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Evaluation of collagen in atherosclerotic plaques: the use of two coherent laser-based imaging methods

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Abstract

Acute coronary events such as myocardial infarction are frequently caused by the rupture of unstable atherosclerotic plaque. Collagen plays a key role in determining plaque stability. Methods to measure plaque collagen content are invaluable in detecting unstable atherosclerotic plaques. Recently, novel coherent laser-based imaging techniques, such as polarization-sensitive optical coherence tomography (PSOCT) and laser speckle imaging (LSI) have been investigated, and they provide a wealth of information related to collagen content and plaque stability. Additionally, given their potential for intravascular use, these technologies will be invaluable for improving our understanding of the natural history of plaque development and rupture and, hence, enable the detection of unstable plaques. In this article we review recent developments in these techniques and potential challenges in translating these methods into intra-arterial use in patients.

Keywords

Lasers; Atherosclerosis; Collagen; Smooth muscle cells; Optical coherence tomography; Laser speckle imaging

Introduction

The unstable atherosclerotic plaque

Despite widespread efforts towards its detection and therapy, cardiovascular disease still remains the leading cause of mortality in industrialized societies. The rupture of unstable coronary atherosclerotic plaque frequently precedes a majority of thrombus-mediated acute cardiovascular events [1]. A type of atherosclerotic plaque, termed the necrotic core fibroatheroma (NCFA), comprises the majority of coronary lesions implicated in acute cardiovascular events. A NCFA consists of a fibrous cap, which shields an underlying necrotic lipid pool from contact with coronary blood [2,3]. Autopsy studies suggest that the typical hallmarks of unstable plaques are the presence of a thin fibrous cap, a large compliant necrotic core, and activated macrophages near the fibrous cap [2,3]. When the fibrous cap ruptures, the thrombogenic contents of the lipid pool become exposed to blood, resulting in occlusive coronary thrombosis that initiates the onset of an acute coronary event.

Collagen in atherosclerotic plaques

Collagen is an important component of the extracellular matrix of the arterial wall. Evidence suggests that the amount and organization of matrix collagen is associated with the mechanical stability of the fibrous cap (Fig. 1a,b). The collagen molecule consists of three procollagen chains, which are assembled in a triple helix structure. There are at least 19 different types of collagens in the vessel wall, of which type I and type III collagen fibers are predominant and are responsible for imparting tensile strength and elasticity [4]. Smooth muscle cells (SMCs) in atherosclerotic plaques are responsible for synthesizing collagen (Fig. 1c,d). In the normal artery, SMCs are present in the media and perform a contractile role. In atherosclerotic plaques, SMCs migrate from the media to the intimal layer, resulting in an increase in collagen content [5]. Inflammatory cells such as macrophages in inflamed NCFA release matrix metalloproteinases (MMPs), causing the proteolysis of matrix collagen accompanied by the apoptosis of intimal SMCs, which impedes collagen synthesis [5]. This dynamic imbalance between collagen synthesis and degradation causes a net reduction in collagen content in the fibrous cap, which may predispose a plaque to rupture. A recent study suggests that increased MMP expression yields thinner and more disorganized collagen fibers, which may be associated with decreased mechanical stability of the fibrous cap [6]. Plaque stabilization with lipid-lowering therapy reverses this process, in both the cap and lipid pool, by restoring collagen production and reversing the effects of collagen degradation [7]. Because of the significance of collagen in the pathophysiology of plaque rupture, methods capable of evaluating fibrous cap collagen in vivo could facilitate the detection of unstable lesions prior to the occurrence of an acute coronary event.

Laser-based methods to evaluate plaque collagen

A variety of catheter-based imaging methods, such as intravascular ultrasound (IVUS), thermography, angiography, and intravascular MRI, has been investigated for the characterization of coronary plaque [8–11]. IVUS elastography facilitates plaque characterization and potentially allows evaluation of plaque collagen by evaluating local strains within the plaque [12]. The use of laser sources for optical diagnostics has opened new possibilities for the evaluation of collagen in atherosclerotic plaques at image resolutions higher than those of the above techniques. The ability to guide light via intra-arterial catheters equipped with miniaturized optical fibers renders optical diagnostic techniques suitable for intracoronary use in patients. Spectroscopic techniques, such as diffuse reflectance near-infrared (NIR) spectroscopy, Raman spectroscopy, and fluorescence spectroscopy, have been successfully demonstrated in the determination of the chemical composition of atherosclerotic plaques and for the evaluation of plaque collagen [13–16]. Used in conjunction with other intra-arterial imaging techniques, such as optical coherence tomography (OCT), IVUS, or angiography, spectral data obtained from the arterial wall may provide valuable information about both, anatomical and biochemical determinants associated with plaque rupture [17]. Recently, two novel, coherent laser-based imaging techniques, polarization-sensitive optical coherence tomography (PSOCT) and laser speckle imaging (LSI), have been investigated, and they provide a wealth of information related to both plaque collagen content and stability. In this manuscript we describe recent developments in these two techniques and potential challenges in translating these methods into intra-arterial use in patients.

Polarization-sensitive OCT

OCT is a high-resolution (~10 μm) imaging method, somewhat analogous to ultrasound, which provides information about tissue microstructure [18]. With OCT, spatially coherent, broadband, laser light reflected from tissue is interfered with by a reference beam. Interference is detected only when the reference and sample arm path lengths match to within the coherence length of light, which is inversely proportional to the bandwidth of the broadband light. In this

manner, reflectance as a function of depth (A-line) may be obtained by the measurement of the amplitude of the interference pattern as the path length of the reference arm is scanned. For a cross-sectional image to be generated, A-lines are obtained as the beam is scanned across the tissue. Recent ex vivo and in vivo studies have demonstrated the high sensitivity and specificity of OCT for plaque characterization, identification of thin-cap fibroatheromas (TCFAs), and quantification of macrophage content [19–22].

PSOCT enhances conventional OCT by measuring tissue birefringence [23–25], a material property that is elevated in tissues containing proteins with an ordered structure, such as organized collagen and SMC actin–myosin in atherosclerotic plaques. Birefringence measurements, using PS-OCT, were reported in biological tissue, and a loss of birefringence was demonstrated in a collagen-rich tendon sample in response to laser heating [23]. Over the years, PSOCT technology has been further developed, and measurements in other birefringent tissues have been reported for clinical applications in ophthalmology [26,27], dermatology [28], otolaryngology [29], and dentistry [30], to name a few. Advanced systems have been subsequently introduced with analyses based either on the use of Stokes vectors to describe the polarization state of light, [24] or on Mueller matrix formalisms [31] to describe the polarization-related properties of the sample.

Methods to measure the polarization state of light backscattered from tissue have recently been investigated for the evaluation of collagen in atherosclerotic plaques ex vivo. A recent study demonstrated a qualitative assessment of plaque collagen, using OCT, by displaying resulting changes in back-reflected light achieved by altering the incident polarization state [25]. In this study, a single-detector OCT system was used, and changes in tissue reflectivity due to polarization effects were operator evaluated by the manual changing of the position of the polarization controllers in the reference arm. Polarization changes within the tissue were shown to be associated with the presence of collagen in plaques. Kuo et al. demonstrated the use of a free-space PSOCT system in which the polarization state of light incident on the sample could be precisely controlled, along with a dual detector to measure orthogonal polarization states of light reflected from tissue [32]. In this method, the phase difference between the horizontal and vertical components of the detected interference fringes at each depth within tissue was used to calculate birefringence (Fig. 2). Their results showed that the measurement of birefringence from PSOCT images enhanced the discrimination between normal intima, fibrous, lipid-rich and calcific plaques when compared to the measurement of intensity alone [32].

New optical fiber-based PSOCT systems have been developed to facilitate easier imaging access for biological samples and for potential in vivo use [33,34]. These systems have accounted for the random birefringence of optical fibers, by using multiple incident polarization states and generalized analysis algorithms to ensure correct determination of tissue birefringence independent of the polarization state of light incident on tissue, a fundamental requirement for intracoronary imaging. A fiber-optic PSOCT system has been developed for measuring birefringence in tissue. The system design and the mathematical algorithms to generate PSOCT images have been previously described in detail [34,35]. Briefly, by scanning the incident beam across the sample and calculating phase retardation angles using the analyses of Stokes vectors [35], the (double-pass) phase retardation at each point in depth introduced by the sample was obtained and displayed as a gray scale image with black corresponding to 0° (at the tissue surface shown in red) and white corresponding to 180° of phase retardation [36]. This system has been recently investigated for evaluating birefringence in atherosclerotic plaques [37]. The study showed that PSOCT birefringence was highly related to total collagen content, collagen fiber thickness and SMC content in atherosclerotic plaques (Fig. 3). In NCFAs, PSOCT birefringence highly correlated with collagen content of fibrous caps, potentially providing an important indicator of plaque stability. A strong positive correlation

was demonstrated between PSOCT birefringence and thick collagen fiber content ($R=0.76$, $P<0.0001$) [37]. These results may be influenced by other sources of birefringence due to the compositional heterogeneity in atherosclerotic plaques, such as the presence of cholesterol crystals and SMCs. PSOCT birefringence demonstrated a high correlation with SMC content ($R=0.74$, $P<0.0001$) [37]. Further analysis of PSOCT images may allow a more precise determination of collagen content to be made. However, taken together, these results indicate that PSOCT birefringence may provide a powerful new index of plaque stability related to the amount of abundant thick collagen fibers and/or SMCs. PSOCT is unique in that it provides images of birefringence, which are co-registered with high-resolution cross-sectional images of plaque morphology obtained by conventional OCT. PSOCT provides additional information about the composition of plaques and NCFA fibrous caps, where low birefringence likely indicates increased instability associated with low collagen content. As in conventional intracoronary OCT, PSOCT images may be obtained through catheters, and the development of next-generation high-speed PSOCT technology for intracoronary use in patients is underway.

PSOCT: Current technology challenges

Key technological challenges are currently being met to advance the applicability of PSOCT to intracoronary diagnosis in patients. Firstly, in an intracoronary PSOCT catheter using an optical fiber, a dynamically changing polarization state during imaging may result from the induced stress birefringence in the optical fiber during rotational scanning and catheter motion over the cardiac cycle. It was recently shown that these effects can be minimized by the isolation of tissue birefringence from stress birefringence induced in the optical fiber (Fig. 4) [38]. The authors achieved this isolation by analyzing depth-resolved polarization states with respect to the instantaneous (not averaged) surface polarization states, for each A-line within the image [38]. Secondly, due to high scattering and absorption of blood, intracoronary OCT is currently conducted in conjunction with saline flushing that adequately purges blood from the field of view during imaging. However, at current PSOCT imaging rates (4–8 frames/s) imaging of long coronary segments will be limited by the large saline load required. Next-generation high-speed PSOCT systems are under development that allow comprehensive screening of long coronary segments in tens of seconds during administration of a reduced saline load [39]. Another limitation arises from the depth of penetration (~2 mm) of light through tissue that limits evaluation of coronary remodeling. In addition, current systems suffer from limited ranging depth over which the returning signal can be measured, which would make it difficult for one to visualize the entire coronary circumference when the catheter is located eccentrically within the vessel lumen. Adaptive auto-ranging techniques have been suggested as a solution, by the adaptive readjustment of the reference arm galvanometer's position to follow the lumen's curvature [40]. New-generation systems are being developed that have greater ranging depth, which overcomes this problem [39]. Finally, speckle in the PSOCT images introduces noise in the calculation of Stokes parameters and, consequently, in measurement of birefringence. Techniques to reduce speckle noise will further improve the performance of PSOCT in determining birefringence, particularly in key microstructural features such as thin fibrous caps.

Laser speckle imaging

When a scattering medium such as tissue is imaged with highly coherent light from a laser, a granular pattern of multiple bright and dark spots becomes apparent in the image, which bears no perceptible relationship to the macroscopic structure of the object (Fig. 5a). These random intensity patterns, known as speckle, result from the interference of light returning from the highly scattering medium. Laser speckle is inherent in optical imaging systems which utilize laser sources and is a dominant factor influencing the signal-to-noise ratio and image quality.

However, the time-dependent dynamic characteristics of laser speckle provide a wealth of information that can enable a number of medical diagnostic applications. By analyzing time-averaged laser speckle patterns obtained from moving scatterers such as blood cells, one can evaluate blood perfusion in tissue [41].

Additionally, laser speckle provides information about the mechanical properties of the tissue. In a medium such as tissue, suspended particles undergo Brownian motion, which is directly related to the viscoelastic properties of the medium [42]. Consequently, in NCFAs, due to the relatively low-viscosity of lipid, particles within a compliant necrotic core exhibit more rapid Brownian motion than do those in the stiffer fibrous regions of the plaque. Since scatterer motion causes a modulation of the laser speckle pattern, the measurement of temporal intensity variations provides information about the intrinsic viscoelastic properties of the plaque, which can be used to determine plaque morphology and collagen content. Using these principles, an ex vivo study has recently been conducted to demonstrate that the measurement of the decorrelation time constant of intensity modulations of time-varying laser speckle patterns provides a highly sensitive and specific method for the characterization of atherosclerotic plaques and the measurement of collagen content [43]. Sensitivity and specificity of 100% and 93%, respectively, were reported for the detection of unstable atherosclerotic plaques by LSI. In addition, total plaque collagen measured by polarized light microscopy of picrosirius red-stained sections was highly correlated with the decorrelation time constants ($R=0.73$, $P<0.0001$). These correlations may be influenced by contributions of other plaque constituents with similar mechanical properties, such as SMCs or elastin. In addition to single point measurements, LSI conducted by scanning the beam provided the spatial distribution of decorrelation time constants over the plaque. (Fig. 5b) These maps may aid in the detection of potential focal weak spots within the plaque associated with low collagen content. By combining the analysis of spatial and temporal information from laser speckle patterns with a diffusion theory and Monte Carlo model to describe light propagation through tissue, a follow-up study demonstrated that it is possible to measure a parameter that is highly correlated with the thickness of the plaque's fibrous cap [44]. Using this technique in conjunction with beam scanning, one may potentially obtain depth-resolved measurements to measure collagen content within the fibrous cap, providing a critical predictor of plaque rupture. Since LSI utilizes inexpensive light sources and fiber bundles, this technique may potentially provide a low-cost alternative for the evaluation of coronary plaque.

LSI: Current technology challenges

The development of miniaturized intravascular catheters utilizing optical fiber bundles is currently underway and would allow the use of LSI as an independent tool or as a powerful adjunct to other optical techniques such as angioscopy. Significant technical challenges need to be overcome to translate LSI technology into in vivo intracoronary application. Firstly, optical fiber bundles for intracoronary LSI catheters will need careful optimization and selection to minimize the effect of light leakage between individual optical fibers (crosstalk), which may corrupt laser speckle data. Secondly, as with other optical imaging techniques, the presence of blood could hinder accurate imaging of the arterial wall. Intracoronary saline flushing may be used in conjunction with LSI, which has been successfully implemented in OCT in vivo and angioscopy procedures to enable unobstructed imaging of the coronary wall [20]. Thirdly, vessel wall pulsation and catheter motion during the cardiac cycle may influence the modulation of laser speckle patterns. It has been shown that LSI retains high sensitivity and specificity in detecting unstable plaques, even under high rates of arterial stretch [43], potentially providing high diagnostic efficacy during vessel pulsation. In addition, the relatively short acquisition time (~40 ms) required for LSI would give, during the resting phase of the cardiac cycle (minimal catheter motion), a temporal window sufficient for one to obtain diagnostic quality speckle data for reliable measurements in vivo.

Summary and conclusions

Histopathologic studies have demonstrated that the site of a ruptured plaque often shows a fibrous cap with diminished collagen content. The overexpression of collagenases further alters the plaque's mechanical properties by yielding thinner, disorganized, collagen fibers [6]. Given the important role that collagen plays in determining plaque stability, methods to measure plaque collagen content are invaluable in detecting unstable atherosclerotic plaques. Coherent laser-based imaging methods such as PSOC and LSI show great promise in quantifying plaque collagen content. Yet, important technological challenges still remain in the development of intravascular catheters and systems for in vivo imaging. Additionally, a great deal still needs to be learned about the disease itself to determine the role of these techniques in routine clinical practice. Studies need to be conducted to address the role of systemic and local predictors of the risk of plaque rupture. If the results of these studies show that focal plaque stabilization provides clinical benefits, therapeutic intervention could be guided by information such as that provided by PSOC and LSI, potentially improving patients outcome.

Coherent laser-based imaging techniques such as PSOC and LSI are unique in that they provide images that are co-registered with collagen composition of atherosclerotic plaques. Beyond the measurement of cap thickness, these methods provide additional information about the collagen content of plaques and NCFA fibrous caps, which are, presumably, indicative of plaque stability. Given the potential significance of the information provided by these techniques and their potential to be applied intravascularly, we anticipate that these technologies will be useful for improving our understanding of the mechanisms of plaque progression and rupture and for the detection of unstable plaques before the occurrence of an acute coronary event.

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Fig. 1.

a, b Picrosirius red (PSR)-stained histological sections of an NCFa showing the presence of a fibrous cap with depleted collagen content, indicative of an unstable plaque (**a**) and an atherosclerotic fibrous plaque with high collagen content (**b**). *Scale bar=250 μm.* **c, d** Alpha-smooth muscle actin (SMA)-stained sections showing the presence of smooth muscle cells in NCFAs. The fibrous cap in **c** shows diminished α -SMA staining indicative of low SMC content, and in **d** shows large numbers of SMCs. *Scale bar=500 μm.* *FC* fibrous cap, *NC* necrotic core

Fig. 2. Lipid-rich fibroatheroma with thick fibrous cap. **a** Trichrome-stained histology section. **b** Picrosirius red (PSR)-stained histology section. **c** Backscatter-intensity image. **d** Phase-retardation image (linear color scale degrees). **e** Fast-axis angle (linear color scale degrees). Reprinted from [32] with permission from the Optical Society of America. *CF* collagen fibers, *L* lipid

Fig. 3.

OCT and PSOCT images of fibrous plaques. **a, e, i** OCT images of fibrous plaques and **b, j** PSOCT images of fibrous plaques showing high birefringence as seen by the rapid transition of the image from black to white, corresponding to 0–180° phase retardation. **c** Picrosirius red (PSR)-stained histology section, showing orange-red fibers (thicker fibers) under polarized light microscopy. **d, h** Trichrome-stained histology images. **f** PSOCT image of fibrous plaque showing black region corresponding to low birefringence below the luminal surface. **g** Corresponding PSR-stained histology section showing yellow-green (thinner fibers) under polarized light microscopy. **k** Corresponding PSR-stained section shows low collagen content in the plaque under polarized light microscopy. **l** Corresponding histology section stained for α -smooth muscle actin shows numerous SMCs within the fibrous plaque. Bars=500 μ m. Permission requested from Elsevier to modify figure from [37]

Fig. 4. **a** OCT image, and **b** PSOCT image, of human coronary obtained with an intracoronary imaging catheter. **c** Trichrome-stained section. Reproduced from [38] with permission from the Optical Society of America

Fig. 5.

a Schematic showing coherent laser light propagating through an NCFA. Interference between photons after multiple scattering events returning from different regions within the tissue results in a laser speckle pattern. **b** The color map shows the distribution of speckle decorrelation time constant across a plaque. The presence of a lipid-rich plaque with clearly demarcated borders is evident in the color map and is corroborated by the accompanying gross pathology photograph