commercially available bone grafts that are not osteoinductive

to elucidate which molecular factors might be responsible for causing ectopic bone formation in certain classes of bone grafts.

Conclusions

The results from the present study demonstrate a large number of significantly upregulated genes that are transcribed during the early phases of osteoblast adhesion to a novel BCP bone graft with osteoinductive potential. The involvement of key chondrogenic genes seems to hint that bone grafts capable of spontaneously inducing ectopic bone formation may implicate endochondral ossification. Further investigations in the triggered pathways involved in the process of triggered bone formation are necessary to understand the key inductive properties of these novel BCP scaffolds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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