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Elemental composition, morphology and mechanical properties of calcified deposits obtained from abdominal aortic aneurysms

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Abstract

Calcified deposits exist in almost all abdominal aortic aneurysms (AAAs). The significant difference in stiffness between these hard deposits and the compliant arterial wall may result in local stress concentrations and increase the risk of aneurysm rupture. Calcium deposits may also complicate AAA repair by hindering the attachment of a graft or stent-graft to the arterial wall or cause vessel wall injury at the site of balloon dilation or vascular clamp placement. Knowledge of the composition and properties of calcified deposits helps in understanding the risks associated with their presence. This work presents results of elemental composition, microscopic morphology, and mechanical property measurements of human calcified deposits obtained from within AAAs. The elemental analyses indicate the deposits are composed primarily of calcium phosphate with other assorted constituents. Microscopy investigations show a variety of microstructures within the deposits. The mechanical property measurements indicate an average elastic modulus in the range of cortical bone and an average hardness similar to nickel and iron.

Keywords: Calcium deposit; Mechanical properties; Elemental analysis; Electron microscopy; Abdominal aortic aneurysm

1. Introduction

Calcium deposits are hard aggregates of calcium-rich particles that form in blood vessels. The biochemical mechanisms of vascular calcium deposit formation are complex and not completely known [1,2]. A histological study of excised aortas by Stary [3] indicated that calcium granules form intracellularly and are released into the extracellular matrix through cell death. Stary observed such granules, approximately 5–10 μm in size, in the cytoplasm of smooth muscle cells. The released calcium granules aggregate in the arteries and grow into larger structures. In an earlier work, Bobryshev et al. [4] used electron microscopy to study aortic calcification. Their observations indicate that aortic calcification requires some pre-existing extracellular structural base, such as unesterified cholesterol. Proudfoot and Shanahan [2] also note that there is good evidence from gene studies to suggest that vascular calcification is a regulated process similar to the calcification in bone.

Regardless of the mechanisms of calcification formation, the presence of vascular calcium deposits poses serious health risks. One major concern is that a deposit will detach from the arterial wall and lodge in a smaller artery or arteriole further downstream [5,6]. A detached calcification originating in a carotid artery may relocate to a cerebral artery and lead to transient ischemic attacks or stroke. Detached deposits from coronary arteries are known to cause heart attacks. Detachment in other areas of the body may result in local cell and/or tissue death.

This work focuses on calcifications located within abdominal aortic aneurysms (AAAs). People above 50 years

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of age are at the greatest risk of developing an AAA. Aortic calcification is also more common in older people. In a study of aortic computed tomography (CT) scans from 257 patients, male and female, and with no known aortic diseases, Dixon et al. [7] reported that calcification was identified in some patients as early as their fourth decade. Of patients in their sixth decade or older, 77% had some degree of calcification. Aortic calcification is even more prevalent in patients with AAAs. Pillari et al. [8] studied CT scans of AAAs from 55 patients, male and female, ages 60–86, and observed some degree of calcification in each.

Previous research by Fillinger et al. [9,10] has demonstrated that aortic aneurysm wall stress is strongly associated with aneurysm rupture risk in abdominal aortic aneurysms. Calcified deposits within an abdominal aortic aneurysm may increase the stress in the aneurysmal wall, and hence increase the risk of aneurysm rupture. The considerable difference in stiffness between the compliant arterial tissue and a hard deposit results in the latter acting as a stress raiser; the deposit limits the deformation of the wall tissue on which it is adhered while causing an increase in the strain of the neighboring tissue. This effect has been demonstrated in AAA finite element studies by Inzoli et al. [11] and Marra et al. [12]. Calcium deposits may also complicate AAA repair by hindering the attachment of a graft or stent-graft to the arterial wall, or cause vessel wall injury at the site of balloon dilation or vascular clamp placement [13].

Knowledge of the composition and properties of calcified deposits is beneficial to understanding the risks associated with their presence. This work presents results of elemental composition, microscopic morphology, and mechanical property measurements of human calcified deposits obtained from within AAAs.

Chemical composition studies of aortic calcium deposits have been performed previously, although only on specimens from non-aneurysmal aortas. Schmid et al. [14] analyzed deposits using various procedures including atomic absorption spectroscopy, alkalimetric titration, thermal conductivity measurements, and X-ray diffraction. They observed that both large and small deposits had essentially the same composition, consisting of 71 wt.% apatite,[1] 9 wt.% carbonate, and 15 wt.% protein. Tomazic [1] also conducted various chemical analyses of aortic deposits and detected primarily calcium phosphates with assorted inorganic constituents including sodium, magnesium, fluoride, and carbonate. X-ray diffraction patterns of the deposits indicated a crystalline structure characteristic of apatite. Tomazic also reported that the amount of organic matter in native (non-deproteinized) deposits was 20–30 wt.%.

[1] Wopenka and Pasteris [15] caution that the word apatite refers to a kind of calcium phosphate with a specific crystal structure and should not be used as an identifier unless such a structure is confirmed. Wopenka and Pasteris also argue that some biological phosphates are mistakenly labeled as apatites.

The microscopic structures of non-aneurysmal aortic calcified deposits have been previously studied by Schmid et al. [14] using scanning electron microscopy (SEM). They observed five different structures in their specimens: (1) smooth-surfaced spheres, (2) spheres of spindle-like, radially arranged particles, (3) networks and bundles of fibers, (4) irregularly-shaped particles with "fuzzy" surfaces, and (5) flat, smooth plates. Similar observations were made by Tomazic in a later SEM study of aortic calcifications.

No known information has been published regarding the mechanical properties of vascular calcified deposits. The mechanical properties of mineral apatites have been reported by Grenoble et al. [16], who used ultrasonic experimental procedures to characterize these materials. The lack of published information on the mechanical properties of calcified deposits may be due to their relatively small size. The properties presented in this work were measured using a nanoindentation system specifically designed for testing very small specimens. Nanoindentation measurements have been reported previously for bone by Rho and Pharr [17]. While the elastic modulus values presented by Rho and Pharr do agree well with bulk bone measurements, it should be noted that properties obtained from nanoindentation testing relate to the microstructural scale of the testing specimen, and may deviate from bulk properties.

2. Materials

Twelve calcified deposit specimens, from 12 different patients, were studied. The patients ranged in age from 53 to 85 and included both genders. All of the specimens studied were excised from patients during planned or emergency open aneurysm repair surgeries, and from arterial wall tissue that would otherwise have been discarded. No specimens were removed from patients specifically for this work. All calcified specimens were removed manually from the intimal side of the excised aneurysm wall. Most specimens were plate-like, but irregular in shape, and varied in size. Specimen thicknesses ranged from about 0.7 mm to about 2 mm (prior to preparation for testing), and the largest specimen dimensions were approximately 8 mm.

3. Methods and results

Approval to use patient tissues for research purposes was obtained before starting this study from the institutional review board (Committee for the Protection of Human Subjects, 8/20/2001) and all subjects provided informed consent.

3.1. Elemental analysis

Elemental analyses were performed on four calcified deposit specimens. The specimens were dried in air for several days and then ground flat using a series of increasingly

ID	Title	Pages
2553	Elemental composition, morphology and mechanical properties of calcified deposits obtained from abdominal aortic aneurysms	6