Morphology, distribution, mineral density and volume fraction of human calcified costal cartilage

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Abstract

This study examines the properties of calcifying human costal cartilage and adjacent rib bone using qualitative and quantitative micro-computed tomography analysis. Calcifications are categorized with respect to location, microstructure, shape, and contiguity using a novel classification scheme and quantified in terms of mineral density, volume fraction, and length of infiltration from the costo-chondral junction (CCJ). Calcifications were present throughout the cartilage by location and ranged from small diffuse calcifications to nodes, rods, plates, and even large complex structures that exhibited a microstructural morphology similar to a cross-section of diaphyseal bone, with a dense shell surrounding a trabecular core. Solid microstructure was most common for calcifications (44.5%), and the morphologies were found to vary with location, with rods and plates being most prevalent at the periphery (91.7% of all rods, 98.4% of all plates). The average mineral density of the calcifications over all locations and morphologies was 658.8 ± 86.36, compared with 662.7 ± 50.37 mgHA cm⁻³ for the adjacent rib bone. The calcification volume fraction (6.54 ± 4.71%) was less than the volume fraction of rib bone (21.62 ± 6.44%).

1. Introduction

The hyaline costal cartilage is a flexible linkage connecting the bony ribs to the sternum. It is known to calcify (also referred to as ossify) in humans [1–3]. The presence of this calcification has been correlated with aging [4–6], and has been described in the forensics literature in limited detail using chest radiographs [7,8]. The literature is lacking a detailed description of the microstructure and composition of these calcifications. Such a description is needed because calcification of the costal cartilage has consequences for thoracic biomechanics under physiological [9–11] and external (e.g. traumatic) loading [12,13]. A detailed understanding of the morphology, distribution, and mineral density of calcified costal cartilage may increase the sensitivity of forensic analyses, enhance modeling of load distribution, stress, and strain within the rib cage under mechanical loading, and provide an insight into the mechanisms of calcification. The objective of this study was to characterize quantitative and qualitative characteristics of calcified costal cartilage and adjacent rib bone on a length scale of approximately 30 μm using micro-computed tomography (microCT) of human tissue spanning a wide range of adult ages.

2. Methods

2.1. Specimen selection, preparation, and imaging

All cadaveric tissue used in this study was obtained and tested in accordance with the 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. Clinical thoracic CT scans (nominal in-plane resolutions of 1 mm) from a convenience sample of available cadaveric tissue in our laboratory were reviewed and 11 donors (age range 23–79 years) were identified that exhibited gross calcification levels. Donors over a wide age range were selected so that a limited assessment of age trends could be made, including a 23-year-old male who served as a baseline “non-calcified” donor, as the CT scan showed no apparent calcification. Specimens (5–7 cm long) that exhibited some calcification from the CT scans were selected and harvested and include 1–2 cm of the adjacent rib bone along with the costal cartilage. Most specimens were...
taken from rib levels 2–6, as the costo-chondral regions for these ribs were relatively straight and could easily fit into the scanner bore. During extraction, specimens found to be damaged (rib or cartilage fracture) near the costo-chondral junction (CCJ) were excluded. Donor information and the test matrix of specimens used for this study are found in Table 1. Specimens were scanned in air using a Scanco vivaCT40 microCT scanner (Scanco Medical, Brüttisellen, Switzerland) at 30 μm isotropic resolution (45 kVp, 110 μA) using Scanco’s 45 kVp phantom calibration setting. Image data were exported as DICOMs for analysis.

Three-dimensional reconstructions of every specimen were assembled using Voxar 3D (Toshiba Medical Visualization Systems, Edinburgh, UK). The reference location of the CCJ was defined as the most lateral point of the concave transition from costal cartilage to rib bone, and was unique for each specimen (Fig. 1). Hard tissue lateral of the CCJ was defined as rib bone and hard tissue medial of the CCJ was defined as calcification. Note that under this scheme the small amount of bone contiguous with the rib bone, which forms the cup of the CCJ, was defined as calcification. This cup is on the order of 5 mm deep, so calcification observations in that region are confounded by the presence of rib bone around the periphery of the specimen.

2.2. Classification scheme

Qualitative observations of the microstructure in the calcification regions motivated the development of a classification scheme describing patterns in terms of location, microstructure, shape, and contiguity to the CCJ. Each individual contiguous piece of calcification present was classified and counted as one occurrence.

The location classifications were the periphery of the cartilage on the superior (Sup), inferior (Inf), anterior (Ant), or posterior (Post) aspects, or interior (Int, defined as more than 1 mm from the periphery). The location was determined from the centroid of the calcification. When the contiguous calcification extended around multiple sides of the periphery it was termed a Shell.

The microstructure was classified as solid (S, not exhibiting organized structure), lattice (L, containing organized microstructure), or mixed (M, a combination of solid and lattice regions). Calcifications with distinct solid and lattice regions that were contiguous at any point were classified as mixed, and included calcifications with a well-defined outer surface (solid-like) and a lattice inner structure. Examples of calcifications classified with the different microstructures and shapes are shown in Figs. 2–4.

The shapes of calcified regions were classified as rods (R), plates (P), nodes (N), complex (C), or clustered (CL). Rods had a major dimension at least three times both of the minor dimensions (Fig. 2). Plates had two dimensions at least three times the third dimension (i.e. the thickness) (Fig. 3). Nodes were small calcifications that did not have a particular major dimension and had no dimensions greater than 5 mm (Fig. 2). Complex calcifications were any calcifications that did not fit the prior classifications (highly irregular in shape) and were relatively large, with a dimension greater than 5.0 mm. Clustered calcifications (CL) were the presence of five or more small calcifications (less than 1.0 mm) contained within a 10 mm diameter sphere (Fig. 2). Calcifications were classified by contiguity as either attached to the CCJ (A) or floating in the cartilage (F).

The presence of diffuse calcification, defined as the presence of 10 or more small calcifications (less than 1.0 mm) scattered throughout the specimen, were classified on a per-specimen basis. If at least 10 small calcifications were concentrated throughout the periphery, the specimen was classified as diffuse-peripheral (D-P). If 10 or more small calcifications were concentrated in the interior, it was classified as diffuse-interior (D-I). If 10 or more small calcifications were present throughout the entire specimen, but without having the required 10 concentrated in either location (peripheral or interior), it was classified as diffuse (D). A specimen

<table>
<thead>
<tr>
<th>Specimen matrix: 54 CCJ samples taken from 11 specimens spanning rib levels 1–9.</th>
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<tbody>
<tr>
<td>Sex</td>
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<tr>
<td>Age</td>
</tr>
<tr>
<td>Rib 1</td>
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<td>Rib 2</td>
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<td>Rib 3</td>
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<td>Rib 4</td>
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<td>Rib 8</td>
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<td>Rib 9</td>
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Fig. 1. Transverse section of costo-chondral junction showing the rib bone, costal cartilage, and CCJ cup. Scale bar 5.0 mm.
meeting the criteria for both D-P and D-I received both classifications.

Excluding the diffuse cases, a four term code was developed for each region of calcification, where the first term defines the location, the second the microstructure, the third the shape, and the fourth the contiguity. So, for example, an interior, solid, node, floating within the cartilage would be described by the code Int.S.N.F. The codes were then ranked by frequency as a quantitative descriptor of the nature of the most frequently observed calcifications.

2.3. Quantitative microCT analysis

Quantitative analysis was performed on cross-sectional image slices of each specimen to calculate the areas/volumes of cartilage and hard tissue, the volume fractions, and the mineral density of thresholded hard tissues. This analysis provided these local measurements for each cross-section along the length of the specimen, which were separated into those regions lateral (rib bone) and medial (cartilage calcification) to the CCJ (example in Fig. 8). Unless otherwise stated, measurement values presented are over the entire volume of the respective region (rib bone or calcification). Local measurements refer to those calculated for a particular cross-section along the length of the specimen (i.e. local calcification mineral density).

The specimen local cross-sectional area was defined for each slice by summing the area of all pixels having a density above 0.95 mgHA cm$^{-3}$. Hard tissues (bone and calcification) were distinguished from cartilage by thresholding at 285 mgHA cm$^{-3}$. With no existing literature threshold value for calcifying costal cartilage,
this value was determined based on visual inspection by reducing the threshold to the point above which cartilaginous tissues were beginning to be captured. The local mineralized volume fraction was defined as the local cross-sectional area of the hard tissues divided by the local cross-sectional area of the specimen and was used to calculate the average volume fraction for the rib and calcification regions.

The local mineral density was calculated by averaging the mineral density values of the thresholded hard tissues pixels within each slice. The rib bone and calcification mineral density for each specimen were taken as the average over all the thresholded pixels within their respective region (medial or lateral of the CCJ). A “mineralization ratio” was defined for each specimen as the ratio of the calcification mineral density to the mineral density of the adjacent rib bone. The local maximum/minimum mineral densities in the rib bone and calcification were found within their respective regions for each specimen. Calcification infiltration was defined as the distance from the CCJ to the medial extremity of the longest contiguous calcification extending from the CCJ into the cartilage for a given specimen.

3. Results

3.1. Observed classes of calcification

The distributions of the calcifications found in the costal cartilage from all specimens, excluding the diffuse cases, are reported in Fig. 5, and totaled 362 occurrences. The three occurrences of Shell, making up 0.83% of all calcifications, were not included in this figure and their characteristics resulted in them all being coded as Shell.M.C.A.

The five most common forms of calcification were Int.L.N.F. (6.91%), Sup.S.N.F. (4.97%), Ant.S.N.F. (4.70%), Sup.S.R.F. (4.14%) and Int.S.N.F. (3.87%). Calcifications were more likely to be floating (83.7%) than attached (16.3%). The most common microstructure observed was solid (44.5%), followed by mixed (35.64%), and lattice (19.9%). Many of the mixed calcifications were observed to have the solid region on the peripheral face and the lattice region on the interior face. Nodes were the most common calcification shape (38.67%) and were distributed throughout the costal cartilage, with 32.8% of all nodes located in the interior. Rods (16.6%) were most commonly found at the periphery (91.7% of all rods), with 45% of all rods being located in a superior location. Plates (16.9%) were all located at the periphery (98.4% of all plates), with the exception of a single plate located in the interior, shown in Fig. 3. Most of the peripheral plates were found in an inferior location (42.5% of all plates). The complex shape made up 19.6%, with the majority being located in the interior (40% of all complex). Clustered calcifications were the least common (8.3%).

The presence of diffuse calcification occurred in 83.3% of all specimens. D-P calcifications were the most common (81.48%). D-I calcification occurred in 14.81% of specimens and D calcifications were present in only one specimen (1.85%). D-I calcifications did not occur alone in any specimens and were only present in specimens that also contained D-P calcifications.

Certain patterns of calcification morphology were observed to be similar to diaphysial bone, containing a solid outer core and trabecular-like inner structure. These were present in four specimens from the 61-year-old female donor (Fig. 3) and were found in the interior of the cartilage. These large calcifications generally made up a significant volume fraction in the costal cartilage. Three were classified as Int.M.C.A, and one as Int.M.P.A. No other donors from this study exhibited such a large, organized level of calcification.

3.2. Quantitative microCT analysis

The results from the quantitative analysis are summarized in Table 2. The mean rib mineral density (661.9 mgHA cm$^{-3}$) was not significantly different from that of the calcification.
The mineralization ratio had a mean of 1.001, indicating that on average, for a particular specimen, the costal cartilage calcification and the adjacent rib bone exhibited similar mineral densities (Fig. 6). While the mineralization ratio exhibited an increasing trend with age ($P < 0.001$, $r^2 = 0.281$), the mineral density of the calcification was not significantly correlated with that of the adjacent rib bone ($P = 0.422$).

Localized regions along the length of the specimen showed differences in mineral density between the rib and calcification. The mean maximum local rib mineral density was 704.1 mgHA cm$^{-3}$, compared with a mean maximum local calcification density of 902.4 mgHA cm$^{-3}$. The local mineral density of calcification exhibited a maximum of 1209.8 mgHA cm$^{-3}$, greater than in the rib, where the highest local mineral density was 814.2 mgHA cm$^{-3}$ (Fig. 7). A quantitative description of a high density calcification is shown in Fig. 8 and cross-sections of a dense calcification are shown in Fig. 4. The minimum local mineral density of a calcification (287.2 mgHA cm$^{-3}$) was biased by the threshold value, but was still different from the minimum local mineral density observed in the rib (700.0 mgHA cm$^{-3}$).

The mean volume fraction of the rib bone (21.62% VF) was higher than that of the calcification (6.54% VF) and had a significant decreasing trend with increasing age ($P < 0.05$, $r^2 = 0.116$). The calcification volume fraction was not correlated with the rib volume fraction ($P = 0.093$, $r^2 = 0.060$) nor the rib mineral density ($P = 0.214$, $r^2 = 0.024$). Large calcifications, like that shown in

![Fig. 5.](image-url) Distribution of percentage occurrence for the different types of calcifications exhibited in the costal cartilage.

### Table 2
Summary of the results of the quantitative analysis.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Skew</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rib mineral density (mgHA cm$^{-3}$)</td>
<td>661.9 ± 50.04</td>
<td>659.4</td>
<td>-0.245</td>
<td>746.0</td>
<td>554.9</td>
</tr>
<tr>
<td>Calcification mineral density (mgHA cm$^{-3}$)</td>
<td>660.9 ± 88.02</td>
<td>678.9</td>
<td>-0.858</td>
<td>825.6</td>
<td>408.7</td>
</tr>
<tr>
<td>Mineralization ratio</td>
<td>1.001 ± 0.169</td>
<td>1.006</td>
<td>0.273</td>
<td>1.402</td>
<td>0.633</td>
</tr>
<tr>
<td>Maximum local rib mineral density (mgHA cm$^{-3}$)</td>
<td>704.1 ± 63.27</td>
<td>720.6</td>
<td>-0.495</td>
<td>814.2</td>
<td>565.9</td>
</tr>
<tr>
<td>Minimum local rib mineral density (mgHA cm$^{-3}$)</td>
<td>615.2 ± 47.14</td>
<td>612.0</td>
<td>0.618</td>
<td>734.6</td>
<td>524.6</td>
</tr>
<tr>
<td>Maximum local calcification mineral density (mgHA cm$^{-3}$)</td>
<td>902.4 ± 121.6</td>
<td>876.6</td>
<td>0.662</td>
<td>1209.8</td>
<td>700.0</td>
</tr>
<tr>
<td>Minimum local calcification mineral density (mgHA cm$^{-3}$)</td>
<td>287.2 ± 126.25</td>
<td>290.3</td>
<td>1.349</td>
<td>638.6</td>
<td>287.2</td>
</tr>
<tr>
<td>Rib volume fraction (% VF)</td>
<td>21.62 ± 6.44</td>
<td>21.45</td>
<td>0.216</td>
<td>34.4</td>
<td>10.39</td>
</tr>
<tr>
<td>Calcification volume fraction (% VF)</td>
<td>6.54 ± 4.71</td>
<td>4.60</td>
<td>1.554</td>
<td>20.19</td>
<td>2.02</td>
</tr>
<tr>
<td>Calcification infiltration (mm)</td>
<td>18.94 ± 11.59</td>
<td>15.36</td>
<td>0.929</td>
<td>47.40</td>
<td>6.18</td>
</tr>
</tbody>
</table>
Fig. 3, exhibited comparable mineralized volume fractions to rib bone, with the maximum average volume fraction of the calcification in a specimen being 20.19% VF (Fig. 9). The local volume fraction and local cross-sectional volume of calcification as a function of distance from the CCJ are shown in Fig. 10. Moving medially towards the CCJ from the rib bone, the local volume fraction decreases while the amount of mineralized rib bone remains fairly constant.

The mean calcification infiltration from the CCJ was 19.21 ± 11.65 mm (Fig. 11). Note that in cases where there were minimal levels of calcification present around the CCJ, the only tissue contributing to this length is the peripheral hard tissue, contiguous with the rib bone, that forms the CCJ cup.

4. Discussion

This study reports high resolution images of adult calcified costal cartilage and quantitative analysis of these structures at the microstructural level. There has been only one other quantitative microstructural study of the costal–chondral junction, which investigated the developing chondral growth plate in infants [14]. A classification scheme is proposed to describe the location, microstructure, shape, and contiguity of the observed calcifications. One key qualitative finding of this study is a structured morphology in many of the calcifications that resembles the cross-section of diaphysial long bones, with a thick, dense shell and a core of struts and other structures similar in appearance to trabeculae (Fig. 3). These formations within the costal cartilage suggests the potential for costal cartilage to be used as autologous bone scaffolds [15,16], as the costal cartilage is considered expendable and has been extensively used in nasal and aural reconstruction surgery [17,18].

The purpose of this study was to document the nature of the calcifications, not to determine their mechanisms of formation. Costal cartilage calcification thus remains idiopathic, [19,20] but certain morphologies observed in this study suggest plausible mechanisms. The prevalence of plates and rods at the periphery may suggest intramembranous ossification, while the lattice and bone-like calcification in the interior suggest endochondral ossification [21]. Furthermore, while on average the mineral density of the calcifications is similar to that of rib bone, the presence of extremely dense isolated calcification outliers suggests other mechanisms involved in their formation.

The trends of decreasing rib bone volume fraction and mineralization with age are consistent with the current literature on bone loss with aging. While the mineralization ratio appeared to increase with age, it was probably an artifact resulting from decreasing rib bone volume with age and not calcification mineral density increasing with age. The rib mineral density was not a good predictor of calcification mineral density, nor was the rib volume fraction a good predictor of calcification volume fraction. The degree to which costal cartilage calcification could be a compensatory mechanism or related to a change in stress pattern related to decreased rib bone modulus is unknown and worthy of further study.

All specimens exhibited some levels of calcification, even if only diffuse calcification that was not apparent from the CT scans.
Calcification in the costal cartilage affects its mechanics as a continuum and as a structure, especially when high levels of structured calcification are present (Fig. 3), and has important consequences for physiological modeling of pulmonary mechanics and for structural modeling of the thoracic response to external loads, such as those applied by a seatbelt [12,13,22]. Even though there are no significant changes in cartilage material properties with aging and increased calcification [23], the presence of calcification leads to an effective stiffening of the costal cartilage [24], which increases the stress in the adjacent bone when an external load is applied [13]. The length of contiguous calcification infiltrating from the CCJ changes the effective length of the rib and could alter the structural response. The observed changes in cross-sectional area while maintaining the mineralized volume fraction when approaching the CCJ from a medial direction can be incorporated in models of the thorax.

The finding that isolated calcifications can have a higher mineral density than the adjacent rib bone implies that these calcifications have a higher elastic modulus [25,26]. The similar average mineral densities imply that the material properties of rib cortical bone [27] would be a reasonable starting point for developing models of calcified costal cartilage. These models can be further refined using the mineral density ranges along with the mineralized volume fractions presented in this study as guidelines to model the aging thorax and calcifying costal cartilage on multiple length scales.

The size of the donor population (54 specimens harvested from 11 donors) in this study should be considered in any interpretation of the findings. The focus of this study was the detail of the observed calcifications, not the degree to which these trends represent the population at large. Studies with less detailed analyses but larger sample sizes have shown significantly increasing volumes of calcification with aging and strongly gender-specific calcification patterns. That age trend did not attain statistical
significance in our study and no attempt was made to stratify the specimens by gender.

5. Conclusion

This study performed a detailed analysis of calcifying human costal cartilage, providing information on the morphologies and their distribution throughout the cartilage, as well as mineral densities and mineralized volume fraction, to serve as guidelines in developing biomechanical and material models of calcified cartilage. While localized regions of calcifications had higher mineral densities, on average they had similar mineral densities to rib bone. The mineralized volume fractions of costal cartilage ranged from low levels of diffuse calcifications to approaching those found in rib bone. Costal cartilage calcifications with morphologies similar to those in diaphyseal bone suggest the potential for costal cartilage to serve as an autologous bone scaffold, warranting further investigation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2010.10.019.

Appendix B. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 1–4 and 6–9, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2010.10.019.

References