Early stage mineralization in tissue engineering mapped by high resolution X-ray microdiffraction

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The specific routes of biominalization in nature are here explored using a tissue engineering approach in which bone is formed in porous ceramic constructs seeded with bone marrow stromal cells and implanted in vivo. Unlike previous studies this model system reproduces mammalian bone formation, here investigated at high temporal resolution. Different mineralization stages were monitored at different distances from the scaffold interface so that their spatial analysis corresponded to temporal monitoring of the bone growth and mineralization processes. The micrometer spatial resolution achieved by our diffraction technique ensured highly accurate reconstruction of the different temporal mineralization steps and provided some insight into the challenging issue of the mineral deposit first formed at the organic–mineral interface. Our results indicated that in the first stage of biominalization organic tissue provides bioavailable calcium and phosphate ions, ensuring a constant reservoir of amorphous calcium phosphate (ACP) during hydroxyapatite (HA) nanocrystal formation. In this regard we suggest a new role of ACP in HA formation, with a continuous organic–mineral transition assisted by a dynamic pool of ACP. After HA nanocrystals formed, the scaffold and collagen act as templates for nanocrystal arrangement on the microscopic and nanometric scales, respectively.

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1. Introduction

Bone has always received considerable attention in different fields such as biology, medicine, and materials science due to its interest to the medical world and its unique properties from a chemical and physical point of view. Indeed, besides the medical applications, e.g. dealing with tissue regeneration in the case of fractures and various injuries [1], it provides an important and intriguing model system to investigate the mechanisms involved in the formation of hierarchical self-assembled organic–inorganic composites [2–5]. Nowadays it is well known that bone, as well as other biominalized tissues, show complex structures developed and organized on multiple length scales, from the molecular to the macroscopic level [6]. More specifically, on the nanometric scale the highly specialized organic matrix of collagen microfibrils seems to direct the formation of a calcium phosphate crystal phase, made up of nanosized hydroxyapatite (HA) platelets which are oriented parallel to the fibril axis [7–9]. Together with this matrix, various highly acidic non-collagenous proteins, although accounting for about only 10% of the total amount of organic components, are believed to strongly affect the mineralization process in many different ways, both in solution and by binding to the surface of mineral particles. Depending on their concentration, they can stabilize crystal precursors, induce or inhibit crystal nucleation, and control crystal growth in terms of size and shape, so contributing to the determination of the structural mineral phase and biomechanical properties of the tissue [10,11]. In addition to these organic components, a mineral calcium phosphate precursor is believed to play a fundamental role in the growth of HA nanocrystals, although there is no consensus as to the nature of this phase and its temporal evolution. Various metastable crystalline phases (such as octocalcium phosphate (OCP) [12,13] and brushite [14]) have been suggested as transitory precursors of HA in bone and tooth formation. However, the most recent studies seem to consider the formation of amorphous calcium phosphate (ACP) as the precursor of HA as a favored mechanism compared with the classical crystal growth from...