Sensors and Actuators B 219 (2015) 283–293

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Compact, non-invasive frequency domain lifetime differentiation of collagens and elastin

Rui Liu[a,1], Zhengtuo Zhao[a,1], Luwei Zou[a], Qiyin Fang[b], Lin Chen[c], Alan Argento[a], Joe F. Lo[a,*]

[a] Department of Mechanical Engineering, University of Michigan-Dearborn, Dearborn, MI, USA
[b] Department of Engineering Physics, McMaster University, Hamilton, ON, Canada
[c] Center for Wound Repair & Regeneration, University of Illinois-Chicago, Chicago, IL, USA

ARTICLE INFO

Article history:
Received 13 December 2014
Received in revised form 25 March 2015
Accepted 28 April 2015
Available online 18 May 2015

Keywords:
Collagen I
Collagen III
Elastin
Frequency domain
Fluorescence lifetime
Extra cellular matrix

ABSTRACT

Changes in the composition of type I and type III collagen in tissue can shed light on various diseases. However, many of the current collagen detection techniques require invasive and destructive tissue sampling. In this study, a low cost, low complexity light emitting diode (LED) based system was developed to realize both non-invasive detection and specific discrimination of collagen and elastin variations in tissue based on fluorescence lifetimes. Modulated LED excitation was applied to frequency domain (FD) fluorescence lifetime spectroscopy to calculate tissue autofluorescence lifetimes. Using this method, fluorescence lifetimes from collagen type I versus type III were clearly separated at 3.95 ns and 5.01 ns, respectively, distinct from the elastin lifetime at 6.78 ns. The probe was tested on bovine ocular tissues, with cornea showing much shorter average lifetime of 4.27 ns than sclera at 7.48 ns. Furthermore, measurements of an 8 mm murine skin wound at 14 days post-wounding also showed distinct, longer average lifetimes at 9.74 ns versus normal skin at 6.72 ns. This FD tissue detection technique can potentially offer a way to examine tissue structures and discern the underlying pathology nondestructively.

1. Introduction

Collagen is the main load-bearing structural protein in the extra cellular matrix (ECM) of biological tissue. The quantity, type, and orientation of collagen greatly influence the way tissues carry stress, stretch, and maintain integrity. Elastin reinforces the collagen network and provides resilience to tissues. To study the collagen and elastin composition in biological tissues, and their changes in diseases, a non-invasive detection technique is required. In this study, a general method is developed to detect and differentiate ECM proteins. Here, detections were demonstrated with type I collagen, type III collagen and elastin in the context of their measurements in skin wounds and ocular tissues [1–3]. The method uses a low cost, low complexity light emitting diode (LED) system to realize both non-invasive detection and specific discrimination of collagen and elastin variations in tissue. Modulated LED excitation was applied to frequency domain (FD) fluorescence lifetime spectroscopy to determine tissue autofluorescence lifetimes (Fig. 1). Using the novel LED-based system, fluorescence lifetimes from collagen type I, collagen type III and elastin can be clearly distinguished. These frequency domain-based lifetimes, especially the direct comparison of type I and III collagen, are reported here for the first time, in contrast to time domain measurements in the literature. The extension of the method to detect other collagen types and ECM components is also discussed. Due to the low cost of LED and photodiode components, low complexity of the fiber optic probe, and the possibility of integrating phase and demodulation analyses on an integrated circuit, the developed technique is well suited to portable applications. Applications in implantable sensors, field diagnostics, and clinical monitoring can benefit from this technique to measure collagen and elastin distributions in biological tissues over time.

1.1. Collagen and ECM composition of skin wounds and ocular tissue

In skin wounds, collagen remodeling occurring underneath a closing wound cannot be easily assessed by visual monitoring. The primary structural component of normal dermis is mostly collagen,

* Corresponding author at: Department of Mechanical Engineering, University of Michigan-Dearborn, HPEC 1332, 4901 Evergreen Road, Dearborn, MI 48128, USA. Tel.: +1 313 593 0913.
E-mail address: jfjlo@umich.edu (J.F. Lo).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.snb.2015.04.124
0925-4005/

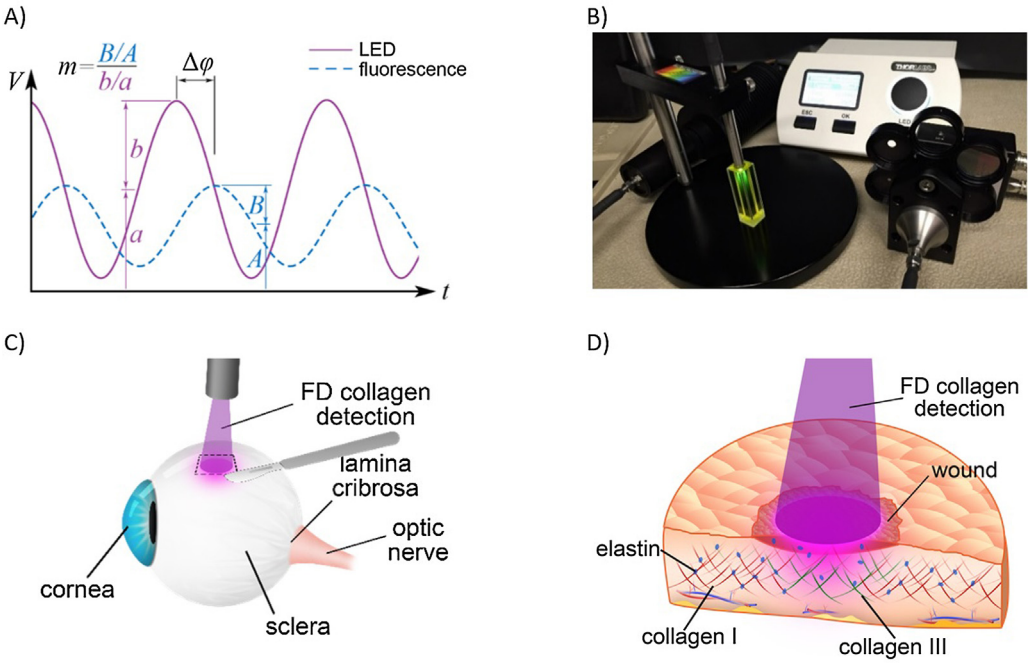

Fig. 1. Fiber based frequency domain collagen and elastin detection system. (A) Frequency domain fluorescence lifetime is based on the phase shift delay and amplitude demodulation of the fluorescence emission when a sample is excited by a modulated light source. (B) The components of the systems are compact and cost-effective. This system can be applied to (C) non-invasive probing of ocular tissue, and (D) non-contact monitoring of wound healing. (Note that ocular and skin tissues were cut into samples and measured in this study.)

with elastin, glycoproteins, and glycosaminoglycans also present. During the proliferation phase, fibroblasts proliferate in the wound and produce ECM, especially collagens, to replace the provisional matrix formed in homeostasis/inflammatory stage of the earlier wound healing process to establish scar tissue [4]. Normal skin and wounds that have undergone collagen remodeling contain more mature cross-linked collagen I fibers, while immature wounds contain more parallel collagen III fibers [5,6]. Elastin is another major protein component of the ECM produced by fibroblasts and smooth muscle cells, which provide resilience to the skin and other tissues. It is abnormally expressed during skin wound healing which partially contributes to the impaired breaking strength of scars compared with unwounded skin [7]. Restoration of normal ECM architecture is necessary for skin wounds to gain the tensile strength that is required to maintain tissue integrity [1]. Full thickness murine wounds have been used as important models to study wound healing, including collagen remodeling processes.

In the eye, the type and orientation of collagen fibers vary by region and are closely related to the eye's functions [2,8,9]. For example, in the peripapillary region where the optic nerve exits the sclera, there is a predominant circumferential fiber alignment [10] that likely functions to control deformation and stress in the optic nerve head tissues. Similar to skin and other connective tissues, sclera is primarily composed of a matrix containing collagens, elastin [11], and proteoglycans [12]. The result is a tissue having strength and viscoelastic properties that help to protect the eye from severe loads, and also from brief elevations in intraocular pressure due to eye movements and other events such as rubbing. In the cornea, the lamellae are composed of collagen fibrils of uniform diameter running parallel to one another; however, in the sclera the collagen fibrils have varying diameter with an irregular branching pattern [13,14]. Human eye tissue contains about 90% type I collagen and less than 5% type III collagen [2]. Levels of type III collagen in the bovine cornea average less than 1% in animals older than 3 months [15,16]. Disorders in the ocular structure play a role in diseases such as glaucoma [17,18] which manifests as damage to the tissues of the optic nerve head, and the corneal thinning disease keratoconus [19]. Structural problems of the sclera have also been implicated in myopia [20]. Abnormal elastin has been found in the lamina cribrosa tissue, which bridges the opening in the sclera at the optic nerve head, of glaucomatous eyes [20]. A non-invasive assay of tissue would aid research into eye disorders such as these as well, as skin wounds discussed above, and could eventually be used as a diagnostic tool.

1.2. Existing ECM detection techniques

Several techniques are currently used to measure tissue ECM compositions, such as histological staining, histological autofluorescence, and non-invasive tissue optical biopsy. Tissue histology has well-established analysis and quantification methods in techniques such as immunofluorescence [21,22] and picrosirius staining microscopy [23,24]. However, histology requires invasive sampling and destructive sectioning to produce samples for measurement. On the other hand, laser-induced tissue fluorescence and microscopy techniques have been developed for non-invasive tissue monitoring, but have not enabled differentiation of collagen types specifically [25–27]. Laser fluorescence and microscopy instrumentation is also prohibitively costly and complicated for widespread biomedical application.

1.3. Characteristics of collagen and tissue autofluorescence

Autofluorescence from collagen (i.e. without staining) is commonly observed in cell and tissue microscopy. The major collagen fluorophores are lysine derived pyridinium, tyrosine, and phenylalanine groups [28–31], which can be affected by crosslinking, glycation, and their compositions in different types of collagens [32–34]. Collagen autofluorescence, in combination with other endogenous fluorophores, can provide differentiation between normal and cancerous tissues [35,36], promising tumor demarcation in minimally invasive surgeries [37,38]. Moreover, collagen