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Osteoclast resorption of beta-tricalcium phosphate controlled by surface architecture

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ABSTRACT

A resorbable bone graft substitute should mimic native bone in its capacity to support bone formation and be remodeled by osteoclasts (OCl) or other multinucleated cells such as foreign body giant cells (FBGC). We hypothesize that by changing the scale of surface architecture of beta-tricalcium phosphate (TCP), cellular resorption can be influenced. $CD14^+$ monocyte precursors were isolated from human peripheral blood ($n = 4$ independent donors) and differentiated into OCl or FBGC on the surface of TCP discs comprising either submicron- or micron-scale surface topographical features (TCPs and TCPb, respectively). On submicrostructured TCPs, OCl survived, fused, differentiated, and extensively resorbed the substrate; however, on microstructured TCPb, OCl survival, TRAP activation, and fusion were attenuated. Importantly, no resorption was observed on microstructured TCPb. By confocal microscopy, OCl formed on TCPs contained numerous actin rings allowing for resorption, but not on TCPb. In comparison, FBGC could not resorb either TCP material, suggesting that osteoclast-specific machinery is necessary to resorb TCP. By tuning surface architecture, it appears possible to control osteoclast resorption of calcium phosphate. This approach presents a useful strategy in the design of resorbable bone graft substitutes.

1. Introduction

If a bone graft substitute is meant to be replaced by new bone tissue in a bony defect, then it must be resorbable. Unlike non-resorbable materials, resorbable materials avoid the local loss of bone density associated with stress shielding that stems from the disproportionally high loadbearing of an implant versus the surrounding bone. Moreover, resorbable bone graft substitutes, such as calcium phosphate (CaP), should allow for remodeling by bone resorbing osteoclasts [1], important for normal bone homeostasis, bone coupling, and osteogenesis [2–5].

The modes by which a CaP bone graft substitute can be resorbed in the body are generally categorized as cell-mediated (often termed bioresorption or biodegradation) or passive, such as dissolution, erosion, and mechanical fragmentation [6]. Related to these, two primary strategies for controlling the resorption rate of CaP include modifying the physical architecture and tuning the ceramic chemistry [7]. The first strategy is to incorporate an interconnected network of pores or channels large enough for cell infiltration (>20 μm) to promote blood perfusion and capillary ingrowth to supply and sustain cells needed for bioresorption and osteogenesis. Additionally, increasing the pore area enhances the surface area allowing faster dissolution. The second strategy is to alter the ceramic crystalline chemistry to thermodynamically favor dissolution in the body. For instance, CaP composed of beta-tricalcium phosphate (TCP) tends to dissolve more readily than hydroxyapatite (HA) and is therefore preferred where resorbability is desired [1].

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Table 1
Physical characterization of TCP.

Physical parameters	TCPs	TCPb
Average grain diameter (μm)	0.95 ± 0.27	3.66 ± 1.05
Average pore diameter (μm)	0.63 ± 0.33	1.78 ± 0.85
Average peak-to-valley roughness, R_a (μm)	0.126 ± 0.003	1.287 ± 0.011
Root-mean-square peak-to-valley roughness, R_{RMS} (μm)	0.158 ± 0.003	1.597 ± 0.011
Porosity (%)	69.6	72.0
Total pore area (m^2/g)	1.477	0.769

Other material properties may also determine CaP resorbability such as the physical surface architecture, i.e., the size and amount of surface micropores (0.1–10 μm) and grains [8,9]. However, Bohner (2012) recently emphasized that no studies have evaluated these factors while maintaining other material properties equal to clearly determine their effects on bioresorption or if there is an optimal design criterion [1]. More recently, results reported by our group described that despite possessing equivalent ceramic chemistry, macropores, and total porosity, TCP with submicron-scale surface grains and pores was shown to be resorbed by ~24% after implantation for 12 weeks in the dorsal muscle of dogs (an osteoinduction model) but TCP with micron-scale surface grains and pores was appreciably un-resorbed [10]. Multinucleated osteoclast-like cells were substantially identified on TCP with submicron-scale surface architecture, whereas multinucleated cells could scarcely be found on the un-resorbed TCP with micron-scale surface architecture. *In vitro*, neither TCP passively dissolved in culture medium containing serum, suggesting that multinucleated cell-mediated resorption is likely the principal mode by which these materials are degraded *in vivo*. However, no resorption was observed *in vitro*, most likely due to the limitations of using the RAW264.7 osteoclast model cell line. These materials induced ectopic bone formation to similar extents that they were resorbed: TCPs formed ~20% in the free area while TCPb induced none, suggesting that TCP resorption and bone induction may go hand in hand in a mechanism akin to bone coupling, as others have previously postulated [11,12].

Table 2
QPCR primer sequences.

Gene target	Sequence (5′ - 3′)	Product size (bp)	Accession ID
UBC	gcggtgaacgccgatgattat tttgccttgacattctcgatgg	202	ENSG00000150991
TATAb	ggtctgggaaaatggtgtgc gctggaaaacccaacttctg	100	ENSG00000112592
HPRT	tgaccttgatttattttgcatacc cgagcaagacgttcagtcct	101	ENSG00000165704
Cyc1	gcatggtggtgaggactacg ggccaggaaagtaggggttg	106	ENSG00000179091
GAPDH	tgggtgtgaaccatgagaagtatg ggtgcaggaggcattgct	61	ENSG00000111640
CATK	ccatatgtgggacaggaagagagtt tgcatcaatggccacagaga	149	ENSG00000143387
TRAP	cacaatctgcagtacctgcaagat cccatagtggaagcgcagata	128	ENSG00000102575
CAII	tggactggccgttctaggtatt tcttgccctttgttttaatggaa	100	ENSG00000104267
AE2	ttgtgggcctctccatagttatc gatcccgttaagggaggtgact	103	ENSG00000164889
TCIRG1	gctgccaaccacttgagctt caaagtgcacgtggttgaaga	114	ENSG00000110719
DC-STAMP	attttctcagtgagcaagcagtttc agaatcatggataatatcttgagttcctt	101	ENSG00000164935
VCAM-1	acaaagttggctcacaattaagaagtt tgcaaaatagagcacgagaagct	100	ENSG00000162692
ITGB1	tttccattggagatgaggttca cgtaaagcccagaggcctaa	100	ENSG00000150093
ITGB2	cgacggccgctgtca tgttgttttcagccagcttgtg	100	ENSG00000160255
ITGB3	aggctggcaggcattgtc agccccaaagagggataatcc	100	ENSG00000259207
ITGA4	ctttccagacagccaggagaa gggcactccatagcaacca	116	ENSG00000115232
ITGAV	tacagcaggtccccaagtcact aattcagattcatcccgcagat	100	ENSG00000138448

The identity and function of such multinucleated cells surrounding CaP *in vivo* is the subject of debate with some groups speculating that they are fused macrophages (i.e., foreign body giant cells – FBGC) [13,14], while other groups asserting that they are specialized bone-resorbing osteoclasts (OCl) [3,15,16]. Actually, it is plausible that both cell types are present depending the chemical composition of the material, how it was fabricated (i.e., sintering temperature), and where it is implanted [6,15,17]. Following the host response, monocyte/macrophages will normally infiltrate the implantation site and adhere to the implant, providing a common precursor pool for both FBGC and OCl on the material surface [18,19]. Though many groups have demonstrated *in vitro* that OCl possess the cellular machinery necessary for resorption of CaP [20–23] and that a bony surface is not necessary for osteoclast activation [24], whether different surface structure can substantially influence this process and whether FBGC can also resorb CaP is unclear.

Beyond material resorption, multinucleated cells such as OCl may be important for the osteogenic properties of CaP, in particular a class of microstructured CaP that can induce ectopic bone formation without exogenous stem cells or growth factors. For instance, depleting OCl by bisphosphonates has been repeatedly shown to obstruct ectopic bone formation by osteoinductive CaP [12,25]. The reason for this may be due to the powerful osteogenic signals that OCl express and secrete (e.g., S1P, Wnts, BMPs, and CTHRC1) [3,26–28]. Therefore, characterizing what material parameters are promotive of OCl formation, survival, and function may also bear impact on the osteogenic capacity of a CaP bone graft substitute.

Following up on our previous findings that the scale of surface architecture plays a determinant role in both the resorbability of TCP and the presence of multinucleated cells *in vivo* [10], we speculated that surface architecture might directly affect the formation and resorptive function of these cells. In essence, we hypothesized that it might be possible to control multinucleated cell formation and resorption by changing the scale of surface structure. To test this, we cultured human peripheral blood monocytes on the surface of the same two TCP with either submicron- or micron-scale surface features and differentiated them into either OCl or FBGC by adding specific cytokines. The effects of different TCP surface architecture on multinucleated cell formation, survival, and resorption were analyzed using a variety of *in vitro* assays and techniques.

2. Materials and methods

2.1. Preparation and characterization of TCP with micron- and submicron-scale surface architecture

Dense TCP discs were fabricated and characterized as previously described [10]. TCP powders were synthesized by mixing calcium hydroxide and phosphoric acid (Sigma–Aldrich) at a Ca/P ratio of 1.50. TCP powders with small (TCPs) or big (TCPb) grains and micropores in the final ceramics were prepared by wet precipitation. The powders were foamed with diluted H_2O_2 (0.1%) (Merck) at 60 °C to form microporous green bodies and then dried. The dry green bodies were subsequently sintered at 1050 °C or 1100 °C for 8 h to achieve small or big grains for TCPs and TCPb, respectively. Microporous discs (⌀9 × 1 mm) were machined from the ceramic bodies using a lathe and a diamond saw microtome (Leica SP1600). Discs were ultrasonically cleaned in successive baths of acetone, ethanol, and deionized water, dried at 60 °C, and then heat sterilized at 160 °C for 2 h for cell culture. Crystal