Atherosclerotic Plaque Imaging using Phase-Contrast X-Ray Computed Tomography

Cardiovascular diseases are main life-threatening diseases in advanced countries. In particular, acute myocardial infarction often leads to sudden cardiac death. Prediction and prevention of this disease are strongly required in cardiovascular fields.

Recent studies revealed that there are mainly two different kinds of coronary atherosclerotic plaque: stable and unstable plaques. Stable plaque (Fig. 1(a)) consists of mainly collagen and smooth muscle cells. On the other hand, unstable plaque (Fig. 1(b)) consists of large lipid deposition, many infiltrating macrophages, and a thin collagen and smooth muscle cell layer (fibrous cap) that covers the lipid pool core. Unstable plaque often induces inflammatory changes near thin fibrous cap, and activated proteases weaken the fibrous cap and induce sudden rupture of unstable plaque. The lipid core contains many thrombogenic factors; therefore, the rupture of unstable plaque subsequently obstructs coronary blood flow, resulting in acute myocardial infarction. Clinical evidence suggests that plaque components rather than the severity of luminal stenosis are important predictors of plaque stability and clinical events.
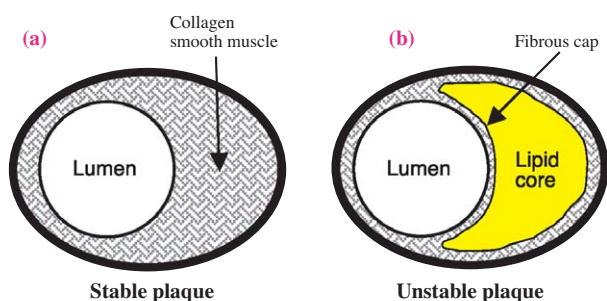

Fig. 1. Schematic diagram showing comparison of the characteristics of "stable" (a) and "unstable" (b) plaques.

Invasive investigations using a catheter technique can reveal the plaque volume and components. However, reliable noninvasive imaging modalities for the characterization of plaque components are clinically desirable. Currently, two emerging and promising techniques, namely, computed tomography (CT) and magnetic resonance imaging (MRI), are widely used by the medical community because they are noninvasive and have the potential to evaluate luminal stenosis and characterize plaque components. Technical developments over the past several years, especially the introduction of multislice CT scanners, have made coronary CT angiography quite reliable. However, the discrimination of noncalcified plaque components remains difficult. Noncalcified

atherosclerotic plaques mainly consist of deposited lipids, inflammatory cells, smooth muscle cells, and collagen. The present clinical X-ray CT is based on absorption-contrast X-ray imaging, in which images are generated by the differences in X-ray absorption as determined by the linear attenuation coefficient. The differences in the X-ray absorption by biological soft tissues are very small, and therefore, the present X-ray CT is not highly sensitive in differentiating plaque components.

X-rays have the nature of waves, and the shift of the wave when it passes through an object is called the X-ray phase shift. Phase-contrast X-ray imaging has great potential to reveal the structures inside soft tissues because the sensitivity of this method to light elements is almost 1000 times greater than that of the absorption-contrast X-ray method. The purposes of this study were to image atherosclerotic lesions by phase-contrast X-ray CT [1] and to investigate whether this method could identify differences in plaque components that would lead to the detection of unstable atherosclerotic plaques.

The interferometric phase-contrast X-ray imaging experiments were performed at beamline **BL20XU** [2]. A sample cell filled with water was inserted into the beam path. Interference patterns were detected using a charge-coupled device (CCD) detector with a 10-μm luminescent screen and lens coupling. The CCD chip

Phase contrast CT

Lipid (Sudan-III)

Collagen (Masson's trichrome)

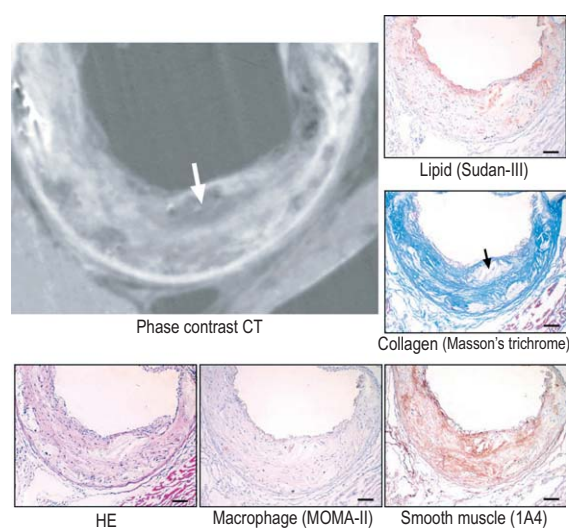HE   Macrophage (MOMA-II)   Smooth muscle (1A4)

Fig. 2. Representative atherosclerotic lesion in the aortic sinus of the ApoE-KO mouse fed a normal diet. This lesion was investigated using phase-contrast CT; HE, Sudan-III, and collagen (Masson's trichrome) staining; and anti-macrophage (MOMA-II) and anti-smooth muscle (1A4) immunohistochemistry. Scale bars indicate 100 μm.
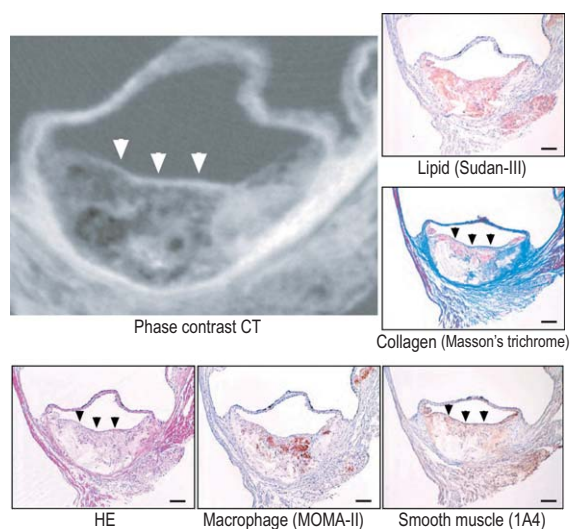
Fig. 3. Representative atherosclerotic lesion in the aortic sinus of the ApoE-KO mouse fed a high cholesterol diet for eight weeks. This lesion was investigated using phase contrast CT; HE, Sudan-III, and collagen (Masson's trichrome) staining; and anti-macrophage (MOMA-II) and anti-smooth muscle (1A4) immunohistochemistry. Scale bars indicate 100 μm.

had 3.14 μm × 3.14 μm pixels. The X-ray energy was set at 12.4 keV.

Atherosclerotic model mice, apolipoprotein E-deficient mice (ApoE-KO mice), were fed a normal diet or a high cholesterol diet. A high cholesterol diet was used to induce unstable like atherosclerotic lesions in mice.

Atherosclerotic lesions were fixed, and *ex vivo* investigations were performed by phase-contrast X-ray CT and histological analysis. Figure 2 shows a representative atherosclerotic plaque in a normal diet-fed ApoE-KO mouse and Fig. 3 that in a high cholesterol diet-fed ApoE-KO mouse. In the normal diet-fed ApoE-KO mouse (Fig. 2), the lipid plaque component was small and limited to its luminal surface. Almost the entire plaque area was positively stained by Masson's trichrome, showing that collagen is the main component of this plaque. Immunohistochemical analysis using 1A4 demonstrated the infiltration of smooth muscle cells into the plaque. Phase-contrast CT images as well as histological analyses enable the characterization of plaque components. The low refractive index (dδ) area in one of the CT images (arrow) corresponded   to the area of low collagen content, as revealed by Masson's trichrome staining.

On the other hand, in the high cholesterol diet-fed ApoE-KO mouse (Fig. 3), Sudan-III staining showed that atherosclerotic plaque formed a large lipid pool. A strong invasion of many macrophages was indicated by MOMA-II immunohistochemistry. The low-dδ area

in the CT image also corresponded to the less or no collagen-containing area, and it was filled with a large amount of lipid. Phase-contrast CT imaging can reveal the differences in atherosclerotic plaque components, particularly the lipid component.

For the quantitative analysis of the mass density of plaque components, the refractive indices dδ of these three components were measured. The measured dδ values and their association with mass densities are shown in Fig. 4. Phase-contrast X-ray CT can directly estimate tissue-mass density and reveal very small differences in mass density among the atherosclerotic plaque components.

The phase-contrast X-ray CT technique can achieve a higher sensitivity than absorption-contrast X-ray CT imaging for the evaluation of biological soft tissues and provide a new quantitative parameter - the "tissue-mass density."
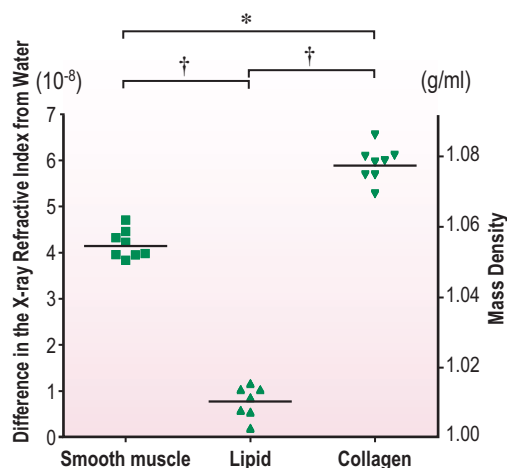

Fig. 4. The mean dδ values and specific mass densities of the various components of atherosclerotic plaques. *, P<.05; †, P<.01.

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