SHORT COMMUNICATION

An initial evaluation of analyser-based phase-contrast X-ray imaging of carotid plaque microstructure

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ABSTRACT. Carotid artery plaque instability can result in rupture and lead to ischaemic stroke. Stability of plaques appears to be a function of composition. Current non-invasive imaging techniques are limited in their ability to classify distinct histological regions within plaques. Phase-contrast (PC) X-ray imaging methods are an emerging class of techniques that have shown promise for identifying soft-tissue features without use of exogenous contrast agents. This is the first study to apply analyser-based X-ray PC imaging in CT mode to provide three-dimensional (3D) images of excised atherosclerotic plaques. The results provide proof of principle for this technique as a promising method for analysis of carotid plaque microstructure. Multiple image radiography CT (MIR-CT), a tomographic implementation of X-ray PC imaging that employs crystal optics, was employed to image excised carotid plaques. MIR-CT imaging yields three complementary images of the plaque's 3D X-ray absorption, refraction and scatter properties. These images were compared with histological sections of the tissue. X-ray PC images were able to identify the interface between the plaque and the medial wall. In addition, lipid-rich and highly vascularized regions were visible in the images as well as features depicting inflammation. This preliminary research shows MIR-CT imaging can reveal details about plaque structure not provided by traditional absorption-based X-ray imaging and appears to identify specific histological regions within plaques. This is the first study to apply analyser-based X-ray PC imaging to human carotid artery plaques to identify distinct soft-tissue regions.

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Carotid artery plaque formation and associated vessel stenosis are leading risk factors for ischaemic stroke. Instability of these plaques is a precursor of acute thromboembolic events that lead to strokes. Patients who are symptomatic with at least 50% stenosis are candidates for carotid endarterectomy (CEA), but it is not clear that CEA is the best option for asymptomatic patients [1]. Plaque vulnerability in these patients appears to be a function of composition, architecture and degree of stenosis. Specifically, plaques with a high lipid content and large necrotic core are more prone to rupture while those with high fibrosis and calcification are more stable. Currently, a few available non-invasive imaging modalities can be used to evaluate soft-tissue

composition. However, these methods are limited by either poor spatial resolution, depth of tissue penetration or the use of exogenous contrast agents. There is a significant need for improved non-invasive, high-resolution imaging techniques that can identify plaque composition without the use of contrast agents.

A number of methods have been investigated for imaging of carotid plaques. B-mode ultrasound imaging provides information on plaque echogenicity and structure. Heterogeneity in the ultrasound appears to depend on plaque composition and may be related to stability [2–5] but cannot be directly related to histological features. In addition, plaques can produce different echosignals depending on location and orientation relative to the ultrasound transducer. MRI can provide good soft-tissue contrast, and has shown potential for identifying distinct histological features and quantifying plaque size [6–9]. Exploiting multiple MRI contrast mechanisms, including T_1 weighted, T_2 weighted, proton density, diffusion weighted and magnetisation transfer ratio and others

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enables identification of regions of calcification, fibrous tissue, lipid necrotic cores, intraplaque haemorrhages and fibrous caps [10–14]. These multimodal MRI techniques have been employed to map major plaque components [15]. However, MRI still has relatively limited spatial resolution. Molecular approaches have been explored in which contrast agents are targeted to regions of inflammation within a plaque to enhance contrast of specific regions when imaging with optical methods, such as spectral X-ray CT [16] or MRI [17]. An abundance of matrix-metalloproteinases is found in vulnerable plaques prone to rupture, allowing for pathophysiological information to be obtained [17]. However, these targeting approaches limit the number and diversity of microstructural features that can be imaged simultaneously. Conventional X-ray-based methods provide high spatial resolution and allow for identification of calcifications. However, soft-tissue structures are the primary contributors to plaque destabilisation and cannot be reliably detected using conventional radiography.

X-ray phase-contrast (PC) imaging exploits contrast mechanisms based on the X-ray refractive and scattering properties of tissue, which can provide higher image contrast at diagnostic X-ray energies than absorption contrast. Studies of plaque features in small animal models have been performed using X-ray interferometric implementation of X-ray PC imaging. Images of the samples' refractive index distribution were produced and related to tissues mass density, which provided information regarding the relative tissue composition of the plaques [18, 19]. While these reports reveal the potential of PC X-ray imaging, the interferometric method utilised has a small field of view and is difficult to implement in clinical environments owing to the dependence on synchrotron radiation, and therefore has little hope for routine clinical application.

Multiple image radiography (MIR) is an analyser-based X-ray PC imaging method that has shown promise for imaging articular cartilage and breast tumours because of its high sensitivity, which permits it to reveal subtle soft-tissue structures [20–22]. It can be employed to image a large field of view and is currently being developed for use with benchtop X-ray sources for laboratory and clinical use [22, 23]. MIR produces three separate images that depict the X-ray absorption, X-ray refraction and ultra-small-angle X-ray scatter (USAXS) properties of tissue. When implemented in CT mode (which will be referred to as MIR-CT), three-dimensional (3D) images of these three complementary tissue properties are produced that can provide a detailed characterisation of tissue features and microstructure.

In this work, we provide evidence of the ability of MIR-CT to reveal potentially informative microstructural tissue features in carotid plaques. Features present in the MIR-CT images were compared with histology to determine whether they provide accurate 3D information regarding soft-tissue composition. This is the first study to investigate MIR-CT imaging of atherosclerotic plaques and provides a proof of principle of the potential advantages of MIR-CT plaque imaging.

Methods

Samples

Patients with advanced carotid vascular disease requiring endarterectomy were identified and consented. This study did not influence patient selection or treatment protocol. Patients were subjected to routine ultrasound analysis prior to CEA. All procedures were approved by the Hines VA Hospital Institutional Review Board (Hines IL). Following excision, plaques were fixed in formalin and underwent routine gross pathological examination. Samples were then stored in formalin prior to imaging, which has a minimal effect on tissue properties determined by analyser-based systems [24].

MIR-CT imaging and image reconstruction

The plaque specimens obtained at CEA were imaged using an MIR-CT imaging system at Brookhaven National Laboratory (Figure 1) [25]. An X-ray detector (VHR 11, charge-coupled device, Photonic Science Limited, Millham Mountfield, UK) sensor was employed to capture X-rays and possessed a detector pixel pitch of 9 μm . Additional details regarding this imaging system and the data acquisition procedures can be found in Zhong et al [25]. A beam energy of 20 keV and a [333] crystal reflection were utilised. The measurement data were acquired at 11 analyser crystal positions ranging from -4 to $+4$ μrad with 0.8- μrad increments. 500 tomographic intensity measurements were acquired over a 180° angular range for each analyser-crystal orientation. The acquisition time at each orientation was 1 s.

At each tomographic view angle, three planar MIR images that represent projected absorption, refraction and USAXS properties of the plaque were computed from the measured data as described previously [26, 27]. The absorption image is similar to a conventional radiograph but is free of undesired scatter. The USAXS quantifies the increase in the angular divergence of the beam caused by the presence of multiple beam refraction from subpixel-sized scatters. These sets of projection images were employed with a parallel-beam filtered back projection algorithm [26] to obtain volumetric images of the three tissue properties. All image analysis and reconstruction were performed using Matlab R2009b (MathWorks, Natick, MA) while V3D was used as the rendering software.

Histology

Following imaging, the plaque specimen was dehydrated (Leica TP1020; Leica Microsystems GmbH, Wetzlar, Germany) using routine histological methods and paraffin embedded in the same planar orientation as the X-ray projection views. This facilitated registration of the histology to the MIR-CT slices. 20 serial sections (5 μm) were cut using a Leica RM2200 microtome (Leica Microsystems GmbH) and alternately stained for haematoxylin and eosin (H&E) or Masson trichrome.

Stained sections were imaged using an Axiovert 200 inverted microscope (Carl Zeiss MicroImaging, Inc., Göttingen, Germany) equipped with an AxioCam

Figure 1. Schematic of the multiple image radiography imaging system at Brookhaven National Laboratory [25].

MRC5 colour digital camera (Carl Zeiss Microscopy, LLC Thornwood, NY). The camera and computer-controlled X–Y–Z stage allowed tiling of multiple images to build high-resolution images of entire sections. Axiovision 4.2 image analysis software (Carl Zeiss Meditec AG) allowed for automated control of all aspects of acquisition and processing. Tissue sections were registered to slices through the MIR-CT images based on gross morphological features that are easily identifiable in all MIR-CT images and histological sections. An experienced cardiovascular pathologist characterised histological sections of the plaque regions (FJS).

Results

On *in vivo* ultrasound analysis prior to CEA, plaques were described as homogeneous and without calcifications. However, MIR radiographs revealed multiple calcifications in nearly all samples. Examination of the three volumetric MIR images obtained from the two CT scans and histology revealed a complex, heterogeneous plaque structure with calcifications.

MIR-CT and histology

After reconstruction, 3D images representing the absorption, refraction and USAXS signatures of the plaque were rendered using maximum intensity projection to facilitate visualisation. Conventional X-ray radiography allows for identification of calcifications only. However, the MIR absorption image is not degraded by refraction and scatter and is therefore much more sensitive to details within the soft tissue (Figure 2a). The 3D MIR-CT refraction image displays the overall morphology of the plaque, borders of the plaque and interfaces between different soft-tissue regions (Figure 2b). The 3D USAXS image reveals areas of high contrast within the plaque consistent with the absorption image (Figure 2c). The three volumetric images were fused and colour-coded to form a 3D rendering representing all properties in a single image (Figure 2d).

The CT images were subsequently compared with histological stains (Figure 3a,b). The refraction image

(Figure 3c) contained the most structural detail. The overall outline of the plaque is easily seen and the interface between the medial wall and an acellular region (black arrow) can be clearly identified. The highly cellularised regions (green and yellow arrows) are areas of inflammation with neovascularization. These regions produced high image texture in the refraction image (Figure 3c) as well as bright intensities in both USAXS and absorption images (Figure 3d,e). Areas of inflammation can be identified within the medial wall (yellow arrow) and fibrous cap (green arrow). Additional regions can be observed in another plaque region (Figure 4). The interface between the media wall and plaque can be seen (orange arrows). Necrotic debris, with a mild macrophage infiltrate, lipid/cholesterol clefts and focal calcification, is present (yellow oval) (Figure 4a,b). This region has the highest intensity in both the USAXS and the absorption images (Figure 4d,e), while it results in the most image texture within the refraction image (Figure 4c). A residual injured arterial wall (green box) is also visible in all images.

Discussion

This was the first study that investigated the use of analyser-based X-ray PC imaging for imaging human carotid artery plaques. Three separate 3D renderings of the plaque were generated corresponding to the X-ray absorption, refraction and USAXS properties of the plaque. These three properties were compared with H&E and Masson trichrome staining of the tissue, and features in image data corresponding to distinct tissue features were found to correlate with histology. First, the interface between the plaque and the media wall was clearly identified in the refraction images. This could allow for quantification of the volume and per cent stenosis of plaques within vessels. Second, regions of inflammation produced specific signatures in all three MIR images. In the refraction images, these regions were seen as areas of high image texture, while in the absorption and USAXS images these regions were higher intensity than background soft tissue. Lipid-rich regions within the plaques also gave distinct image signatures that allowed for differentiation from background plaque

Figure 2. Three-dimensional rendering of multiple image radiography CT of carotid artery plaque. (a) Absorption signature, (b) refraction signature, (c) ultra-small-angle X-ray scatter (USAXS) signature and (d) Composite of absorption, refraction and USAXS.

structure. Research has shown that these areas may contribute to plaque instability, making them more prone to rupture [28, 29]. The ability to identify and calculate the size of these regions non-invasively would be very useful for diagnosis and treatment.

Others have applied an interferometric X-ray PC imaging method to image mouse carotid artery plaques *ex vivo*. They were able to define mass density values from the phase map which were used to identify and quantify regions of smooth muscle, collagen and lipid [18, 19]. Similar soft-tissue types were identified in the two different X-ray PC imaging techniques, but the interferometric technique can be implemented only using synchrotron

radiation. While this MIR imaging study was performed *ex vivo* at a synchrotron, benchtop forms of analyser-based imaging are under development. This initial example of imaging carotid artery plaque with MIR-CT shows the potential value of the technique. As benchtop and clinical systems are developed, this imaging method shows promise for *in vivo* imaging of carotid artery plaques. Future research is needed to correlate PC image signatures with corresponding histology so specific plaque features can be detected. This preliminary research shows that MIR-CT imaging can reveal carotid plaque microstructure and should be investigated further as a viable plaque imaging technique.

Figure 3. Corresponding histology and multiple image radiography CT of plaque. (a) Haematoxylin and eosin, (b) Masson trichrome, (c) refraction, (d) ultra-small-angle X-ray scatter and (e) absorption images. Black arrow indicates medial wall, white and hollow black arrows indicate areas of neovascularisation and inflammation.

Figure 4. Corresponding histology and multiple image radiography CT of plaque. Haematoxylin and eosin, (b) Masson trichrome, (c) refraction, (d) ultra-small-angle X-ray scatter and (e) absorption images. The interface between the media wall and plaque can be seen (arrows). Necrotic debris, with a mild macrophage infiltrate, lipid/cholesterol clefts and focal calcification, is present (ovals). A residual injured arterial wall (boxes) is also visible.

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