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In vitro mineralization of functional polymers

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Abstract

This review covers studies in which simulated body fluid (SBF) has been used either to evaluate *in vitro* the bone bonding ability of a material or to produce a biomimetic coat on a material surface. The review focuses on materials with grafted functional polymer chains and functional polymer substrates. It is restricted to the phosphate, phosphonate, carboxylate and sulfonate functional groups as these mimic the anionic groups which are found on biological molecules implicated in mammalian bone mineralization. In this regard, the review covers articles from 1990 to mid-2015.

Keywords: Simulated body fluid; Graft copolymers; Polymers with anionic groups; *In vitro* mineralization

Contents

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1. Introduction

Mammalian biomineralization occurs predominantly in the skeletal system, resulting in bone, dentin and enamel tissues [1]. These tissues are composite materials composed of water, inorganic and organic components, of which the inorganic component of bone is a calcium phosphate (CaP) mineral very similar to the mineral known as hydroxyapatite (HAP), $Ca_5(PO_4)_3(OH)$. The introduction of other ions into the lattice structure changes the calcium/phosphate ratio, with carbonate ion inclusion between 3% and 5% being the most commonly encountered [2]. Biomaterials with applications in bone repair and regeneration as well as implants interfacing with bone tissue (*e.g.* biomaterials for articular cartilage repair) are required to form a mechanically strong bond to bone tissue. This in turn requires the biomaterial to mineralize, a process that can be evaluated relatively simply *in vitro*. In addition to biomaterials which are intended to mineralize, the study of *in vitro* mineralization is also important for materials where a mineralization event is detrimental to the application such as biomaterials intended for use as contact lenses or vascular grafts. This review will give an overview of biological macromolecules that have been implicated in mammalian bone mineralization and possible mechanisms by which this is achieved through anionic functional groups. The methods that have been used to both functionalise existing materials and fabricate new materials with functional groups, with the aim of mimicking the mineralization abilities of the biological macromolecules, will be reviewed. This will be followed by a description of the so-called simulated body fluid (SBF) and its application to mineralization studies. Finally, detail will be provided on mineralization outcomes of substrates that have been modified with functional groups. This review will not cover hydrogel biomaterials (ie. crosslinked hydrophilic networks) and the reader is referred to an excellent review on this topic [3].

2. Biological molecules in mammalian bone mineralization

Organisms use various strategies to control mineralization processes and form mineralized tissue. Through biosynthesis the organism can form sites where mineralization may occur, both within the cell (intracellular) and outside of the cell (extracellular). Simple molecules within the cell, such as lipids, are capable of self-organizing to form intracellular vesicles [4]. Furthermore, cells are capable of synthesizing macromolecules which can be excreted from the cell and these macromolecules are also capable of self-organising into structures called extracellular macromolecular frameworks [5]. In this case mineralization may occur within internal arrays of spaces or at the surface of the framework. The self-organized structures formed by the organism allow it to exercise several levels of control over the mineralization process [5]. The organic component of bone is predominantly the insoluble framework macromolecule collagen (85–90%), whilst the remainder non-collagenous proteins function as the soluble control macromolecules [6]. The collagen matrix of bone is secreted by osteoblasts [7] and subsequently mineralized with HAP forming in the gap zones [8]. However, collagen itself does not have any direct effect on HAP formation [9], and soluble control macromolecules have thus been implicated in mineralization of the collagen matrix. As such the macromolecule bone sialoprotein (BSP) has been implicated in enhancement of bone mineral formation while Osteocalcin (OC), Osteopontin (OPN) and Chondroitin-4-sulfate (Ch4S) have been attributed inhibitory mineralization effects.

Bone sialoprotein (BSP) is expressed by osteoblasts and osteocytes [10], during the mineralization of bone extracellular matrix [11,12]. It is a 70–80 kDa glycoprotein [13] which has been identified as an important soluble macromolecule in bone and cementum tissues, implicated in HAP nucleation [12]. BSP is rich in carboxylate (regions of glutamic acid) and phosphate (phosphoserine residues), along with some sulphated regions. The regions rich in glutamic acid residues are conserved across many mammalian species [12]. BSP bound in an agarose gel has been observed to induce HAP nucleation in undersaturated solutions at concentrations as low as 0.3 μg/mL [14]. The carboxylate groups of BSP have been shown to be required for HAP nucleation [15,16] while the presence of the phosphate groups lower the amount of BSP required for nucleation by one order of magnitude [17].

Osteocalcin (OC) is the most abundant non-collagenous protein found in bone and is an important regulator of mineralization [18]. Elevated levels of OC have been observed in conjunction with several bone diseases associated with deficient mineralization (*e.g.* rickets and osteoporosis) [19]. In computational simulation a number of γ-carboxyglutamic acid and aspartic acid residues located on one face of the protein [20] have been associated with binding of OC to the 100 face of HAP [21] and these residues are conserved across many species [19].

Osteopontin (OPN) is a 44–75 kDa phosphoglycoprotein featuring a polyaspartate region [22]. It is derived from multiple sources in the body, including bone and smooth muscle cells [23,24]. It inhibits pathological calcification (*e.g.* calcification of vascular tissue and formation of urinary stones [25,26]) and has been implicated in controlling mineralization in bone [27]. It is a potent inhibitor of HAP nucleation and growth *in vitro* which has been directly linked to the carboxylate groups of the aspartic acid region [28,29], however, it is the phosphate groups of this protein which has the most pronounced effect on its inhibitory effect, as the loss of these groups through modification negates HAP growth inhibition [28–30].

Chondroitin-4-sulfate (Ch4S) is a glycosaminoglycan (GAG) composed primarily of repeating disaccharide units of N-acetylgalactosamine sulfate and glucuronic acid [31], connected to the aggrecan core protein. Its high negative charge is thought to play a role in the binding of Ch4S to the surface of HAP thereby inhibiting growth [32]. In addition, it has been found that Ch4S adsorbed to a surface lowered the nucleation barrier by improving the interfacial structure [33]. Recent work on HAP growth in the presence of anionic polymers showed that the highly sulphated GAG heparin caused a significant