Deriving Ash Fraction from microCT Bone Scans

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INTRODUCTION:

Ash fraction (A_f) is a commonly calculated gravimetric parameter and is defined as ashed mass of a bone sample divided by the dry mass of the same sample. Ash fraction, sometimes referred to as percent mineralization, is considered the gold standard for determining the amount of mineralization in a bone sample and has been used to estimate bone material properties such as elastic modulus [6,10]. Unfortunately, calculation of ash fraction requires destructively ashing the specimen to remove all non-mineral constituents to quantify the mass of the mineral (i.e. ash). A non-destructive process involving micro-computed tomography (microCT) has been recently used to quantify the mineral density distributions (defined as the degree of mineralization of bone, DMB) from specimens in-vivo. Drum et al. [1] showed high correlations (Pearson's correlation coefficient = 0.93) between the mean quantitative CT density (in units of mg/ml tri-calcium phosphate) measurements and the destructively derived percent mineralization for five third metacarpal bones taken from two racing and three non-racing horses. Although microCT derived DMB values have been correlated with values of ash fraction, there is no theoretical formulation for directly comparing the two metrics. The purpose of this study is to propose a theoretical derivation for determining voxel-specific ash fraction from microCT scan data and bone constituent properties. The effects of selected parameters used in the derivation of ash fraction are evaluated.

METHODS:

Using a manufacturer-specific calibration curve, microCT derived voxel-based linear attenuation values can be converted to an equivalent hydroxyapatite density (i.e. gHA/cm^3). This mineral density, in conjunction with knowledge about the underlying constituents of bone, can be used to directly calculate a voxel-specific ash fraction. It is assumed here that bone tissue is comprised of ash, organics, and water [9] and that the volume fraction of the organics constituent is constant [8,11]. Table 1 summarizes the symbols used in this section.

***Table 1**. Symbols used in derivation*

Symbol	Units	Definition
m_a	grams	Mass of the ash constituent
m_o	grams	Mass of the organics constituent
R_o	unitless	Volume fraction of the organics constituent
ρ_{HA}	gHA/cm^3	MicroCT derived mineral density
ρ_o	g/cm^3	Organics constituent density
V_t	cm^3	Volume of a single microCT voxel
V_o	cm^3	Volume of the organics constituent
A_f	unitless	Ash fraction

Using the following relationships of:

$$m_a = \rho_{HA} \cdot V_t ,$$

$$m_o = \rho_o \cdot V_o = \rho_o \cdot R_o \cdot V_t ,$$

and knowledge that dry mass of a bone sample is the sum of the organics and ash mass consitiuents, ash fraction (A_f) can be defined as:

$$A_f = m_a/(m_a + m_o) = \rho_{HA}/(\rho_{HA} + \rho_o \cdot R_o) . \quad \text{Eq. 1}$$

The voxel-based ash fraction in Equation 1 is a function of ρ_{HA}, ρ_o, and R_o. The microCT mineral density (ρ_{HA}) is the output metric from the microCT scanner. The density of the organics constituent (ρ_o) and the volume fraction of the organics constituent (R_o) are the remaining parameters that influence the derived ash fraction. Baseline model parameters of $\rho_o = 1.4$ and $R_o = 0.36$ are defined from Martin [1]. The effect on ash fraction from varying R_o between 0.3 and 0.4 for two values of ρ_o of 1.1 [2] and 1.4 g/cm^3 [8] are also evaluated.

RESULTS:

Ash fraction for the baseline and varied parameter models are depicted in Figure 1. At a mineral density of 1.2 gHA/cm^3, the baseline model yields an ash fraction of 0.70, slightly larger than common values that have been previously reported of 0.65 +/- 0.03 [9] and 0.67 [11] for fully mineralized bone. The nonlinear ash fraction versus mineral density relationship is governed by the assumed mass of the organic constituent (appearing in the denominator of Eq. 1), with larger values of the organic constituent mass corresponding to smaller magnitudes of ash fraction for the same microCT HA density. For the mineral density of 1.2 gHA/cm^3, the predicted ash fraction for all the models ranged from 0.68 to 0.78. The predicted ash fraction at 0.4 gHA/cm^3 ranged from 0.42 to 0.55, with the baseline model resulting in a value of 0.44.

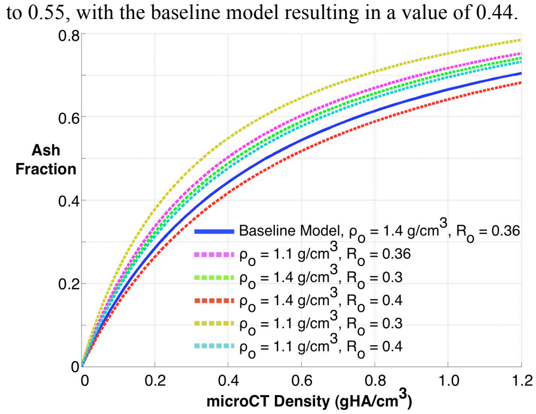

***Figure 1**. Ash fraction as a function microCT HA density.*

DISCUSSION:

The purpose of this study was to present a theoretical relationship relating microCT voxel-based mineral density to ash fraction and quantify the effect of assumed parameter values. The microCT derived ash fraction presented here is similar to previous values used in the literature. In developing an equation relating dry tissue density and ash fraction, Hernandez et al. [1] assumed fully mineralized bone had an ash fraction of 0.7. The baseline model used here yielded the same ash fraction for a mineral density of 1.2 gHA/cm^3, which has been previously used to represent fully mineralized bone [7]. Deviations in the organics constituent parameters propagated to smaller percentage changes of the predicted ash fraction. Assuming a unit voxel, a 70% increase in the contribution of organic mass, observed when comparing two parameter sets ($\rho_o = 1.1$, $R_o = 0.3$; $m_o = 0.33$ versus $\rho_o = 1.4$, $R_o = 0.4$; $m_o = 0.56$), resulted in a 15% change in ash fraction for bone tissue with a measured mineral density of 1.2 gHA/cm^3.

The variation of ash fraction (Figure 1) due to the selected model parameter deviations assumed from observed ranges in the literature may be a conservative estimate on the overall model sensitivity. The lower range of the organics constituent density (ρ_o of 1.1 g/cm^3) was from a single source [2] and was significantly lower than all other reported organic constituent densities, which were within 5% of 1.4 g/cm^3 [1,4,5,8]. If the organics constituent density is assumed to be 1.4 g/cm^3, the variation of the organics volume fraction (0.3 to 0.4) for a mineral density of 1.2 gHA/cm^3 results in less than a 6% deviation in ash fraction from the baseline model. An experimental study is currently being conducted to validate the efficacy of the theoretical formulation and the inherent assumption that microCT imaging can accurately quantify the mineral content for reliable prediction of ash fraction using the procedure described here.

SIGNIFICANCE:

Ash fraction has been the gold standard metric for determining tissue mineralization and is the independent measure used in many derived relationships (e.g., bone stiffness). The proposed derivation allows for the calculation of a comparative voxel-by-voxel ash fraction utilizing non-destructive microCT scan data.

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