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BONE FORMATION: THE RULES FOR FABRICATING A COMPOSITE CERAMIC

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ABSTRACT

Bone, teeth and shells are complex composite ceramics which are fabricated at low temperature by living organisms. The detailed understanding of this fabrication process is required if we are to attempt to mimic this low temperature assembly process. The guiding principles and major components are outlined with the intent of establishing non-vital fabrication schemes to form a complex composite ceramic consisting of an organix matrix inorganic crystalline phase.

INTRODUCTION

Bone is referred to as a "hard tissue" and is a natural material with unusual physical and mechanical properties. This unusually strong material is capable of holding up the body's weight while also withstanding acute impacts of many-fold greater force than the body weight. Importantly, this natural material, bone, can bend without shattering and can be flexible without breaking within defined limits. Although we often focus on bone's ability to break, a piece of bone will not shatter like a dish nor crumble like a piece of chalk, even though its inorganic phase is comparable to these two brittle materials.

Bone is a *composite ceramic* which includes both organic fibers which are comparable to steel cables in reinforced cement and an inorganic crystalline phase comparable to any heat-tempered ceramic [1,2]. Unlike high temperature ceramics which are fabricated at 800 to 1600°C, bones or teeth form at low temperatures (35 to 40°C), with other hard substances comparable to bone, such as sea shells, forming at even lower temperatures (10-15°C). As a complex composite ceramic composed of organic fibers and a crystalline phase, the unique physical and mechanical properties of bone are a direct result of the atomic and molecular interactions intrinsic to this unusual composite material.

The question and challenge can be raised which asks whether we can formulate a ceramic resembling bone which is made up of an organic-inorganic composite. It is clear that this must be accomplished at relatively low temperatures to ensure the retention of the integrity of the organic component.

The Rules

Bone is made up of an oriented matrix which is secreted by bone-forming cells: the osteoblasts [3,4]. This organic matrix is made up of structural molecules which serve as a scaffolding and which are laid down in a very precise, oriented pattern of fibrils into and onto which the inorganic crystalline phase forms [1,2]. The formation of the first crystals of inorganic salt of calcium phosphate is referred to as the initiation or nucleation site which appear at regular intervals along this complex organic scaffolding, collagen, laid down by osteoblasts. Once nucleation has occurred, the next major process involves the continuation of crystalline growth from the nucleation sites outward along the fabric of the organic matrix and eventually between the molecules which serve as scaffolding. As crystal growth continues and forms a dense, inorganic matrix, there is a loss of organic components which are designed to reserve space in this matrix for ever-expanding the inorganic phase [1,2,5,6].

The important landmarks, the organizational RULES, can be deduced and are as follows:

1. Oriented multicomponent organic matrix secreted by osteoblasts.
2. Structural molecules which serve as scaffolding and nucleation sites.
3. Initiation or nucleation of the inorganic crystalline phase.
4. The continuation of crystal growth with simultaneous rearrangement or elimination of components of the organic matrix.

Bone Formation

Bone is formed by a secretory cell referred to as an osteoblast [3,4]. Osteoblasts can be found as a cobblestone sheet of cells with no unique cell-cell contacts. However, the unique arrangement of cells is directly responsible for the fabric of bone; these secretory cells, the osteoblasts, secrete a discrete organic matrix from their face while keeping their back to the vascular or capillary network. The orientor of this secretory reaction is the vasculature while the unique secretory products formed by osteoblasts define the organic matrix of bone and directly control the formation of inorganic crystalline material by also facilitating the localization of both calcium and phosphate molecules [3,4].

The unique, differentiated secretory cell, the osteoblast, is derived in a sequence of individual steps starting with an undifferentiated progenitor cell or stem cell and culminating in the formation of a non-dividing secretory osteoblast [7,8,9]. This sequence of changing cellular events involved in the differentiation of secretory osteoblasts is referred to as a lineage. This lineage, depicted in Fig. 1, can be deduced by a variety of experimental means [7,8]. Importantly, this sequential set of cellular and molecular transitions which characterize each separate lineage stage are the same in embryonic and adult material. This implies that in adult, there must be osteogenic stem cells which are in near and convenient repositories and exist poised to form the secretory osteoblast.

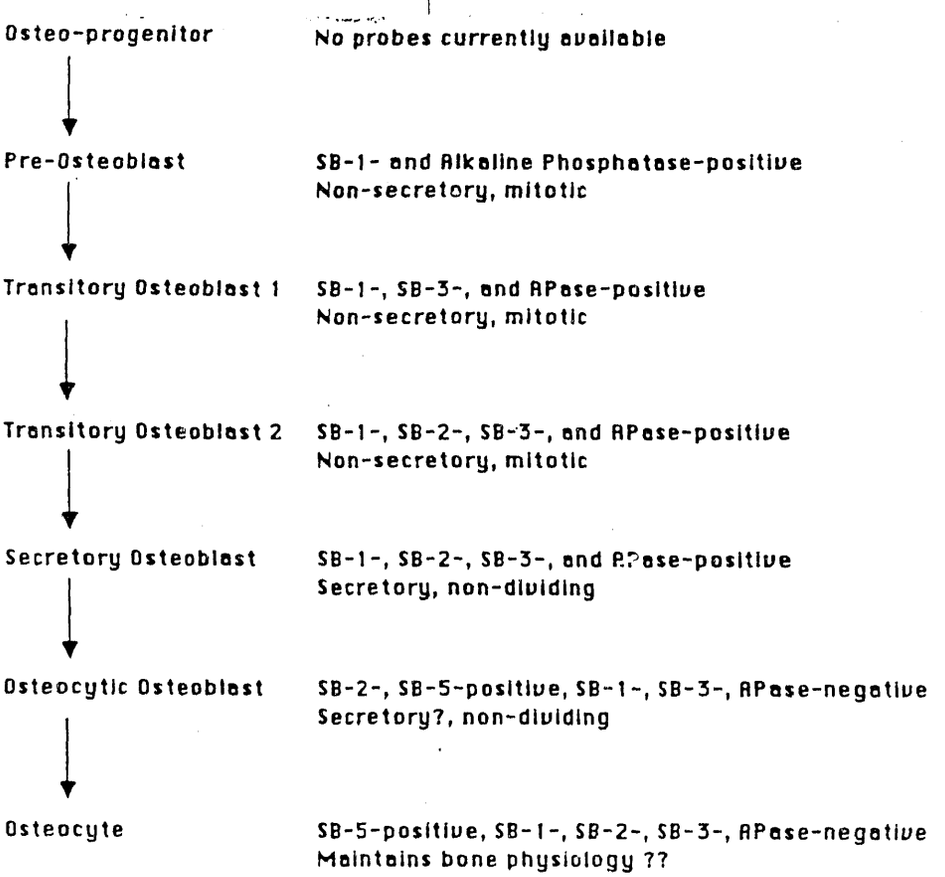

Figure 1: Lineage Map for Osteogenic Cells.
A series of proposed steps is pictured in the development and differentiation of bone-forming cells, *osteoblasts*, and cells involved in the maintenance of bone, *osteocytes*. The monoclonal antibodies SB1, 2, 3 and 5 which bind to discrete subsets of osteogenic cells are used to identify these lineage transitions [7,8,9]. It is clear from these studies that differentiated cells arise from a series of cellular transitions which are dependent on the local environment and the intrinsic capabilities of the cells.

Once the lineage-related steps in the differentiation of osteoblasts have been completed, the osteoblast ceases dividing and initiates bone formation by secreting a highly organized and oriented extracellular matrix. These osteoblasts, as aligned sheets of cells, produce oriented matrices which are then subsequently mineralized.

THE MOLECULES

A variety of unique molecules come together to form the organic phase of bone. This organic matrix is secreted by the differentiated osteoblast which eventually controls the mineralization of this matrix. The inorganic phase of bone is composed of calcium and phosphate which are brought to the nucleation and crystal growth site by unique carrier molecules or substrates which can be altered to generate and concentrate both calcium and phosphate ions. For example, many phosphates are chemically bonded to the hydroxyl of serine residues in a bone-specific protein or proteins [10,11]. The enzyme alkaline phosphatase can cleave these phosphates off the proteins to effect locally high concentrations of phosphate ions at sites of mineral deposition.

The organic phase is made up of a large number of unique molecules all of which are not yet completely known. The major molecules found in mineralizing organic phase of bone are as follows:

1. *Type I collagen* is a well characterized molecule which forms a large rigid triple-stranded rod of large cross-sectional diameter in bone [1,12]. These large collagen fibrils of bone have within them unique gap regions which are hypothesized to be the nucleation site for calcium phosphate crystal formation. As in the case of the cartilage type II collagen, smaller collagens, including type IX and XII, are associated with these fibrils and are thought to regulate the fibril size and the interactive properties of these fibrils with the extracellular matrix [13].

2. *Phosphoproteins* are a unique class of molecules which contain between 8 and 20 moles of phosphoserine per molecule [10,11]. These phosphoproteins are intimately associated with the initiation and continued crystal growth and have been hypothesized to serve a variety of functions. First, it has been shown that these bone phosphoproteins can bind calcium and may serve to orient calcium ions at the initiation of crystal growth. In addition, these phosphoproteins can serve as a source of inorganic phosphate which can be enzymically released by phosphatases and may directly add to the calcium phosphate crystal growth. Lastly, it may be that these phosphoproteins serve to inhibit mineral deposition and crystal growth and their removal may stimulate the formation of calcium phosphate salts associated with the mineral phase of bone.

3. *Proteoglycans* are complex organic molecules made up of a protein core to which large repeating disaccharide chains are bound [14,15]. These repeating disaccharide chains, referred to as glycosaminoglycans, are highly (negatively) charged and may serve to inhibit crystal growth by their high negative charge. Importantly, these proteoglycan molecules structure many times their mass of water and may serve to reserve the extracellular space for future calcium phosphate crystal growth. With the removal of the

proteoglycan from these domains, large spaces would then be filled with inorganic crystals which had been nucleated and expanding on collagen molecules.

4. *Alkaline phosphatase* is an enzyme which is always associated with mineralizing tissue [9,16]. The exact role of this enzyme which cleaves phosphate from organic donor molecules is not precisely known. It may be that alkaline phosphatase serves to liberate phosphate from organic donors at the site of crystal growth and thereby provide relatively high concentrations of phosphate at the site of mineral deposition.

The combination of these organic molecules is called the *matrix*. Such matrices which contain substantial amounts of these molecules may or may not mineralize. Clearly other relatively minor organic molecules are involved in the mineralization phenomena although simple model systems containing just collagen with reservoirs of calcium and phosphate are sufficient to form hydroxyapatite inclusion within the collagen matrix. Also teeth, shell, sea urchin spines, and other natural composite ceramics contain other predominant organic molecules [17]. The different chemistries and physical properties of these matrices serve to organize the mineral phases to produce hard tissues of different strengths and brittleness. The detailed structures and molecular anatomy of these materials are only now being elucidated. This information will allow a more detailed "rule book" to be written with regard to the control of biological mineralization.

THE 1990's

The future research objectives of this laboratory and others are designed to attempt to understand the details of process and key components of biological mineralization in order to attempt to mimic this complex phenomenon and to produce a composite organic ceramic with characteristics similar to bone but in the absence of living cells. Several approaches are now being explored which involve attempting to understand how to align isolated osteoblasts into sheets of cells which secrete sheets of mineralizable matrix. Alternately, natural matrices such as those found in turkey tendon or ligament are being used in attempts to concentrate inorganic molecules on or in these natural matrices to form bone-like substances [18]. In addition, artificial matrices which involve collagen gels are being used in attempt to understand the characteristics and features which will allow calcium phosphate to form in the organic matrix within a collagen gel [19]. And lastly, one can suggest that when the rules of the road are understood in greater detail, that "designer" matrices can be fabricated which both support and control mineral deposition to create an artificial composite ceramic with the physical characteristics of bone without the interface of living cells.

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