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Segmentation of elastic fibres in images of vessel wall sections stained with Weigert's resorcin–fuchsin

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ABSTRACT

Background and objective: The elastic fibres are an essential component of the extracellular matrix in blood vessel walls that allows a long-range of deformability and passive recoil without energy input. The quantitative determination of elastic fibres will provide information on the state of the vascular wall and to determine the role and behaviour of this key structural element in different physiological and pathological vascular processes.
Methods: We present a segmentation method to identify and quantify elastic fibres based on a local threshold technique and some morphological characteristics measured on the segmented objects that facilitate the discrimination between elastic fibres and other image components. The morphological characteristics analysed are the thickness and the length of an object.
Results: The segmentation method was evaluated using an image database of vein sections stained with Weigert's resorcin–fuchsin. The performance results are based on a ground truth generated manually resulting in values of sensitivity greater than 80% with the exception in two samples, and specificity values above 90% for all samples. Medical specialists carried out a visual evaluation where the observations indicate a general agreement on the segmentation results' visual quality, and the consistency between the methodology proposed and the subjective observation of the doctors for the evaluation of pathological changes in vessel wall.
Conclusions: The proposed methodology provides more objective measurements than the qualitative methods traditionally used in the histological analysis, with a significant potential for this method to be used as a diagnostic aid for many other vascular pathological conditions and in similar tissues such as skin and mucous membranes.

1. Introduction

The observation and measurement methods used on histological samples in published studies vary widely. Traditionally, vessel wall structures quantification has been performed using both biochemical [1] and stereological methods [2], and lately by techniques of digital image analysis and processing.

Digital image analysis allows more objective, sensitive and accurate quantitative assessments than visual qualitative methods. These techniques are applied to images to improve their quality, interpretation or provide tools to extract information from them [3]. The methods of image segmentation provide an initial approximation of the structure of interest based on morphological characteristics such as size, shape, colour, etc., and later the quantification of these elements [4]. But histological images may be affected by various factors that hinder analysis, such as overlying tissues,

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folds, variations in colour and brightness, blur areas, etc., which complicates the task of identifying the structures of interest.

Elastic fibres are one of the most studied components of the vascular wall by histological methods. Elastic fibres are an essential component of the extracellular matrix in blood vessel walls that allows a long-range of deformability and passive recoil without energy input. In certain pathological conditions some enzymes in the extracellular matrix are able to cleave elastic fibre molecules and they are scattered in the vascular wall.

Elastic fibres are composed of an inner core of amorphous crosslinked elastin, surrounded by an outer microfibrillar mantle [5]. Elastin has elastomeric properties from which derives its role in the structural integrity and functioning of large arteries and veins. In addition, it is a critical autocrine factor that regulates the vascular smooth muscle cell life cycle and smooth muscle tissue organization having effects over the smooth muscle cell and their proliferation [6–8]. Elastic fibres are degraded and fragmented with age and disease, leading to an increased stiffness of the vessel wall. Age-related alterations in the mechanical properties of the vascular system have profound effects on human morbidity and mortality [9]. Quantitative determination of elastic fibres will allow us to know the state of the vascular wall and to establish the role and behaviour of this key structural element of the extracellular matrix in different physiological and pathological vascular processes, determining its statistical and clinical significance.

In histological studies the more specific stain techniques for elastic fibres are Orcein, Verhoeff, Weigert's resorcin-fuchsin and Aldehyde-fuchsin method. The Weigert's resorcin-fuchsin method can be combined with eosin or trichrome methods like van Gieson to increase the number of structures stained.

Although digital image analysis is applied in a growing number of studies in many areas of biology and medicine, to the best of our knowledge, few studies address the quantitative analysis of elastic fibres by using these techniques in the study of blood vessels.

In the work of Abe et al. [10] the specimens from 38 patients were stained with Elastica van Gieson and elastic fibres were segmented using a technique known as colour slicing where pixels within a specific colour range are segmented. User defines the colour range and the technique is suitable when the colour range of interest is disjoint from the colours of any other image component. Similar technique is used in the study of Elsharawy et al. [11] where specimens from 35 patients were stained with Verhoeff van Gieson. In the works of Regadera et al. [12] and Velasco [13] the authors used the colour deconvolution method [14] implemented by a plugin in the program ImageJ to quantify specimens, from 10 and 30 patients respectively, stained with Orcein, where we hypothesize that they used the built-in H-DAB stain vector provided by the plugin.

Above techniques are not suitable to the particularities of our specimens where the staining hue is not homogeneous across the tissue. In addition, above studies did not take into account the presence of certain artefacts such as tissue folds, spots, etc. that can lead to misinterpretation in the identification of elastic fibres.

We tried the quantification of elastic fibres using samples of health and pathologic magna saphenous vein from patients affected by insufficiency venous disease. In this pathologic condition the venous wall became weakness as a result of structural problems. In the saphena magna vein is possible to distinguish three layers: intima, media and adventitia. The intima is the innermost layer constituted by endothelium, basal lamina and scarce extracellular matrix of connective tissue, the media is the middle layer formed by smooth muscle cells and extracellular matrix, and in the adventitia layer predominates the connective tissue. Structured elastic fibres usually appear between the intima and media layers and between the media and adventitia in health veins. Elastic fibres are also scattered in the different layers mainly in adventitia, and their disposition and amount vary depending on the physiological and pathological conditions. Each of these layers is depicted in Fig. 1.

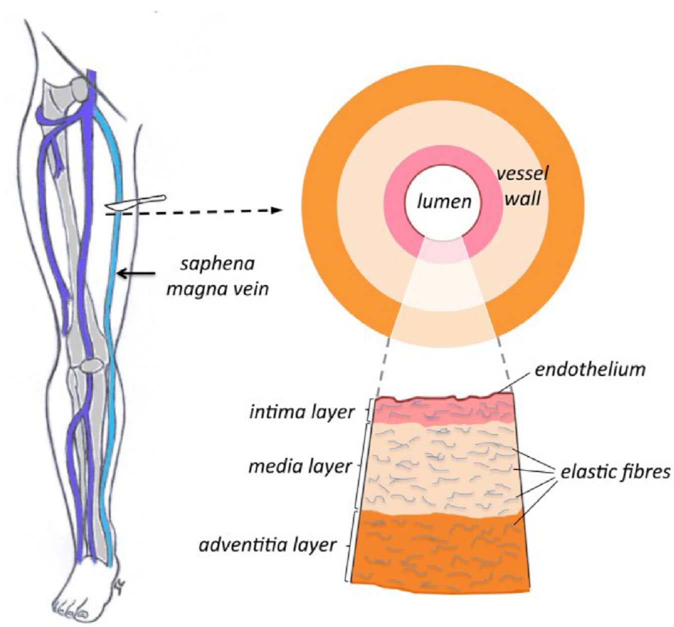

Fig. 1. Location and transverse section of the saphena magna vein.

The vein sections were stained with Weigert's resorcin–fuchsin in which elastic tissue fibres became stained in brown to purple colour. This work proposes a segmentation method to identify and quantify elastic fibres on a vessel wall based on a local threshold technique and some morphological characteristics as thickness and length of the segmented objects that facilitate the discrimination between elastic fibres and other image components. Several experiments were conducted using the hold-out evaluation method, with a validation set to find out an optimal parameter combination for this proposed method, and a test set to measure the performance. Therefore, the innovation is a novel and validated methodology for the segmentation, identification and quantification of the elastic fibres on a vessel wall.

The paper is organized as follows. Section 2 describes the image database used in this work and the proposed segmentation method. Section 3 presents the performance results, Section 4 discusses the experimental results, and the conclusions are drawn in Section 5.

2. Material and methods

This section describes the image database and the image acquisition procedure, followed by the description of the segmentation method to identify and quantify elastic fibres.

2.1. Sample preparation

A total of 31 samples of long saphenous vein were collected from several patients of both sexes and different ages who underwent saphenectomy by venous insufficiency and from cadavers without chronic venous disease, which were subjected to necropsy by legal reasons in the Institute of Legal Medicine of Las Palmas de Gran Canaria. The vein samples were taken from different anatomical regions of the saphena vein: thigh, knee and ankle.

Samples were placed in 10% paraformaldehyde and processed for embedding in paraffin, which were sliced into 7 μm thick sec-