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# Micromechanical modeling of calcifying human costal cartilage using the generalized method of cells

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ABSTRACT

Various tissues in the human body, including cartilage, are known to calcify with aging. There currently is no material model that accounts for the calcification in the costal cartilage, which could affect the overall structural response of the rib cage, and thus change the mechanisms and resistance to injury. The goal of this study is to investigate, through the development of a calcifying cartilage model, whether the calcification morphologies present in the costal cartilage change its effective material properties. A calcified cartilage material model was developed using the morphologies of calcifications obtained from microCT and the relaxed elastic modulus of the human costal cartilage obtained from indentation testing. The homogenized model of calcifying cartilage found that calcifications alter the effective material behavior of the cartilage, and this effect is highly dependent on the microstructural connectivity of the calcification. Calcifications which are not contiguous with the rib bone and constitute 0–18% of the cartilage volume increase the effective elastic modulus from its baseline value of 5 MPa to up to 8 MPa. Calcifications which are attached to the rib bone, which typically constitute 18–25% of the cartilage volume, result in effective moduli of 20–66 MPa, depending on the microstructure, and introduce marked anisotropy into the material. The calcifying cartilage model developed in this study can be incorporated into biomechanical models of the aging thorax to better understand how calcifications in the aging thorax affect the structural response of the rib cage.



## 1. Introduction

Throughout a lifetime, soft tissues of the human body exhibit forms of calcification or ossification. Skeletal development is the major period of endochondral ossification, where a cartilage model is turned into bone [1–3]. Other biological soft tissues can calcify and in certain cases this can result in pathological conditions. Calcification of biological tissues can alter their material properties thus their physiological behavior. Regions of the human body that are known to calcify include: vertebral disks, ligaments, aorta, heart valves, articular cartilage, and costal cartilage [4–10].

The degree of calcification in the costal cartilage is known to increase with age [11–13]. The costal cartilages link the sternum to the ribs and thus serve an important structural role within the thoracic cage during respiration [14]. Calcifications of the costal cartilage, along with other physiological thoracic changes associated with aging (e.g. osteoporosis of the ribs), also alter the mechanical response of the thorax under external loading such as restraining forces during an automotive crash [15]. Older drivers are more prone to sustain chest injuries in automotive crashes [16] and are more likely to die from chest injuries [17]. Rib cage mechanics likely play a role in both of these negative outcomes. Because the aging thorax is also a calcifying thorax, it is important to know the material properties of the calcifying costal cartilage, which in turn, allows for a better understanding of the aging thorax during respiration, under external loading, and following injury.

The material properties of the costal cartilage have been studied at the micrometer [18] and nanometer [19] length scales, and at the whole-structure level during calcification up to 24% volume fraction [20,21]. However, no studies have quantified how the specific morphologies and connectivity of calcifications [10] alter the effective modulus of the costal cartilage at the millimeter length scale—the scale at which typical finite elements of the entire thorax are developed.

Micromechanical analysis, in particular, the method of cells, was developed in the composites field to predict the effective behavior of a multi-phased material when the material properties

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of the individual constituents, their volume fractions, and the between-phase surface interactions are known [22]. In its most basic form, the method of cells models a doubly periodic composite with fiber and matrix constituents. The analysis consists of a representative volume element (RVE) containing 4 subcells—one composed of fiber and three composed of matrix. By imposing continuity of displacements and tractions at the subcell interfaces, along with the constitutive relations, the effective material properties of the particular composite can be estimated. That is, the method of cells can be used to calculate the equivalent material properties of the composite were it to be composed of a single homogeneous, isotropic material. The method of cells was then generalized for an RVE with an arbitrary configuration of inclusions and number of subcells and became known as the generalized method of cells [23].

The generalized method of cells (GMC) has been validated against theoretical elastic solutions and experimental testing of multi-phase composites from previous studies. For uni-directional fiber composites, the GMC method has been found to have good agreement with the elasticity solutions and measure properties of glass/epoxy [24,25] and carbon/epoxy composites [26,27]. The three dimensional formulation for GMC has been validated against measured experimental moduli for short-fiber composites of steel/epoxy, showing fair agreement [28]. In addition, GMC was found to accurately predict the elastic behavior of short fiber composites of metal matrix materials [29]. With these predictive capabilities, GMC has been extensively utilized and continually developed in the composites field [30], and have also been applied for analysis of biological tissues [31].

The goal of this study is to investigate how representative volume fractions and morphologies of calcifications in the costal cartilage alter the effective material properties at this scale. The calcifying costal cartilage is modeled as a two-phase composite (cartilage substrate with calcification inclusions) and micromechanical homogenization analysis using the generalized method of cells is used to estimate effective material properties.

## 2. Materials and methods

### 2.1. Morphology and material property measurements

In order to perform micromechanical modeling, both the arrangement and the material properties of the constituents must be known. MicroCT image data were taken from a repository of microCT scans of the human costal cartilage. Of the 8 cadaveric subjects used in this study, 6 of these came from subject microCT previously analyzed in Lau et al's study [10] and 2 of the subjects were added to the repository afterward. MicroCT scans of human costal cartilage segments were performed with a Scanco vivaCT40 (Scanco Medical, Brüttisellen, Switzerland) scanner to obtain the microstructural composition of the calcifying costal cartilage. The in-plane and slice resolution was 30 μm. The scanner settings were 45 kVP and 177 μA and calibrated with the Scanco phantom. All cadaveric tissue used in this study was obtained and tested in accordance with ethical guidelines established by the Human Usage Review Panel of the National Highway Traffic Safety Administration, and with the approval of the Office of the Vice President for Research and an independent Oversight Committee at the University of Virginia Center for Applied Biomechanics. Cadaveric tissues were fresh frozen and were thawed and hydrated with physiological saline prior to experimentation.

In the micromechanical model, the cartilage phase was modeled as linear elastic, with an elastic modulus $E = 5$ MPa and Poisson's ratio $\nu = 0.499$. The elastic modulus of the cartilage phase was obtained through spherical indentation testing of the cartilage substrate regions using similar methods described in Lau et al's study. [18]; due to the typically-slow loading rates in vivo, the relaxed elastic modulus was used.

Due to the small size of the calcifications, it was difficult to obtain reliable material properties with indentation testing. Instead, the calcification phase was assumed to have material properties similar to those of rib bone because the microCT analysis indicated that they exhibited similar mineral densities of ~650 mg HA/cm$^3$ [10]. The calcification phase was modeled as linear elastic, with an elastic modulus $E = 13.9$ GPa [32] and Poisson's ratio $\nu = 0.35$.

### 2.2. Micromechanical modeling

Many codes for this micromechanical analysis have been incorporated into the software package *Micromechanics Analysis Code with Generalized Method of Cells (MAC/GMC) 4.0*, which was created by the U.S. National Aeronautics and Space Administration (NASA) [33–35]. Permission was obtained from NASA to use this software for modeling calcifying cartilage in this study.

The "User-Defined Triply Periodic Generalized Method of Cells Architecture Code" from the NASA MAC/GMC software was used to obtain the homogenized material properties of calcifying cartilage. This form of the code allows for an arbitrary subcell microstructure and arbitrary constituent material properties. In this study, the calcifying cartilage is modeled as a two-phase composite material consisting of a hard calcification phase embedded in a soft cartilage matrix, and the GMC is used to predict the effective material properties of this composite.

MicroCT image data were used to directly generate volumetric geometries at the native voxel size of the scan. Custom MATLAB (the Mathworks, Natick, MA) code was used to locate and isolate a cubic representative volume element (RVE) of interest for modeling. The calcifications were isolated from the cartilage substrate using a threshold of any voxel density greater than 285 mg HA/cm$^3$ [10] and converted into an input file for the MAC/GMC software. Larger RVEs were down-sampled using a linear bicubic interpolation in order to meet the 32-bit memory limitations of the MAC/GMC software.

A total of 16 different RVE's were extracted from calcified cartilage and analyzed for this study. The volume fraction of calcification (relative to the surrounding cartilage matrix) ranged from baseline (0% calcification) to just over 20% calcification, based on previous observations [18]. In addition, a single RVE was taken from a region of plate-like shell calcification that had a volume fraction of over 50%. These RVE's were chosen to be on the order of a 1–5 mm cube, which is the typical length scale used in larger FE models. Consistent with previous research [10], both lattice-type calcifications—in which the calcification nodes form a porous bone scaffolding—and dense nodal-type calcification—in which the calcification constitutes an amorphous porous region—were modeled. A description of these RVE's is outlined in Table 1 and examples are shown for a lattice type (Fig. 1) and dense nodal type (Fig. 2) calcification. Images for all of these models can be found in the online supplement.

Homogenization was performed using the MAC/GMC 4.0 software running on a Windows 7 64-bit workstation (Intel Core2Quad, 2.5 Ghz, 8 GB Ram). Typical runtime for the $50 \times 50 \times 50$ voxel models was 25–30 min. The homogenization algorithm outputs effective elastic moduli in 3 orthogonal directions.

### 2.3. Sensitivity study-calcification elastic modulus

While the mineral densities of the calcified regions were, on average, comparable to those of rib bone, the microCT analysis indicated localized regions of calcifications with mineral densities