Alpha tricalcium phosphate biomaterials for potential clinical application in bone regeneration

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Abstract

Regeneration of oral and craniofacial tissues is one of the significant developments in treatment modalities. It combines innovative application of basic science, clinical science and engineering technology. Synthetic bone substitutes have been developed in a fast pace and research is ongoing to develop more efficient biomaterials with promising ability to regenerate lost bone structure. Tricalcium phosphate biomaterials are among them. In this review, recent innovations to develop more effective biomaterials are discussed.

Introduction

Oral and craniofacial defects may be uncomfortable to the patient and affect functions such as mastication and articulation such that structure, function, aesthetics and pain must all be managed effectively resulting in treatment challenges that are often more complex than in other parts of the body. In daily clinical practice, the dental surgeons frequently encounter considerable challenge when facing with periapical, periodontal and peri-implant bone defects as well as reduced alveolar bone volume for prosthetic rehabilitations. Recently, innovative application of basic science, clinical science and engineering technology altogether renders the regeneration of lost bone structure. The techniques to repair orofacial defects apply accepted therapies for restoring tissue structure and/or function elsewhere in the body, and include synthetic materials (alloplasts), patient's own tissue (autografts), tissue from other person of the same species (allografts) and animal tissues (xenografts). All these have specific advantages and disadvantages. Although autogenous bone is considered as the gold standard, it has significant limitations, including donor site morbidity, inadequate amount, and inappropriate form. These limitations have prompted increasing research in alternative bone grafts such as allografts and xenografts. Though they are attractive sources, there are several problems encountered in using them, including the risk of disease transmission, immunogenicity, loss of biologic and mechanical properties secondary to its processing, increased cost, and non-availability world-wide due to financial and religious concerns. Consequently, significant efforts are being made to develop ideal synthetic bone graft substitutes. Bone grafts and their substitutes can be divided according to their properties of osteoconduction, osteoinduction, and osteogenesis (Table 1).

Term	Definition	Examples
Osteogenic	Contains Osteoprogenitor cells or stimulates (committed) osteoprogenitor cell proliferation	Bone marrow, bone graft
Osteoconductive	3-D scaffold on which committed osteoprogenitor cells produce bone; supports ingrowth of capillaries and cells from the host bed (fracture ends); guides repair in the location where normal healing will occur if left untreated	Demineralized bone matrix, bone graft, calcium phosphate ceramics
Osteoinductive	Bone morphogenesis: phenotypic conversion of stem cells from a non-osseous environment to chondrocytes and osteoblasts; allows repair in a location that would not normally heal if left untreated; able to induce bone formation at an ectopic (extra-skeletal, non-osseous) site	Bone graft, dentine matrix, demineralized bone matrix, bone morphogenetic proteins (BMPs)

Table 1. Fracture healing and bone morphogenesis terminology (Glowacki, 1992; Kenley, 1993; Winn, 1998; Burg, 2000)

Tissue engineering principles

New therapeutic strategy termed tissue engineering has been developed (Langer, 1993). Tissue engineering is one of the biomedical technologies developed to assist the regeneration of body tissues to treat large size defects that are not possible to self-repair. Three basic component; scaffolds, cell sources and signals (the tissue engineering triad) are necessary to optimize development of a single tissue, hybrid organ or interface.

In vivo tissue regeneration, one of the tissue engineering strategies, applies scaffolds and signal molecules to stimulate local host cells for tissue regeneration. In bone tissue engineering, bone morphogenetic protein-2 (BMP-2) has shown its beneficial effects on bone regeneration (Yasko, 1992; Bostrom, 1998; Schimandle, 1995; Sandhu, 1995). However, there are some problems to be solved such as short shelf life, inefficient delivery to target cells and high price.

Calcium phosphate biomaterials as osteoconductive scaffold

One of the first studies reported using calcium phosphate for bone repair was performed by Albee and Morrison in 1920. In 1970s, calcium phosphates were synthesized, characterized and used to a large extent. The earliest application of calcium phosphate salts was in the form of powders (Ferraro, 1979). Synthetic calcium phosphates are salts of orthophosphoric acid (H_3PO_4), and thus can form compounds that contain H_2PO_4–, HPO_3^{2-} or PO_4^{3-}. The calcium phosphate salts constitute a wide group of compounds (Elliot, 1994). Table 2 summarizes the chemical name, the formula, the abbreviation, and the calcium to phosphorus ratio (Ca/P) of some calcium phosphate compounds. Calcium phosphate salts vary by their composition and their crystal structures, leading to specific physicochemical properties.

Name	Formula	Abbreviation	Ca/P
Dicalcium phosphate anhydrate or monetite	$CaHPO_4$	DCPA	1.00
Dicalcium phosphate dihydrate or brushite	$CaHPO_4.2H_2O$	DCPD	1.00
Octacalcium phosphate	$Ca_8(PO_4)_4(HPO_4)_2.5H_2O$	OCP	1.33
Tricalcium phosphate	$Ca_3(PO_4)_2$	TCP	1.50
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	HA	1.67

Table 2. Main biologically relevant calcium phosphate salts *(From Barrèr et al., International Journal of Nanomedicine, 2006: 1(3) 317–332)*

Calcium phosphate biomaterials such as hydroxyapatite and tricalcium phosphate are commonly used biomaterials because they are highly biocompatible, osteoconductive and gradually resorbed and replaced with bone. Two major distinct phases of anhydrous tricalcium phosphate (TCP) crystals exist: α-TCP and β-TCP phases. The α-TCP crystallizes in the monoclinic space group, and β-TCP crystallizes in the rhombohedral space group. Despite their similar chemical composition, their different crystallographic features confer different resorption features: α-TCP is more soluble than β-TCP, and it is obtained after heating the β-TCP to more than 1170°C. Clinically, α-TCP is a major reagent in the composition of cements as they hydrolyze into apatitic structures, but it is also sold under the form of powder, blocks, or granules, like β-TCP. Hydroxyapatite is the most stable since it crystallizes in hexagonal space group.

Histological and histomorphometrical comparative study of the degradation and osteoconductive characteristics of alpha and beta-tricalcium phosphate in block grafts was carried and reported that α-TCP porous blocks showed higher initial solubility in vivo than β-TCP blocks degradation of α-TCP seemed to be aligned with bone formation (Yamada et al., 2007). Kihara et al. (2006) studied biodegradation process of α-TCP particles and new bone formation in a rabbit cranial defect model and found that α-TCP particles are osteoconductive and space-maintaining. We conducted a preclinical study to assess whether α-TCP can be applicable in vertical bone augmentation (Nyan et al., 2012). We found that alpha tricalcium phosphate prevents soft tissue migration into the space and gradually resorbed and replaced with new bone.

Strategies to promote effective bone regeneration

If pharmacological compounds can upregulate the expression of intrinsic growth factors to stimulate bone growth, the strategy to combine such compounds with an osteoconductive bone substitute as drug carrier scaffold would be more cost-effective for bone regeneration. Ideal drug carrier scaffold should have the ability to deliver the drug at appropriate time & proper dose; the presence of a substratum that will enhance cell recruitment and attachment and potentiate chemotaxis; the presence of a void space to allow for cell migration and to promote angiogenesis and the ability to biodegrade without generating an immune or inflammatory response and without producing toxic waste products (Lieberman et al, 1999).

Alpha TCP with statins

Topically applied simvastatin, a cholesterol-lowering drug, has been shown to stimulate BMP-2 mRNA expression in osteoblasts and promote bone growth (Mundy et al. 1999; Sugiyama et al. 2000; Garrett et al. 2001; Ohnaka et al. 2001; Thylin et al. 2003). A preclinical study was carried out by using alpha tricalcium

phosphate (aTCP) as drug carrier and scaffold which is a highly biocompatible, osteoconductive and degradable biomaterial (Nyan et al., 2008). Various doses of simvastatin were combined with aTCP and grafted in rat calvarial defects. The results indicated that 0.1 mg simvastatin is the optimal dose for maximum bone regeneration of the defects in this study model. To clarify the mechanisms underlying the stimulation of bone regeneration by this combination material, another study was performed to analyze the cellular and molecular changes in bone defects at early time points after the material application (Nyan et al., 2009). The results suggested that simvastatin combined with alpha tricalcium phosphate induced bone regeneration in rat calvarial defects by augmenting osteogenic cell proliferation, migration, recruitment and differentiation in the early phase of bone healing which were associated with increased expression of BMP-2 and TGF-b1. Alpha TCP could release simvastatin sufficiently at the early time, provide spaces into which osteogenic cells migrated and recruited. Moreover, its gradual dissolution allowed a smooth exchange with newly formed bone and we for the first time reported that 0.1mg simvastatin+14mg α-TCP is the best combination for optimal bone regeneration. Simvastatin is a widely prescribed cholesterol lowering drug and has been proven for its safety. It is readily available and less expensive compared with recombinant growth factors. Moreover, it is chemically stable. The results of this preclinical study suggest that the combination of simvastatin with α-TCP would be applicable clinically as an effective, simple and safe biomaterial for reconstructive treatments.

We recently evaluated the osteoconductivity of three different bone substitute materials: α-tricalcium phosphate (α-TCP), β-tricalcium phosphate (β-TCP), and hydroxyapatite (HA), combined with or without simvastatin (Rojbani et al., 2011). In α-TCP group, the amount of newly formed bone was significantly more than both HA and control groups but not significantly yet more than β-TCP group. Degradation of α-TCP was prominent and β-TCP showed slower rate while HA showed the least degradation. Combining the materials with Simvastatin led to increasing in the amount of newly formed bone. These results confirmed that α-TCP, β-TCP, and HA are osteoconductive materials acting as space maintainer for bone formation and that combining these materials with simvastatin stimulates bone regeneration and it also affects degradability of α-TCP and β-TCP. Conclusively, α-TCP has the advantage of higher rate of degradation allowing the more bone formation and combining α-TCP with simvastatin enhances this property.

Alpha TCP with green tea extract EGCG

Epigallocatechin-3-gallate (EGCG), the most abundant and biologically active catechin in green tea, has anti-inflammatory and anticancer properties, the ability to reduce serum lipid and blood pressure and to modulate immune response (Hegarty et al., 2000; Wu et al., 2002; Rahman et al., 2006; Tosetti et al., 2009). Additionally, EGCG was found to induce apoptotic cell death of osteoclast-like multinucleated cells and ameliorated experimentally induced arthritis in mice (Nakagawa et al., 2002; Lin et al., 2009; Lee et al., 2010). Such pharmacological effects of catechins may be useful for prophylaxis or treatment of inflammatory bone disease (Morinobu et al., 2008). Recent in vitro studies show that EGCG increases bone mineral nodules in cell lines. However, there has been no study that investigates the effect of the bone regenerative capacity of EGCG in vivo. Our recent work aimed to investigate effects of the combination of epigallocatechin-3-gallate (EGCG) and a-tricalcium phosphate (a-TCP) on bone regenerative capacity (Rodriguez et al., 2011). The combination of α-TCP particles and 0.2 mg EGCG stimulates maximum bone regeneration and this combination would be potentially effective as bone graft material.

Conclusion

The reported pharmacological approach in tissue engineering using alpha tricalcium phosphate biomaterials would be considered very promising and cost-effective in maxillofacial bone regeneration in periapical, periodontal and peri-implant bone defects as well as reduced alveolar bone volume for prosthetic rehabilitations.

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