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Research Paper

On the effect of calcification volume and configuration on the mechanical behaviour of carotid plaque tissue

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ABSTRACT

Vascular calcification is a complex molecular process that exhibits a number of relatively characteristic morphology patterns in atherosclerotic plaques. Treatment of arterial stenosis by endovascular intervention, involving forceful circumferential expansion of the plaque, can be unpredictable in calcified lesions. The aim of this study was to determine the mechanical stretching mechanisms and define the mechanical limits for circumferentially expanding carotid plaque lesions under the influence of distinct calcification patterns.

Mechanical and structural characterisation was performed on 17 human carotid plaques acquired from patients undergoing endarterectomy procedures. The mechanical properties were determined using uniaxial extension tests that stretch the lesions to complete failure along their circumferential axis. Calcification morphology of mechanically ruptured plaque lesions was characterised using high resolution micro computed tomography imaging. Scanning electron microscopy was used to examine the mechanically induced failure sites and to identify the interface boundary conditions between calcified and non-calcified tissue.

The mechanical tests produced four distinct trends in mechanical behaviour which corresponded to the calcification patterns that structurally defined each mechanical group. Each calcification pattern produced unique mechanical restraining effects on the plaque tissue stretching properties evidenced by the variation in degree of stretch to failure. Resistance to failure appears to rely on interactions between calcification and non-calcified tissue. Scanning electron microscopy examination revealed structural gradations at inter-

Abbreviations: FTIR, Fourier Transform Infrared; ATR, attenuated total reflectance; CT, computed tomography imaging; CVF, calcified volume fraction; SEM, scanning electron microscopy; EDX, Energy dispersive x-ray; lip, lipid; calc, calcification; col, collagen.

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face boundary conditions to facilitate the transfer of stress. This study emphasises the mechanical influence of distinct calcification configurations on plaque expansion properties and highlights the importance of pre-operative lesion characterisation to optimise treatment outcomes.

1. Introduction

Vascular calcification is a highly regulated and complex process that occurs as part of atherosclerotic plaque development (Stary, 2000). The process is initiated by apoptotic smooth muscle cells and the release of matrix vesicles by macrophages responding to pro-inflammatory stimuli which generate microcalcification crystals speckled throughout the necrotic core region adjacent to the internal elastic lamina (Leopold, 2015; Otsuka et al., 2014). An adaptive response mechanism can inhibit the inflammatory stimuli and regulate the mineralisation process forming macrocalcifications which encompass the necrotic core and surrounding tissue regions. The macrocalcifications display a number of relatively characteristic morphology types which parallel the development of the atherosclerotic stenosis (Pugliese et al., 2015; Otsuka et al., 2014; Stary, 2000).

In terms of treating a stenosis, the safe development of carotid artery stenting (CAS) has been impeded by the mechanical challenges posed by dilating calcified lesions. The examination of heavily calcified plaques following CAS using high resolution optical coherence tomography imaging techniques has highlighted a number of procedural issues (Cremonesi et al., 2014). Among these issues is evidence of stent under-expansion and also stent malapposition (Saw, 2014) with thrombus forming around the malapposed stent struts leading to post-procedural cerebral embolization (De Donato et al., 2013). Calcified plaques are also less compliant and require higher radial forces to achieve sufficient luminal gain and overcome the vessel resistance (Tsutsumi et al., 2008). Consequently, calcification has a strong association with iatrogenic arterial dissection (Fitzgerald et al., 1992). Stent fractures can occur due to high regional focal stress points induced by calcification. According to Ling et al. calcified internal carotid arteries are 8 times more likely to experience a stent fracture (Ling et al., 2008). The clinical relationship between a stroke occurring at the time of intervention and lesion calcification is not clear (Saw, 2014). The fundamental issue regarding the progress of treating calcified lesions with stenting is that the mechanical stretching mechanisms involved in circumferentially expanding the lesion, and how this varies under the influence of different calcification morphology types, is unknown.

The endovascular treatment applies a circumferential force to the entire diseased portion of the arterial vessel to restore luminal patency. This procedure triggers a complex whole-plaque mechanical response including contributions from the calcifications embedded within the complex fibrous tissue. The composition and morphology of the plaque are important determinants of mechanical behaviour (Wong et al., 2012). Recent studies have determined the dissimilar material properties of individual plaque components (Teng et al., 2014) while we have previously examined the mechanical properties of the plaque as a whole tissue composite by expanding plaques in their circumferential loading direction to complete failure (Mulvihill et al., 2013; Cunnane et al., 2015). In these latter studies scanning electron microscopy (SEM) revealed calcification deposits present at the failure sites suggesting a negative mechanical effect increasing the propensity to rupture. The global mechanical stretching properties are largely influenced by the structural integrity of the entire lesion which comprises of mutually integrated structural components (Akyildiz et al., 2014). Classifying the global calcification detail in conjunction with experimentally stretching the lesion in a controlled testing manner may be appropriate to evaluate the stretching mechanisms and define mechanical limits of the tissue.

This study aims to examine the influence of specific calcification patterns on the mechanical expansion behaviour and failure properties in human carotid plaque lesions. The mechanical properties of the carotid tissue are determined using uniaxial extension testing and calcification morphology is examined by computed tomography imaging. SEM is also employed to examine the failure sites and the interaction between calcified and non-calcified tissue. An improved understanding of the mechanical influence of calcification on plaque deformation and rupture behaviour during circumferential expansion will have major implications for the treatment of calcified lesions.

2. Methods

2.1. *Sample acquisition*

Carotid plaques were obtained from seventeen endarterectomy patients at the University Hospital Limerick in a manner that conformed to the declaration of Helsinki and was approved by the hospital's Ethical Research Committee. The symptomatic carotid plaque specimens were removed by standard endarterectomy, whereby an arteriotomy was made along the anterolateral side of the common carotid artery and extended distally into the internal carotid artery. Upon excision, the plaques were immediately frozen in phosphate buffer solution (PBS) and stored at −20 °C. The plaques were then thawed at 4 °C overnight, further equilibrated to room temperature in PBS, and subsequently immersed in PBS at 37 °C to equate the tissue back to physiological temperature prior to mechanical testing.