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Structural and dynamical studies of acid-mediated conversion in amorphous-calcium-phosphate based dental composites

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ABSTRACT

Objective. To investigate the complex structural and dynamical conversion process of the amorphous-calcium-phosphate (ACP)-to-apatite transition in ACP based dental composite materials.

Methods. Composite disks were prepared using zirconia hybridized ACP fillers (0.4 mass fraction) and photo-activated Bis-GMA/TEGDMA resin (0.6 mass fraction). We performed an investigation of the solution-mediated ACP-to-apatite conversion mechanism in controlled acidic aqueous environment with *in situ* ultra-small angle X-ray scattering based coherent X-ray photon correlation spectroscopy and *ex situ* X-ray diffraction, as well as other complementary techniques.

Results. We established that the ACP-to-apatite conversion in ACP composites is a two-step process, owing to the sensitivity to local structural changes provided by coherent X-rays. Initially, ACP undergoes a local microstructural rearrangement without losing its amorphous character. We established the catalytic role of the acid and found the time scale of this rearrangement strongly depends on the pH of the solution, which agrees with previous findings about ACP without the polymer matrix being present. In the second step, ACP is converted to an apatitic form with the crystallinity of the formed crystallites being poor. Separately, we also confirmed that in the regular Zr-modified ACP the rate of ACP conversion to hydroxyapatite is slowed significantly compared to unmodified ACP, which is beneficial for targeted slow release of functional calcium and phosphate ions from dental composite materials.

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Significance. For the first time, we were able to follow the complete solution-mediated transition process from ACP to apatite in this class of dental composites in a controlled aqueous environment. A two-step process, suggested previously, was conclusively identified.

1. Introduction

Amorphous calcium phosphate (ACP) is a unique form of calcium phosphate minerals in organisms [1]. As suggested by its name, the atomic structure of ACP lacks the long-range periodic order of crystalline calcium phosphates. ACP, a metastable phase, is formed as the initial solid phase that precipitates from a highly supersaturated calcium phosphate solution [2,3], and is known to be capable of converting to more stable crystalline hydroxyapatite (HAP) phases through a few different transition pathways [4–6].

ACP has drawn much attention since its discovery [7] due to its importance in biomineralization research. For example, ACP has been identified as a component of bone along with crystalline apatites [7]. The content of ACP in bone is found to correlate with the age of bone [8,9]. More recently, several groups of authors have employed Raman spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray absorption near-edge structure micro-spectroscopy, and scanning and transmission electron microscopy to present evidence for ACP being a transient precursor phase to crystalline biominerals in a wide variety of animal systems, including larval and adult echinoderm skeletons [10,11], radular teeth of chitons [12], larval mollusk shells [13], crustacean cuticles [14], and the fin bones of (vertebrate) zebrafish [15,16]. Moreover, studies of nucleation of apatite crystals *in vitro* suggest that transient ACP is a required intermediate step for the formation of HAP nanocrystal [17–19]. Despite all this progress, we note that the role of ACP as a precursor phase in biomineralization remains inconclusive due to lack of unquestionable proof. Regardless of this ambiguity, ACP is currently among the most widely studied and used biomineralization agents.

It has long been recognized that HAP is the primary inorganic component of mineralized tooth tissues [20]. Due to the strong connection between ACP and HAP, ACP compounds have been explored as restorative and adhesive dental materials designed to promote remineralization of mineral deficient teeth [21–24]. For dental applications, ACP has been shown to possess benefits such as better *in vivo* osteoconductivity and biodegradability than tricalcium phosphate and HAP, good bioactivity, and no cytotoxicity [25,26]. ACP has also been shown to increase alkaline phosphatase activities of mesoblasts, enhance cell proliferation and promote cell adhesion [27]. The unique role of ACP during the formation of mineralized tooth tissue makes it a promising candidate for dental materials.

To assess these benefits of ACP and make it more relevant to general dentistry, ACP has been incorporated as a filler phase in bioactive polymer composites [28–31]. In these preventive or restorative dental materials, ACP is encapsulated in a polymer binder, and is capable of slowly releasing in aqueous environments substantial amounts of calcium and phosphate ions in a sustained manner through the transition from ACP to apatitic phases [28,32], where the polymer resin serves to slow down the transition, as well as providing the mechanical integrity of the composite material. These composites have been shown to promote the recovery of mineral deficient tooth structure in *in vitro* situations such as remineralization of artificially produced, caries-like lesions in bovine enamel [33]. The bioactivity of these materials originates from the propensity of ACP, once exposed to oral fluids with fluctuating pH, which include those with acidic low pH, to release calcium and phosphate ions in a sustained manner while spontaneously converting to thermodynamically stable apatitic structures such as HAP [34,35]. It has also been demonstrated that the released calcium and phosphate ions in saliva milieus create local calcium- and phosphate-enriched super-saturation conditions favorable for the regeneration of tooth mineral lost to decay or wear because these ions can be deposited into tooth structures as apatitic mineral, which is similar to the HAP found naturally in tooth and bone [36,37]. These features of the ACP-based composite make them very attractive bioactive dental restoration materials, but the detailed structural aspects of this kinetic transformation have yet to be elucidated.

Our previous studies of ACP-based dental materials have been primarily focused on the design of bioactive, non-degradable, biocompatible polymeric composites derived from dental acrylic resins and ACP fillers rendered by photochemical or chemically activated polymerization [38]. While the unambiguous potential of this class of composite materials has clearly been demonstrated through our efforts and the efforts of others, unlike the transformation from ACP to HAP where there is a reasonable understanding of the conversion process, it still remains unclear how polymer encapsulated ACP converts to crystalline calcium phosphate phases in these composite materials. The objective of this work is to improve the understanding of the structural evolution and the fundamental process that governs ACP stability. In particular, we focus our study on the transition of ACP in acidic environments where it is speculated that acidic oral fluids with fluctuating pH values act to assist the conversion of ACP in these composites materials. In practical terms, this is also important because acidic oral challenges (lactic acid from bacteria generated biofilms, citric acid from foods, *etc.*) are generally accepted as the primary cause of tooth mineral loss in humans [39].

To achieve this set of goals, we primarily employ X-ray techniques including X-ray photon correlation spectroscopy (XPCS), ultra-small angle X-ray scattering (USAXS), and X-ray diffraction (XRD) to evaluate the effect of acidic challenges on