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Diagnosis of Vulnerable Atherosclerotic Plaques by Time-Resolved Fluorescence Spectroscopy and Ultrasound Imaging

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Abstract— **In this study, time-resolved laser-induced fluorescence spectroscopy (TR-LIFS) and ultrasonography were applied to detect vulnerable (high-risk) atherosclerotic plaque. A total of 813 TR-LIFS measurements were taken from carotid plaques of 65 patients, and subsequently analyzed using the Laguerre deconvolution technique. The investigated spots were classified by histopathology as Thin, Fibrotic, Calcified, Low-Inflamed, Inflamed and Necrotic lesions. Spectral and time-resolved parameters (normalized intensity values and Laguerre expansion coefficients) were extracted from the TR-LIFS data. Feature selection for classification was performed by either analysis of variance (ANOVA) or principal component analysis (PCA). A stepwise linear discriminant analysis algorithm was developed for detecting Inflamed and Necrotic lesion, representing the most vulnerable plaques. These vulnerable plaques were detected with high sensitivity (>80%) and specificity (>90%). Ultrasound (US) imaging was obtained in 4 carotid plaques in addition to TR-LIFS examination. Preliminary results indicate that US provides important structural information of the plaques that could be combined with the compositional information obtained by TR-LIFS, to obtain a more accurate diagnosis of vulnerable atherosclerotic plaque.**

Keywords—**Time-resolved fluorescence spectroscopy, Laguerre expansion technique, atherosclerosis.**

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I. INTRODUCTION

Rupture of a high-risk (vulnerable) atherosclerotic plaque is the major cause of fatal sudden cardiac events and strokes [1]. Improve in prevention of plaque rupture and its often deadly consequences will depend on accurate clinical assessment of plaque vulnerability and effective plaque stabilization therapies. Advances in diagnosis and treatment of VP will depend, in turn, on the ability to detect and monitor in-vivo cellular and molecular mechanism involved in plaque vulnerability and/or resulting from therapeutic interventions.

Plaque composition (i.e. lipid content), activity (i.e. inflammation) and morphology (i.e. thin cap over a large lipid core) are key predictor factors for rupture [1]. Thus, optimum methods for VP diagnosis should provide high-resolution structural definition of the plaque (i.e by IVUS, OCT), with compositional and functional definition (i.e. by NMR, Raman, NIR and/or fluorescence spectroscopy) [2].

Time-resolved laser-induced fluorescence spectroscopy (TR-LIFS) has the potential to detect biochemical changes in tissue due to pathological conditions [3]. In this paper, TR-LIFS instrumentation developed for clinical applications is briefly discussed. A computational framework for TR-LIFS based diagnosis is also presented. Within this framework, system identification is applied to extract spectral and time-resolved information from the tissue autofluorescence. Such information is subsequently correlated to the tissue histopathology in order to develop classification algorithms for tissue diagnosis.

The application of TR-LIFS based diagnosis to the detection of inflammation in vulnerable atherosclerotic arteries is reported. Potential synergetic combination of TR-LIFS and intravascular ultrasound (IVUS) for the detection of vulnerable atherosclerotic plaque is evaluated.

II. METHODOLOGY

A. Clinical model

The human carotid atherosclerotic plaque was used as clinical model for diagnosis of plaque vulnerability. TR-LIFS measurements were taken from fresh resected human carotid plaques, obtained from 65 patients undergoing carotid endarterectomy. As part of a preliminary study, 4 of these plaques were further investigated with a high-resolution ultrasound imaging system.

B. Instrumentation

The experiments were conducted with a TR-LIFS prototype system, recently developed by our group and described in detail elsewhere [4]. Briefly, autofluorescence of tissue was induced with a pulsed nitrogen laser (wavelength 337 nm, pulse width 700 ps) using a bifurcated fiber-optic probe. The collected fluorescence was dispersed by an imaging spectrometer/monochromator and detected with a gated multi-channel plate photo-multiplier tube (rise time 180 ps). The fluorescence was temporally resolved using a digital

oscilloscope (bandwidth 1 GHz, sampling rate 5 Gsamples/s) coupled to a preamplifier (bandwidth 1.5 GHz). Ultrasound images were obtained by means of a scanning system with a high-frequency (40 MHz) single-element transducer.

C. Data acquisition

During operation, the diseased artery was opened and the plaque was removed en-bloc according to usual procedure. Immediately after explantation, TR-LIFS measurements, consisted of full spectrum scans ranged from 360 to 550 nm with increments of 5 nm, were obtained from different locations on the plaque. After each measurement sequence, the laser pulse temporal profile was measured. US images from the lumen side were obtained after TR-LIFS in four of the plaques. All investigated specimens undergone routine histological processing. Collagen, elastin, macrophages/foam-cells and smooth muscle cell contents were quantified by two pathologists specialized in cardiovascular diseases.

D. Data Analysis

The TR-LIFS data was deconvolved using the Laguerre expansion technique [5]. The relationship between the fluorescence response y(n) and the laser pulse x(n) is expressed by the convolution equation:

$$y(n) = T \cdot \sum_{m=0}^{M-1} h(m)x(n-m) \qquad (1)$$

The parameter M in (1) determines the extent of the system memory, T is the sampling interval, and h(m) is the intrinsic FIRF. The Laguerre expansion technique uses the orthonormal set of discrete time Laguerre functions $b_j^{\alpha}(n)$ to discretize and expand the fluorescence IRF:

$$h(n) = \sum_{j=0}^{L-1} c_j b_j^{\alpha}(n) \qquad (2)$$

In (2), c_j are the unknown expansion coefficients, which are to be estimated from the input-output data by generalized linear least-square fitting; $b_j^{\alpha}(n)$ denotes the jth order orthonormal discrete time Laguerre function [5]; and L is the number of Laguerre functions used to model the IRF. The Laguerre parameter ($0 < \alpha < 1$) determines the rate of exponential decline of the Laguerre functions.

Once the fluorescence IRF's were estimated, the steady-state spectrum (I_W), was computed by integrating the FIRF along the time domain. To characterize the temporal dynamics of the fluorescence decay, two sets of parameters were used: 1) the average lifetime (τ_A); and 2) the normalized value of the corresponding LEC's ($c_j, j = 0,\ldots,L-1$). Thus, a complete description of the atherosclerotic sample fluorescence emission was given by the variation of these spectroscopic parameters (I_W, τ_A, and $c_j's$) as a function of emission wavelength λ_E (Fig. 1).

E. Classification

The normalized fluorescence intensity (I) and the Laguerre Expansion Coefficients (LEC) at each emission wavelength represent the initial feature space for classification. From this initial highly multidimensional set, a reduced number of features has to be selected. Two feature selection approaches were adopted: a) via analysis of variance (ANOVA) on each discrete TR-LIFS parameters at every wavelength, in which the wavelengths with the lowest P-value were chosen for each parameter to form the new feature space; b) via Principal Component Analysis (PCA) on each TR-LIFS parameter (I, LEC's) as a function of wavelength, in which the first three Principal Component Scores from each PCA transformation form the new feature space.

Based on previous work [6], a stepwise linear discriminant analysis (SLDA) approach was adopted for classification. The two set of features (ANOVA and PCA) were applied independently to optimize the discriminant functions, and the classification accuracy was assessed using a leave-one-out cross-validation approach. Two classification goals were defined aiming to discriminate: a) Thin, Fibrotic/Calcified (as one group) and Necrotic/Inflamed (as one group) lesions; b) Thin, Fibrotic/Calcified/Low-Inflamed (as one group) and Necrotic/Inflamed (as one group) lesions.

III. RESULTS

A. Histology

A total of 813 TR-LIFS measurements were taken ex-vivo from the carotid plaques of 65 patients. Plaques were categorized based intima thickness and composition as: a) Thin (n=28), b) Fibrotic (n=420), c) Calcified (n=38), d) Low-Inflamed (n=273), e) Inflamed (n=34), and f) Necrotic (n=20). The Inflamed and Necrotic lesions corresponded to vulnerable plaques.

B. Spectroscopic Parameters

The normalized spectrum for each plaque group is shown in Fig. 1 (upper left panel). There is significant overlap on the emission spectra of all groups, except for the "Thin" lesions, presenting a significantly higher normalized intensity between 400-500 nm. The spectral signature of the "Thin" lesions reflects the significant contribution of elastin (with peak emission at 440-450 nm) to the overall fluorescence emission of these plaques. The average lifetimes as a function of emission wavelength for each plaque group are also shown in Fig. 1 (upper right panel). The "Thin" lesions present significantly shorter lifetimes at wavelengths below 450 nm, while the "Necrotic" lesions present shorter lifetimes at wavelengths above 500 nm. The short and relatively stable lifetimes (as function of wavelength) of the "Thin" lesions reflects the significant contribution of elastin to the overall fluorescence emission of these plaques.

The Laguerre expansion coefficients (LEC) were also compared among the lesion groups (Fig.1, bottom panels). LEC-0 presented very similar trends as the average lifetimes. The lipid-rich "Inflamed" and "Necrotic" lesions presented significantly higher LEC-2 values at wavelengths above 450 nm. This was of special relevance, since these lesions represent some of the most common forms of vulnerable plaque. It is also interesting to notice that as the lesion progress from stable (Thin, fibrocalcified) to less stable (Low Inflamed) and very unstable (Inflamed/Necrotic), the LEC-2 is able to follow such trend.

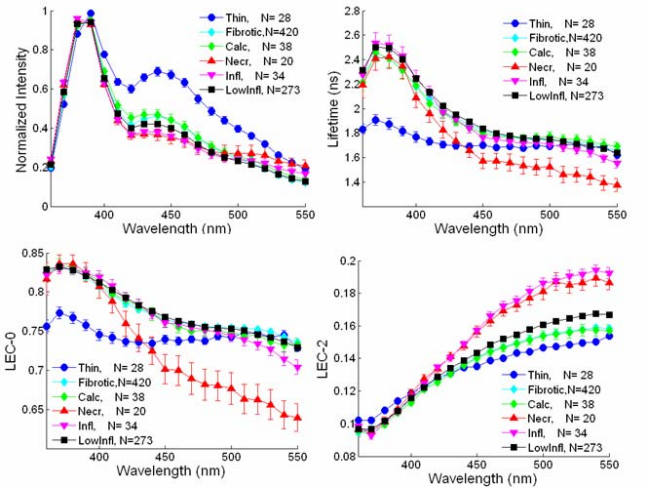

Fig. 1. Sample time-resolve spectrum and average normalized spectra (mean ± SE) for each of the aortic sample types.

C. Classification

The following 10 discrete and statistical (STAT) significant features were selected via ANOVA: I_{440}, τ_{380}, τ_{550}, $LEC0_{380}$, $LEC0_{550}$, $LEC1_{380}$, $LEC1_{550}$, $LEC2_{500}$, $LEC2_{550}$, $LEC3_{380}$. The PCA based feature extraction resulted on 18 features, corresponding to the first three Scores from the PCA analysis of I, τ, LEC0, LEC1, LEC2 and LEC 3. Classification with either ANOVA or PCA features provided similar performance.

When classifying Thin, Fibrotic/Calcified and Necrotic/Inflamed lesions, Thin lesions were discriminated with sensitivity (SE) and specificity (SP) >80%. However, there was an appreciable overlap between Thin and Fibrotic/Calcified lesions. On the other hand, Necrotic/Inflamed lesions could be detected with high SE >80% and even higher SP >90% from the rest of the more stable lesions.

When classifying Thin, Fibrotic/Calcified/Low-Inflamed and Necrotic/Inflamed lesions are shown below, Thin lesions were also discriminated with high SE/SP >80%, although appreciable overlap between Thin and Fibrotic/Calcified/Low-Inflamed lesions was also present. Necrotic/Inflamed lesions could still be detected with SE ~80% and high SP >90% from the rest of the more stable lesions. However, the accuracy of detecting these vulnerable plaques slightly decreased when Low-Inflamed lesions were included in the analysis. Classification results using ANOVA features are shown below.

TABLE I: CLASSIFICATION RESULTS

	TR-LIFS based prediction			SE (%)	SP (%)
	Thin	Fibro/Ca Low-Inf	Nec/ Inflam		
Thin (n=28)	25	3	0	89.3	81.0
Fibro/Ca Low-Inf (n=731)	149	519	63	71.0	81.7
Nec/Inflam (n=54)	0	12	42	**77.8**	**91.7**

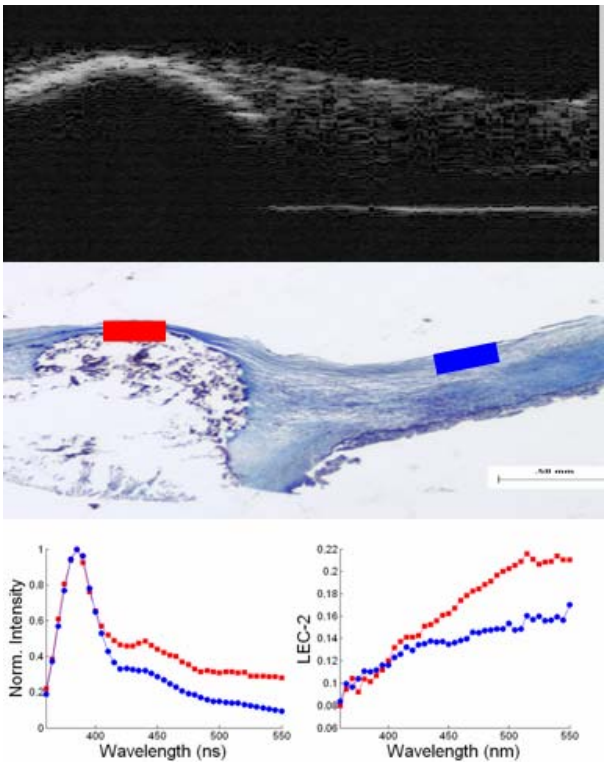

Fig. 2. Correlation of US imaging (top), histopathology (middle), and TR-LIFS features (bottom).

D. Histology, US and TR-LIFS Correlation

A sample US image of a carotid section is shown in Fig. 2 (top). Plaque histology (middle) showed two regions: thin cap fibroatheroma (left), and fibrotic lesion (right). In the US image, the fibroatheroma appears as a hypoechoic region (left side), which could be also mistaken as calcification. The TR-LIFS measurement taken from that area (bottom), however, confirmed that the lesion corresponded to a necrotic and/or inflamed plaque, as indicated by the large value of LEC-2 at higher wavelengths (see Fig. 1). By integrating both US and TR-LIFS information, it was possible to determine that the left region of the plaque

corresponded to a thin cap fibroatheroma. This result suggests the potential of combining US and TR-LIFS information for a more complete plaque characterization. Analysis of US radiofrequency signal (RF) from lipid-rich and fibrotic lesions were also applied, including spectral analysis and integrated backscattering methods [7, 8]. However, no statistical difference was observed from RF parameters derived from lipid-rich and fibrotic lesions.

IV. DISCUSSION

Differences in morphology and biochemical composition of the investigated carotid plaques were reflected in their fluorescence time-resolved spectra. The steady state spectrum from early thin fibrous lesions differentiated from the spectra of more advanced lesions, while the fluorescence decay characteristics from necrotic/inflamed lesions differentiated from fibrous and calcified lesions.

More interesting, the Laguerre expansion coefficient LEC-2 was able to discriminate the inflamed lesions, which is of special clinical interest. This result provides strong indication that the Laguerre expansion coefficients offer a totally new domain of representing the time-resolve information of tissue fluorescence emission in a very compact, accurate, complete and computationally efficient way.

Although in the present study the complete fluorescence emission spectrum was measured, our results indicate that spectroscopic information relevant for discrimination is contained in a reduced number of discrete emission wavelengths (380 nm, 440 nm, 500 nm and 550 nm). This evidence is of special importance, because it allows reducing significantly the acquisition time, making it practicable to perform in-vivo investigations.

Linear discriminant analysis on a few spectral and time-resolved parameters provides a means for detecting early lesions from more advanced lesions, and more important, the presence of necrosis and inflammatory cells (foam cells and lymphocytes) in the plaques, which constitute important markers of plaque vulnerability. Future effort will be place on investigating if more advance classification schemes (i.e. support vector machines, Bayesian neural networks) would improve diagnosis accuracy.

The accuracy of detecting these vulnerable plaques slightly decreased when Low-Inflamed lesions were included in the analysis. This was expected, since the Low-Inflamed lesions represent a large and highly variable group containing focal and/or scattered accumulation of lipids in the form of foam-cells and/or necrosis. Although the hitopathological division of the plaques between Low-Inflamed and Necrotic/Inflamed lesions (with a threshold of 20% inflammation/necrosis) provides a good staring point for investigating the potential of TR-LIFS for detecting vulnerable plaque, other potentially more suitable histopathological criteria should be further evaluated for a more precise assessment of the TR-LIFS based diagnosis accuracy.

Classification by TR-LIFS alone, however, was not able to distinguish fibrotic from fibrocalcified lesions. Similarly, analysis of the US RF data alone did not show difference between lipid-rich and fibrotic lesions in our sample pool, although other studies have shown some promising results [7, 8]. Thus, it is very probably that a combination of TR-LIFS technique with other imaging modalities, such as intravascular ultrasound (IVUS), would allow a better discrimination of the morphological and compositional features of atherosclerotic plaques. The preliminary results presented in this paper suggest that US can provide structural definition of the plaque and identify calcification, while TR-LIFS can detect fibrotic caps, necrosis, and inflammation.

V. CONCLUSION

This study demonstrates the potential of applying TR-LIFS for the detection of important marker of plaque vulnerability, such as inflammation and necrosis. Detection of some other important marker, such as calcification and plaque structure would required alternative and/or complementary imaging modalities. Future research in our group will investigate the potential of combining TR-LIFS and IVUS modalities for the ex-vivo and in-vivo characterization of vulnerable plaques. We believe this new direction in our research efforts may provide better prediction of plaque vulnerability than single-modality approaches.

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