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Mineralization-driven bone tissue evolution follows from fluid-to-solid phase transformations in closed thermodynamic systems ☆

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HIGHLIGHTS

- Extracellular bone tissue mineralizes under closed thermodynamic conditions.
- Fibrillar and extrafibrillar masses are preserved during mineralization.
- Precipitation of mineral ions leads to volume shrinkage of mineralizing bone tissue.
- Neutron diffraction data provide experimental access to fibrillar volume shrinkage.

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ABSTRACT

The fundamental mechanisms that govern bone mineralization have been fairly well evidenced by means of experimental research. However, rules for the evolution of the volume and composition of the bone tissue compartments (such as the mineralized collagen fibrils and the extrafibrillar space in between) have not been provided yet. As an original contribution to this open question, we here test whether mineralizing bone tissue can be represented as a thermodynamically closed system, where crystals precipitate from an ionic solution, while the masses of the fibrillar and extrafibrillar bone tissue compartments are preserved. When translating, based on various experimental and theoretical findings, this mass conservation proposition into diffraction–mass density relations, the latter are remarkably well confirmed by independent experimental data from various sources. Resulting shrinkage and composition rules are deemed beneficial for further progress in bone materials science and biomedical engineering.

1. Introduction

Bone mineralization is a very complicated process where several microns-sized osteoblastic cells release, through budding from their membranes, so-called matrix vesicles (sized tens of nanometers) into the extracellular space (Anderson et al., 2005; Anderson and Reynolds, 1973; Wiesmann et al., 2004). These vesicles carry all molecular components for triggering a multistage process, binding mainly calcium to phosphate ions, which finally results in the precipitation of hydroxyapatite in the form of nanocrystals. The latter then penetrate the vesicles' membranes, and continue to grow into the ("extra-vesicle") extracellular fluid; finally, the originally single vesicle-related crystals fuse into larger crystal clusters of up to hundreds of nanometers size (Cuisinier, 1996). In addition, the osteoblastic cells excrete organic matrix called osteoid (Parfitt, 1983; Skinner and Jahren, 2007), which is composed of 300 nm long and 1 nm thick strand-type collagen molecules. These molecules self-assemble into higher organizational units called fibrils, with typical diameters of tens to hundreds of nanometers, and lengths reaching even millimeters, leaving some extrafibrillar space in between. Within the fibrils, 280 nm long overlap regions (with dense collagen packing), alternate with 40 nm long gap regions (with loose collagen packing), forming a staggered scheme discovered by Hodge and Petruska (1963). Collagen deposition and hydroxyapatite mineralization are separated in time and space (Parfitt, 1983), such that the organic matrix, some ten to twenty days after the deposition, starts to become mineralized. Transmission electron micrographs showed that mineral precipitation occurs both intrafibrillarly and extrafibrillarly, but that, as a rule, the majority of the mineral is found in the extrafibrillar space (Hellmich and Ulm, 2003; Lees and Prostak, 1988; Lees et al., 1994; Prostak and Lees, 1996; McNally et al., 2012; Alexander et al., 2012; Jantou et al., 2009; Jantou-Morris et al., 2010).

This short summary clearly shows that, up to now, bone mineralization has been mainly studied experimentally, aiming at the deciphering of fundamental mechanisms. Wishing to foster the current trend in biomaterial science to develop theoretical and

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Nomenclature

Variables

d	Neutron diffraction spacing
$\mathcal{D}$	Dessication degree
f	Volume fraction
m	Mass
$\mathcal{M}$	Mineralization degree
n	Amount
$\mathcal{R}$	Fluid-to-organic mass ratio
t	Time
V	Volume
ρ	Mass density

Prefix

Δ	Change of variable due to dehydration

Suffices

col	… of collagen
dry	… of dried tissue
ec	… of extracollagenous space
ef	… of extrafibrillar space
ef,fl	… of extrafibrillar fluid
ef,HA	… of extrafibrillar mineral
fib	… of fibrillar space
fib,fl	… of fibrillar fluid
fib,HA	… of fibrillar mineral
fl	… of fluid
HA	… of hydroxyapatite
max	Maximum of …
$tissue$	… of tissue (at the extracellular scale)
w	… of wet tissue

Superscripts

0	at the beginning of the mineralization process
∞	at the end of the mineralization process
dh	partially dehydrated
t	at time instant t, between the beginning and the end of the mineralization process
$\hat{\cdot}$	inside the fibrils
$\check{\cdot}$	in the extrafibrillar space
$\dot{a}$	temporal derivative of a

computational approaches to further pervade the matter, the present paper is concerned with finding mathematically formulated rules behind the aforementioned mineralization process within and outside the collagen fibrils. These rules will be strictly validated against a variety of physically and statistically independent experimental data collected from the rich literature on the topic. While the systems biology of bone, or the hierarchical microstructure of mineralized bone and its emerging mechanical properties have been studied quite successfully by theoretical and computational approaches (Lemaire et al., 2004; Qin and Swain, 2004; Fritsch and Hellmich, 2007; Hellmich et al., 2004; Pivonka et al., 2008), the *evolution* of the fibrillar collagen–mineral nanocomposite in the course of biomineralization has, to the best knowledge of the authors, at mostly faintly been addressed by means of mathematical modeling. As kind of première-type activity, we will test, throughout the remainder of this paper, the following proposition: bone mineralization is a closed thermodynamic process, both at the tissue level and at the fibrillar level, which is expressed by mass density increase and volume reduction.

2. Methods

2.1. Mass conservation during mineralization

We wish to check, whether the structural evolution of bone tissue during mineralization can be explained by means of fluid–solid phase transitions in two thermodynamically closed systems. Therefore, we consider a piece of extracellular bone tissue (at a scale of some tens of micrometers) with properties averaged over a classical bone sample measuring one to a few millimeters, as accessible through standard experimental protocols (Biltz and Pellegrino, 1969; Gong et al., 1964; Lees et al., 1979; Vuong and Hellmich, 2011). As reviewed in the introduction, such tissue is laid down (at time "0") in the form of osteoid, consisting of collagen molecules with mass m^0_{col}, and of a ionic fluid with mass m^0_{fl}. Hence, the overall tissue mass reads as:

$$m^0_{tissue} = m^0_{col} + m^0_{fl} \tag{1}$$

At a higher organizational level (see Fig. 1), the collagen molecules build up fibrils with mass m^0_{fib}, consisting of molecular collagen and intercollagenous fluid (with mass $m^0_{fib,fl}$), so that

$$m^0_{fib} = m^0_{col} + m^0_{fib,fl} \tag{2}$$

The rest of the osteoid tissue consists of fluid-filled extrafibrillar space with mass $m^0_{ef} = m^0_{ef,fl}$. Hence, the initial tissue mass can be alternatively written as

$$\begin{aligned} m^0_{tissue} &= m^0_{fib} + m^0_{ef} \\ &= m^0_{col} + m^0_{fib,fl} + m^0_{ef,fl} \end{aligned} \tag{3}$$

Thereafter, the ions swimming in the fluid start to form hydroxyapatite minerals (referred to by suffix "HA" in the sequel).

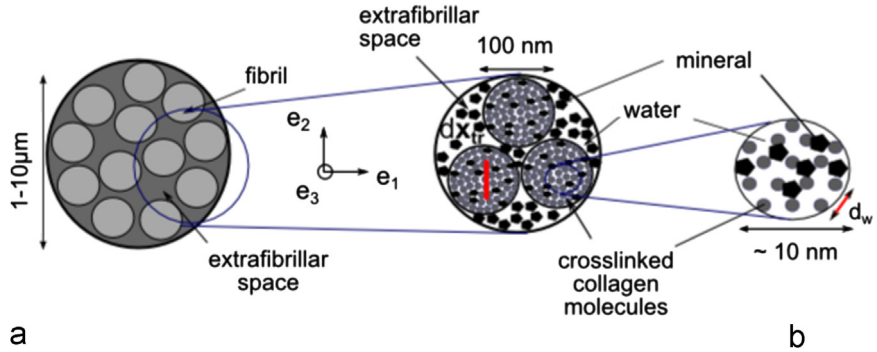

Fig. 1. Scheme concerning hierarchical structure of mineralizing bone tissue: (a) bone tissue and (b) fibrillar space.