Acta Histochemica 126 (2024) 152209

Contents lists available at ScienceDirect

Acta Histochemica

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

The elastic system: A review of elastin-related techniques and hematoxylin-eosin/phloxine applicability for normal and pathological tissue description

Thalles Fernando Rocha Ruiz [a,b,*], Luara Jesus Ferrato [b], Lorena Gabriela de Souza [b], Gervásio Evangelista Brito-Filho [b], Ellen Cristina Rivas Leonel [c], Sebastião Roberto Taboga [b,**]

[a] Department of Structural and Functional Biology, Institute of Biology, University of Campinas – UNICAMP, SP, Brazil
[b] Microscopy and Microanalysis Center, Department of Biological Sciences, Institute of Biosciences, Humanities and Exact Sciences, São Paulo State University – UNESP, SP, Brazil
[c] Animal Molecular and Cellular Biology Group (AMCB), Louvain Institute of Biomolecular Science and Technology (LIBST), Université catholique de Louvain, Place Croix du Sud 4-5, Louvain la Neuve 1348, Belgium

ARTICLE INFO

Keywords:
Elastin
Histotechnology
Connective tissue

ABSTRACT

The elastic system is one of the most developed interstitial elements in connective tissue. With diverse functions, pre-elastic and elastic fibers contribute to the distensibility and malleability of several organs. Also, micro-analyses of the elastic system were obtained by different histological techniques that were employed over years to describe normal and pathological conditions. Compared to conventional stains, hematoxylin-eosin/phloxine (HE/P) under fluorescence and confocal microscopy presented a highly detailed observation of the elastic system in different organs and scenarios. This technique provides a better demarcation of the elastic fibers, favoring their description in relation to their deposition and aggregation in different organs. Also, fibrils with low aggregation or loss of this characteristic are observed in an optimal view in the skin, heart valves, and large-caliber blood vessels. Degradation, fragmentation, and rupture were also well described by the HE/P technique. Several organs, such as the mammary gland, prostate, skin, aorta, and lung, could be described with precision under this technique. In association with non-linear microscopy, the results of the research presented in this paper improved and detailed characteristics of precise pathogenesis. Thus, the HE/P technique presented an interesting efficiency to demonstrate alterations and structures in which the elastic system showed a relevant role, and when compared to other techniques it demonstrated a similar or better result. In addition, it is expected that future studies can reveal more information about the elastin and interactions with specific dyes, thus allowing a greater understanding of the great efficiency of this technique.

1. Introduction

The connective tissue is an organized system in which cell population is mostly composed by fibroblasts. Such cells are responsible for synthesizing a variety of extracellular matrix (ECM) types, depending on its function and localization. Connective tissue proper, for example, has the filling function of attaching different tissues and produces an ECM mostly composed of collagen and elastic fibers. Being the most abundant type of protein in the mammalian organisms, the collagen fibrils are composed by three coiled α-chains, which form fibers with a wide variety in structure and function (Ross and Pawlina, 2016). Elastic fibers, in contrast, consist in an association of amorphous and microfibrillar components that provide a rubber-like network (Rosenbloom et al., 1993; Berk et al., 2012). This network stores potential energy, required for maintaining vital functions such as blood flow during diastole (Faury, 2001; Cocciolone et al., 2019) or air inhalation (Liem, 1988;

List of Acronyms and Abbreviations: HE/P, Hematoxylin-eosin/phloxine; ECM, Extracellular matrix; TEM, Transmission electron microscopy; HE, Hematoxylin-eosin; DUET, Dual-mode Emission Transmission microscopy; IAPP, Idiopathic atrophoderma of Pasini and Pierini.

* Corresponding author at: Department of Structural and Functional Biology, Institute of Biology, University of Campinas – UNICAMP, SP, Brazil.
** Correspondence to: São Paulo State University (Unesp), Department of Biology, Institute of Biosciences, Humanities and Exact Sciences, Cristóvão Colombo St., 2255, Jardim Nazareth, São José do Rio Preto, SP 15054-000, Brazil.
E-mail addresses: thalles.ruiz@unesp.br (T.F.R. Ruiz), sebastiao.taboga@unesp.br (S.R. Taboga).

https://doi.org/10.1016/j.acthis.2024.152209
Received 7 March 2024; Received in revised form 24 September 2024; Accepted 15 October 2024
Available online 22 October 2024
0065-1281/Published by Elsevier GmbH.

Mecham, 2018).

Molecules for elastic fiber synthesis are secreted by fibroblasts, endothelial cells, smooth muscle cells, and keratinocytes (Kajiya et al., 1997; Mecham et al., 1985; Narayanan et al., 1976; Ozsvar et al., 2021; Uitto et al., 2013; Wise et al., 2009). Among these components, two proteins participate in the core structure of elastic fibers (Fig. 1): the fibrillin microfibrils and the elastin central core. Elastic microfibrils are mainly composed of fibrillin-1. This protein is inserted around elastin macromolecules and form a core for elastic fiber aggregation with fibrillin-2 (Kielty et al., 2002; Lockhart-Cairns et al., 2020; Yanagisawa and Davis, 2010). Fibronectin and transglutaminase also participate in microfibril stabilization. Finally, elastogenesis is concluded with the aggregation of tropoelastin molecules to the microfibrils (Fig. 1), by cross-linking that forms desmosine and isodesmosine (Fig. 2) (Schmelzer et al., 2020). Elastin may arrange to form flattened sheets, meshwork or elongated fibers, which are interspersed with collagen fibers and connective tissue cells (Fig. 3). For example, in the loose connective tissue, a 0.2–1.5 μm thick network of elastic fibers is present; in the aorta, the elastic lamina is organized by layers of 1–10 μm sheets (Ushiki, 2002; Yanagisawa and Wagenseil, 2020).

Methods for elastic fiber identification and morphological analysis have been signaled taking in consideration its molecular aspects for studying its physiological or pathological process of degeneration and senescence (Heinz, 2021). Scanning electron (Torre et al., 2021) and atomic force microscopy (Ronchetti et al., 1998; Sambani et al., 2023) are standard techniques that properly allow analysis of the elastic fibers 3D surface. In transmission electron microscopy (TEM) (Fig. 3), tannic acid is applied in the fixative process for a better identification and distinction of elastic fibers from other ECM components (Cisne et al., 2018; Ferraz de Carvalho and König, 1982). In addition, light

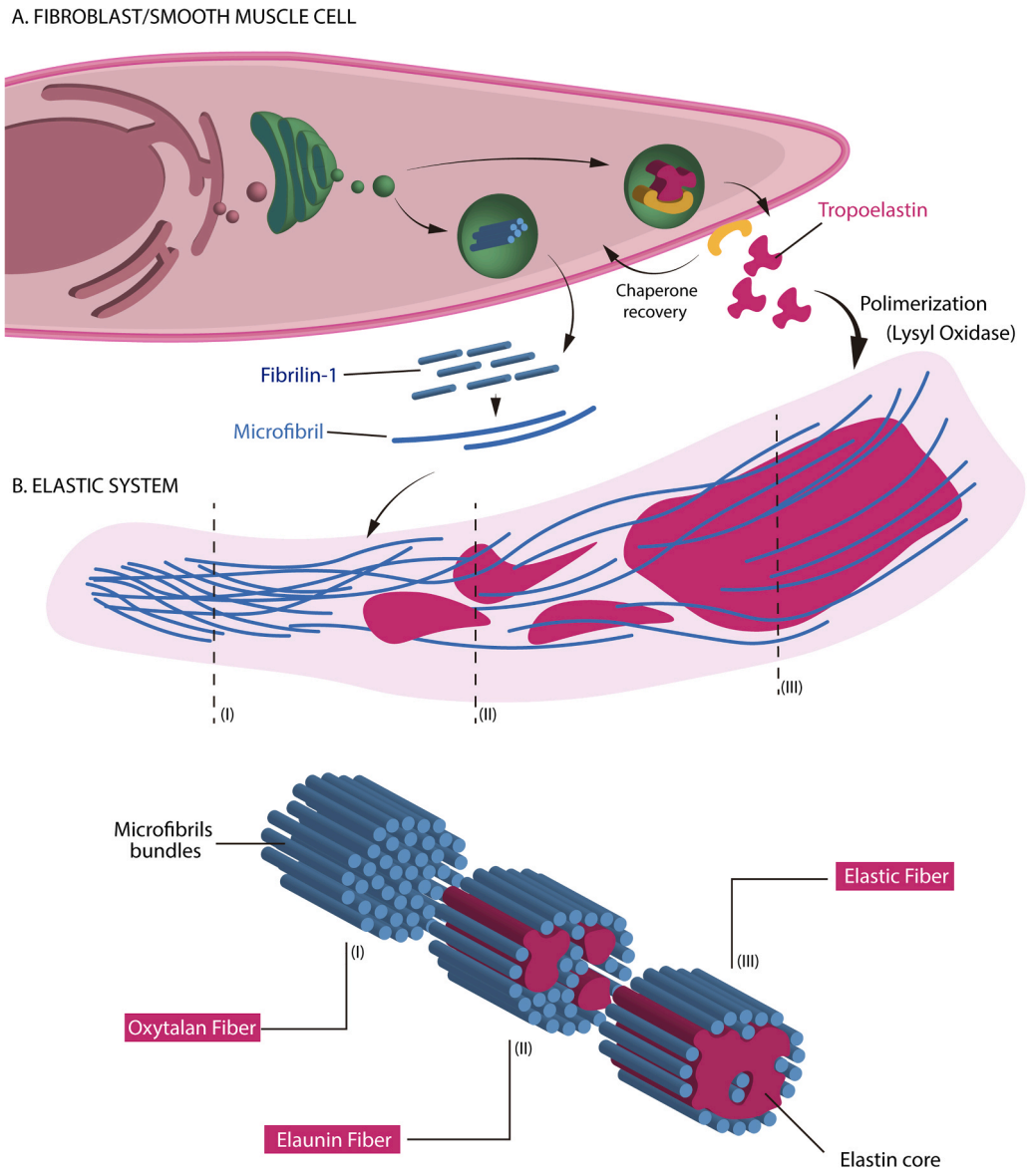

Fig. 1. Elastic fibers synthesis, deposition, and microarchitecture. (A) Intracellular tropoelastin and fibrilin-1 synthesis. The main proteins of elastic fibers, fibrillin-1 and elastin, are synthesized and secreted via common biosynthetic pathway of fibroblast and smooth muscle cells. Fibrillin is secreted and quickly polymerized into microfibrils, which comprises the fibrillar scaffold to elastin deposition. Elastin is pre-formed as tropoelastin associated to a chaperone protein. In the extracellular space, tropoelastin is polymerized in a central region of microfibril bundles, a mechanism catalyzed by lysyl oxidase (LOX), a copper-dependent enzyme. Its deposition is irregular among microfibrils scaffold. (B) Elastic System. Irregular deposition of elastin demonstrates different subtypes of fibers in the elastic system. (I) Oxytalan fibers are pre-formed fibers composed only by fibrillin-microfibrils bundles. (II) Elaunin fibers are called pre-elastic fibers due their low elastin content. (III) Elastic fibers are the main fiber and the mature shape found in elastic system, notably due to its elastin core.

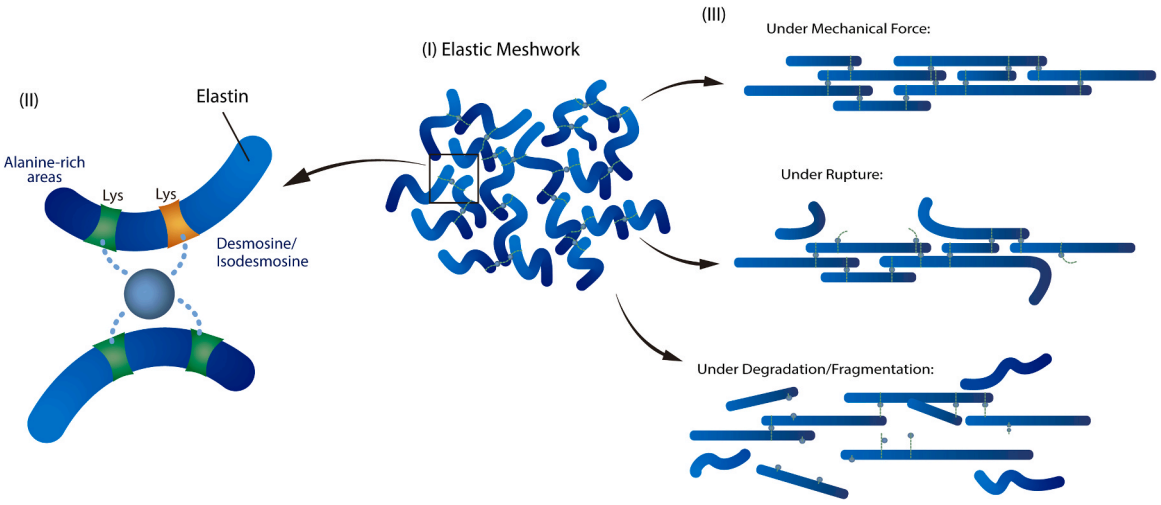

Fig. 2. Elastic fiber meshwork and interaction. (I) After assembling, the function of elastic fibers occurs after its fibril's association to an interconnected network. (II) This interconnection is established between alanine-rich signaling regions in the elastin, in which desmosine or isodesmosine molecules are covalently linked to lysine (Lys) sites. (III) In this way, the elastin fibers are interconnected to form a distensible meshwork or being under rupture, degradation or fragmentation.

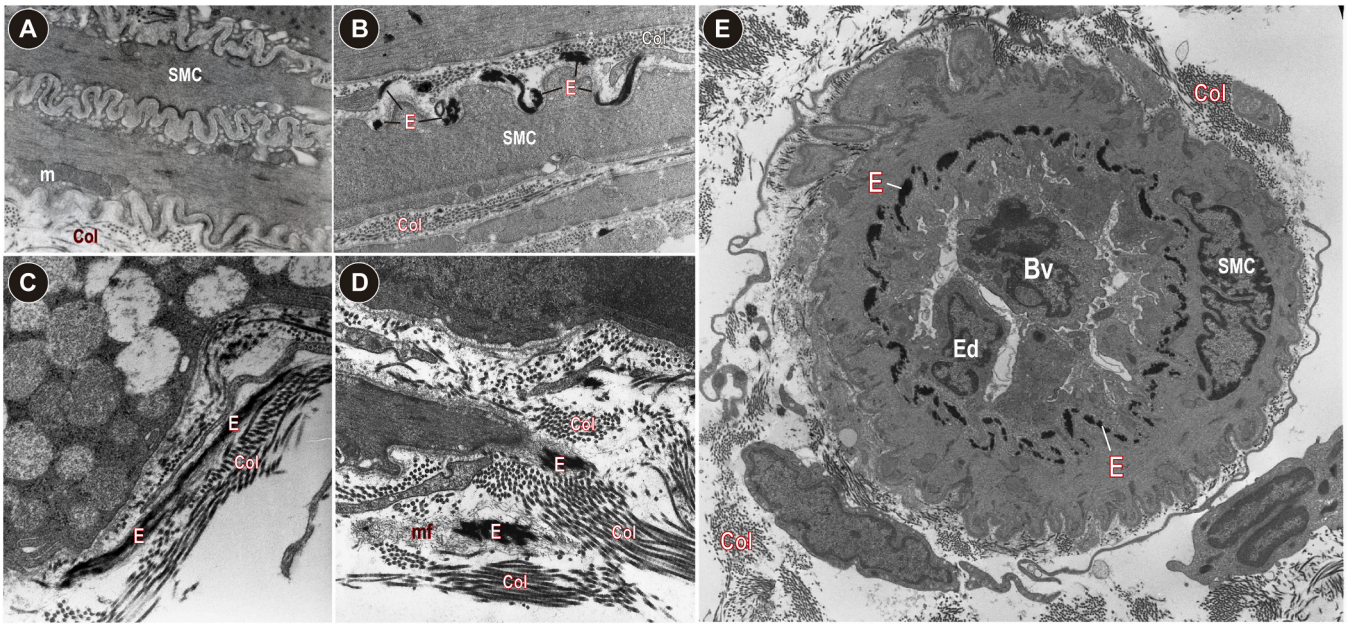

Fig. 3. Elastic fibers under transmission electron microscopy. (A-B) Smooth muscle sheets in prostatic stroma were able to synthesize elastic fibers under stress, such as by castration. In healthy conditions, smooth muscle cells do not present synthetic activity related to elastic system (A). Under prostatic stromal remodeling, these cells can synthetize elastic fibers, observed among cytoplasmic projections (B). (C-D) Elastic fibers in longitudinal and transversal sections; note microfibrils bundles in association with elastic core. (E) Blood vessel highlighting elastic lamina. Elastic fibers are well demonstrated after tannic acid preparation under electron microscopy procedures. *Abbreviations*: E: elastic fiber; Col: collagen; SMC: smooth muscle cells; mf: microfibrils; m: mitochondria; Bv: Blood vessel; Ed: endothelial cells.

microscopy (Fig. 4) is a faster and easier method for performing such analyzes; in this aspect, cytochemical established methods, such as Hart's (Davis and Li, 2017), fuchsin and phloxine, and immunohistochemical techniques (Sawada et al., 2006; Shen et al., 2022) were described.

Moreover, ECM is a complex arrangement of macromolecules that exert not only an accurate biochemical and signaling role among cells and tissues, but also adhesion and mechanical functions (Ross and Pawlina, 2016). In this aspect, the growing focus of the scientific community in the field of tissue engineering, searching for regenerative therapies has raised interest in the composition and mechanisms involved in ECM synthesis and maintenance (Ouni et al., 2020). Allied to that, the fact that tumor development is directly dependent on ECM structural aspects is another element that contributes to the growing interest on the investigation of its morphological aspects (Di Martino et al., 2021). In terms of metastatic features, ECM influences cell motility by mechanisms that may impact polymerization and depolymerization of intracellular – cytoskeletal – filaments (Kim et al., 2016).

The paper of De Carvalho and Taboga (1996) presents the conventional technique of hematoxylin-eosin/phloxin (HE/P) as a relevant tool for analysis of the elastic system in several biological samples (De Carvalho and Taboga, 1996). The HE/P technique allied to fluorescence microscopy (Fig. 4 B-B') has been frequently used to describe normal and histopathological conditions. Since then, many works have been performed based on this technique, and advances in the field have been reached in terms of synthesis (Shemesh et al., 2018) and degradation (Antoniassi et al., 2020; Vilamaior et al., 2003) of elastic fibers. Thus, this review is dedicated to present relevant data in the literature as well

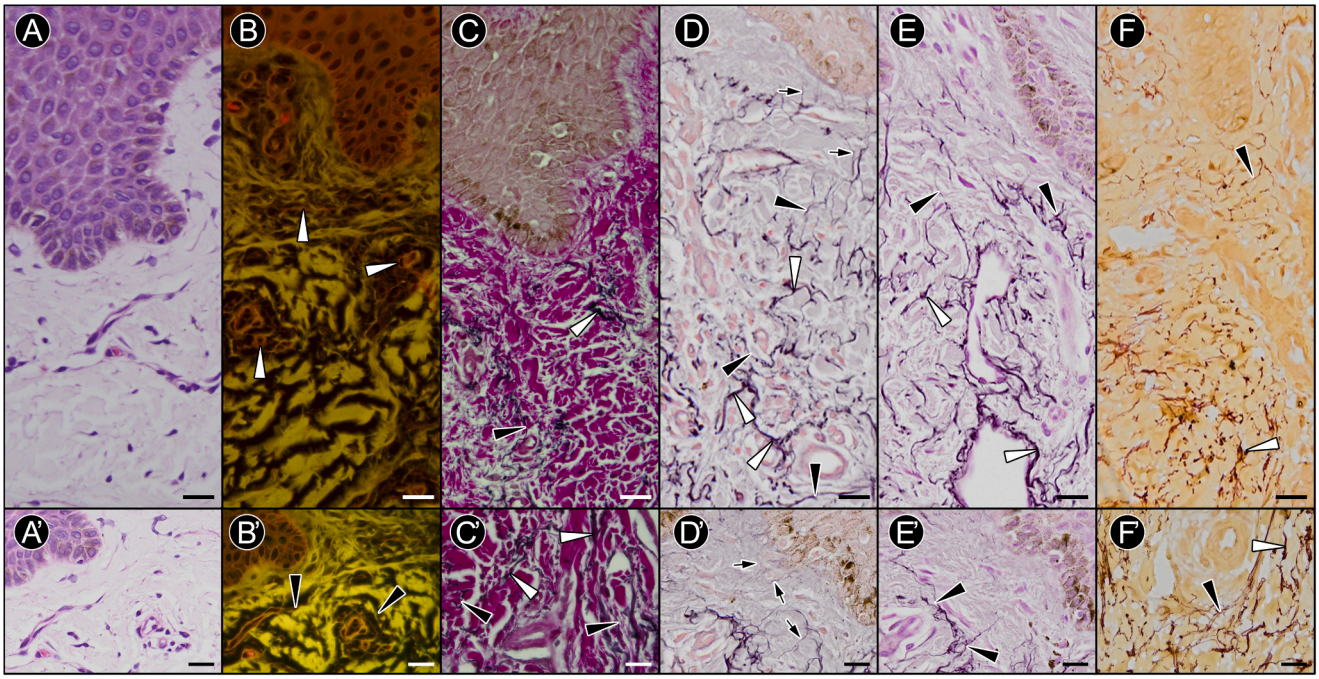

Fig. 4. Histochemical staining methods for elastic system detection. Rodent skin tissue was observed in serial depth sections and stained with different dyes and techniques. (A-A') Hematoxilin-eonsin/phloxine under light microscopy. (B-B') Hematoxilin-eonsin/phloxine under confocal/fluorescent microscopy. The excitation spectrum ranges between 530 and 550 nm, while the emission spectrum is above 590 nm. This technique allows a good differentiation of mature elastic fibers (white arrowheads) from other matrix components, even under 400x (B') magnification. In this technique, elaunin fibers (black arrowheads) are identified by its greenish color. (C-C') The Verhoeff iron-hematoxylin shows a strong staining for elaunin and mature elastic fibers, but it does not distinguish the oxytalan fibers. (D-E') The Weigert's resorcin-fuchsin provides an efficient differentiation for elastic system fiber types. Peroxidation (D-D') allows identification of oxytalan fibers, the thinnest fibers of the elastic system. However, without peroxidation step (E-E'), this histochemical technique evidence matures (white arrowheads) and pre-elastic fibers (black arrowheads). (F-F') Orcein dye exhibits a strong stanning of thicker fibers (elaunin and mature elastic fibers) and facilitates their observation due to a less intense background staining. Elaunin fibers are better observed under a 400x magnification (F').

as the usability of this technique for describing histopathological phenomena. We also aimed to compare it with other commonly applied techniques, since there isn't much current information about it in the literature. Our main goal is to present (1) the techniques also employed for the detection of elastin showing the pros and cons of each one; (2) provide the description of homeostatic (normal physiological) conditions under HE/P technique, allowing an overview of elastic systems of different organs; and (3) demonstrate the use of this technique under histopathological and experimental conditions as a valuable tool.

2. Elastic fibers: cytochemical techniques and properties

2.1. Techniques applied for detection of the elastic system

The elastic fibers are composed by insoluble structures present in the ECM (Sherratt, 2009). In elastic system studies, some staining techniques allow the differentiation of such components (Table 1). In the conventional hematoxylin-eosin (HE) technique (Fig. 4 A-A'), the elastic fibers have an acid feature, being stained by hematoxylin (Table 2). However, this staining does not allow a clear differentiation between the fiber types in the system, since elastic fibers are thinner and may get overshadowed by other more abundant basophilic components (Fullmer and Lillie, 1958).

In the *in vivo* environment, elastic fibers are naturally acidophilic. However, after oxidation they acquire a basic character, which can be assessed by using different substances, such as periodic acid, peracetic acid or potassium permanganate. The oxidation of the fibers is important since some staining techniques are only able to stain the elastic fibers after their anionic change. It is known that most dyes capable of staining pre-formed fibers, such as oxytalan, are cationic dyes (after tissue oxidation), and few dyes are anionic, highlighting only fibrillar elements after elastin deposition (Fullmer and Lillie, 1958). Unlike oxytalan, elaunin fibers are stained by orcein, aldehyde-fuchsin and resorcin-fuchsin stains, even without peracetic acid oxidation, probably since these fibers already present the reactive structures (elastin) even before oxidation (Fullmer and Lillie, 1958). On the other hand, in oxytalan fibers is observed the presence of microfibrils, which are formed by fibrillin connected from disulfide bridges. This requires the occurrence of a pre-oxidation in the tissues, which leads to the breakdown of these bonds, and allows their staining by cationic dyes (Schmelzer and Duca, 2022). It is worth mentioning that each staining has specific limitations, such as lack of specificity and weak staining or the need for differentiation for the reaction to occur, as occurs in most of them (Percival and Radi, 2017).

The staining method by Verhoeff-Van Gieson (Fig. 4 C-C') is widely applied in the identification of elastic fibers (Table 2). However, this method makes it difficult to differentiate thinner fibers (Percival and Radi, 2016). In this technique, a regressive hematoxylin stain is used with ferric chloride and iodine, which will act as a mordant and oxidant, linking the hematoxylin to tissue components. Later, ferric chloride and sodium thiosulfate are applied for differentiation, to increase the affinity of high elastin deposition regions and remove its excess from the tissue (Kazlouskaya et al., 2013; Wulff et al., 2004). The elastic fibers are blue-black/black stained, similarly to cells nuclei (Titford, 2005). The collagen fibers are stained red (Van Gienson), and other cytoplasmic components, yellow (Percival and Radi, 2016). In such staining, hydrochloric acid can be used to increase the selectivity of the staining, since it prevents the dye from binding with pseudo-elastin-a component with similar histochemical and staining properties to those seen in collagen fibers (Puchtler and Waldrop, 1979). Lugol's solution can be applied, which increases the bond lifetime between iron and hematein, since Lugol will act by preventing peroxidation of dyes (Puchtler and

Table 1
Elastic fibers staining.

Techniques/ methods	PROS	CONS	APLICATIONS
Hematoxylin-Eosin	- General staining; - No advanced techniques required.	- No clear differentiation of the different types of fibers, due to dual staining for basophilic and acidophilic components.	- Some specific analyses require changes to the staining protocols; in one such case, hematoxylin staining was associated with immunolabeling in order to identify elastic fibers associated with tumor cells in lung cancer (Shen et al., 2022); - Require employment of microscopy techniques for differential observation of fibbrilar components (confocal microscopy, fluorescence microscopy).
Verhoeff-Van Gieson	- Distinction of ECM fibers (collagens and elastic); - Elastic fibers are strong stained.	- No differentiation among elastic fibers subtypes (oxytalan and elaunin).	- It is widely used in the diagnosis of pathologies, especially cutaneous, and is important for distinguishing some complex diagnostic alterations (Kazlouskaya et al., 2013); - Specific analyses may require changes to the protocol for best results, such as analysis of the fibers of small-caliber vessels (Percival et al.,2016).
Weigert's Resorcin-Fuchsin	- Easy differentiation of the thin elastic fibers; - Selectivity for elastic fibers.	- Preparation and process require long time.	- The use of Weigert's resorcin-fuchsin staining combined with fast green FCF staining proved to be an effective method for detecting elastic fibers in the heart, as it allows them to be distinguished from collagen fibers (Dmitrieva et al., 2022).
Orcein	- Dual tissue staining; - Quantification feasible and precise of elastic fibers.	- Low selectivity and low contrast for thinness subtypes; - Counterstaining required for better visualization of other components; - Not feasible for quantification of elaunin and oxytalan fibers.	- In the diagnosis of Lupus, several analyses are required to confirm the diagnosis, including analysis of the deposition of elastic fibers, where orcein staining is often used, and this analysis is important for the final diagnosis (Asadi Kani et al., 2014). - In quantitative analyses for atherosclerosis, it is important to assess whether the elastic fibers of the artery have ruptured, and orcein staining is commonly used. This stain can be combined with other stains, such as the Martius scarlet blue (MSB) stain, used to analyze fibrin (Gajda et al., 2017).

Waldrop, 1979).

In Weigert's resorcin-fuchsin staining (Fig. 4 D-E'), with no previous treatment, the dye specifically associates to the elastic fibers. After tissue acetylation, sulfation and phosphorylation, the dye is induced to bind structures containing glycogen and polysaccharides, such as collagen, but without changing the staining of elastic fibers (Puchtler and Sweat, 1964) (Table 2). This method stains the elastic fibers black, while muscle fibers lying between the elastic ones do not stain (Akhtar, 2010). It is believed that this elastic fiber binding is not associated with the presence of elastin, but with the polysaccharide esters. The resorcin-fuchsin would bind by hydrogen bonds, which occur between the hydroxyl groups of the dye resorcinol and the oxygen of the ester group of the acetylated polysaccharides (Puchtler and Sweat, 1964).

Weigert's resorcin-fuchsin staining applies iron chloride, which reacts differently when binding elastic and collagen fibers, contributing to their differentiation (Newton, 1966; Puchtler et al., 1961). In this case, collagen strongly reacts with acid dyes, whereas elastin has fewer carboxylic groups, which allows iron to generate a good differentiation between the two. Thus, collagen binds more intensely in the presence of basic fuchsin (Puchtler et al., 1961); the elastic fibers show a stronger reaction to resorcin, being only delimited by the association of basic fuchsin and ferric chloride (Newton, 1966).

Orcein staining (Fig. 4 F-F') is one of the most widely applied techniques for studying elastic fibers, comprising an acid dye with a black staining (Table 2). It is believed that Van der Waals and hydrogen bonds between the dye and the elastin mostly influence this staining (Horobin and James, 1970). In this reaction, an alcoholic solution is used so that the positively charged portions of the orcein are solubilized (Weiss, 1954). However, this staining ends up presenting low selectivity and the elastin staining provides low contrast, which difficult its observation (Humason and Lushbaugh, 1960). In general, the technique produces a black or reddish-black coloration in elastic tissue, and as with Weigert staining, the presence of iron can increase the binding of the fabric to the dye (Lillie et al., 1968).

2.2. *Fluorescence*

Confocal/fluorescence microscopy improves capture resolution and image sharpness, promoting better visualization of cellular and fiber structure and arrangement. The confocal method contributed to the revolution of microscopy soon after 1950, improving the images obtained by fluorescent microscopy (Bayguinov et al., 2018; Paddock and Eliceiri, 2014). Those methods are used in several studies and have multiple applications, such as better understanding of virus biology (Menke et al., 2023; Putlyaeva and Lukyanov, 2021), fiber assembly (Kozel et al., 2005), as well as three dimensional imaging in both live animals (Fibered Confocal Fluorescence Microscopy) and tissue sections

Table 2
Protocols of common stain used for elastic fibers.

Protocol	HE/P (De Carvalho and Taboga, 1996)	Verhoeff-Van Gieson (Verhoeff, 1908)	Weigert's modified (Crescenzi et al., 1991)	Orcein (Lillie et al. 1968)
Dewaxing	- Xylene - Decreasing Alcohol (100 %, 95 %, 80 %, 70 % 50 %) - H_2Od			
Dyes Preparation	- Hematoxylin 1. Hematoxylin - 5 g 2. Alcohol 95 % - 100 mL 3. Aluminum and ammonium sulphate - 20 g 4. H2Od[1] - 1.000 mL 5. Acetic acid - 2–4 mL 6. Mercury oxide - 3 g Boil the solution after each addition of compound - Eosin 1 % 1. Eosin - 1 g 2. H_2Od - 100 mL - Phloxine 1 % 1. Phloxine - 1 g 2. H_2Od - 100 mL The substances should be added in the following order: 1. Eosin 1 % - 25 mL 2. Phloxine 1 % - 2,5 mL 3. Alcohol 95 % - 195 mL 4. Acetic acid – 10 mL	- Verhoeff's hematoxylin: 1. Hematoxylin - 1 g 2. Alcohol absolute - 22 mL 3. Ferric chloride 10 % - 8 mL 4. Lugol - 8 mL - Iron chloride (2 %): 1. Ferric chloride - 1,4 mL 2. H_2Od - 100 mL - Sodium hyposulphite (5 %): 1. Sodium hyposulphite - 5 g 2. H_2Od - 100 mL - Van Gieson or Picro-Ponceau: 1. Ponceau aqueous solution 1–100 mL 2. Picric acid saturated solution - 90 mL 3. Glacial acetic acid - 1–2 mL	- Weigert's ferric hematoxylin: 1. Solution A: Hematoxylin - 1 g Alcohol 95 % –100 mL 1. Solution B: Iron perchloride (30–29 %) - 4 mL Hydrochloric acid - 1 mL H_2Od - 95 mL Combine the two solutions when using - Weigert's resorcin-fuchsin: 1. Basic fuchsin - 2 g 2. Resorcin - 4 g 3. H_2Od - 200 mL 4. Ferric chloride 29 % - 25 mL 5. Alcohol 95 % - 200 mL 6. Hydrochloric acid - 4 mL Dissolve fuchsin in distilled water and bring to the boil. Then add resorcin and boil for 1', adding ferric chloride afterwards. Boil for 1', allow to cool and filter. Dry the precipitate and dissolve in 95 % alcohol, boiling for 1'. Cool and add hydrochloric acid. - Ammoniacal water: one part of Ammonium hydroxide to five parts of distilled water	- Polychrome blue (Unna): 1. Methylene blue - 1 g 2. Potassium carbonate - 1 g 3. H_2Od - 100 mL - Orcein solution: 1. Orcein - 0,25 % 2. Alcohol 70 %. - 100 mL
Staining	- Hematoxylin (3') - H_2Od - Wash in 80 % alcohol - Eosin/Phloxine (wash quickly) - H_2Od	- Verhoeff's hematoxylin (15–30') - H_2O - Ferric chloride for a few minutes - H_2Od - Wash in 95 % alcohol quickly - Hyposulphite (5') - H_2Od - Optional: van Gieson or Picro-Ponceau (1')	- Weigert's ferric hematoxylin (10') - H_2Od - Weigert's resorcin-fuchsin (20') - if they become over-colored, bleach with ammoniacal water - Optional: van Gieson (1') - if they become over-colored, bleach with ammoniacal water - Alcohol with drops of picric acid in saturated aqueous solution	- Polychrome blue (5–15') - Orcein solution - until the fibers turn brown
Staining pattern	- Acidic tissue components - purple-blue - Basic tissue components - pink	- Elastic fibers and nuclei - dark blue-black - Collagen fibers - red - Muscle fibers - yellow	- Elastic fibers and nuclei - dark blue-black - With van Gieson: collagen fibers in red and muscle fibers in yellow	- Elastic fibers – brown
Technical Issues	- Hematoxylin needs to be filtered before use	- Fresh ferric chloride should be used when preparing stain - Van Gieson staining, it is necessary to check with microscope until elastic fibers are stained	- During Hematoxylin and van Gieson staining, it is necessary to check with microscope until elastic fibers are stained - Hematoxylin cannot be stored for a long time	- During the staining it is necessary to check with microscope until fibers are stained - The polychrome solution takes months to become suitable for use

(Al-Gubory, 2019; Elliott, 2020). With that, histological techniques were rethought within this new microscopy generation method: the HE/P method was conceived from observation in both fluorescence and confocal microscopy. More recently, this technique has been also applied in association with modern microscopy techniques known as Dual-mode Emission Transmission microscopy (DUET) (Fereidouni et al., 2019) This is recognized as a simple and fast microscopy technique, with great potential for use in clinical diagnostic approaches when coupled with HE/P.

Furthermore, HE is also associated with fluorescence microscopy for the identification of some fluorophore's molecules, as elastin components of elastic fibers (Bradbeer et al., 1994). Despite this potential, HE combination does not provide precise results, since the fluorescence intensity is not strong enough, compromising the observation of fibers details. Thus, eosin combination with phloxine is often applied for enhancing the fluorescent labeling (Table 3). The phloxine is known as an eosin-like dye, since both present a similar large wavelength range (varying from 490 nm to 530 nm), which enables the observation of the elastic fibers in three different fluorescent colors: blue, yellow and red, when irradiated by light waves of 395 nm to 530 nm (Carvalho and Taboga, 1996). The yellow shift allows a major differentiation of elastic fibers (occurring in a fluorescence peak of 490 nm wavelength), obscuring oxytalan and elaunin fluorescence intensity, and impairing these fiber's specific differentiation (Heo and Song, 2011).

3. The elastin fiber staining under physiological conditions

Description of the elastic system is often related to organs in which the connective tissue plays a crucial role in organ mechanical and physiological aspects, as well as organ organization during embryogenesis. In general, the HE/P technique has been frequently applied for describing these phenomena. This technique allows correlating the distribution of elastic fibers with their micromechanical properties in organs (Akhtar, 2010). Also, the high resolution provided by confocal microscopy allows highlighting nuances of fiber morphology and their form of aggregation in specific tissues and organs (Parasassi et al., 2000). Such dynamics of deposition and aggregation are favored by this technique, in terms of the demarcation of the territory of elastic fibers

Table 3
Common technical issues in fluorescence microscopy and HE/P technique.

Problem	Description	Improvements	he/p technique
Fluorophore Concentration	In thick tissue sections the fluorescence can be unequally spread into the tissue; Lower or no signal.	Confocal microscopy can provide a better visualization; Use thin slides when performing fluorescence microscopy.	Use of confocal microscopy for elastic fibers can improve the complete observation of elastic system; Optimal phloxine addiction avoid high or very low fluorescence.
Photobleaching	Common in major organic fluorophores; The modification of the fluorophore molecule to a non-emissive state.	Store the samples in the dark; Regulate the light intensity when using the samples; Use of photostabilizing buffers.	Samples stained with HE/P generally do not present fading of fluorescence due to phloxine tight aggregation.
Contrast Errors	Underexposure or overexposure of the image, leading to blurry images or overexposure spots.	Use the correct wavelength and reduce or increase the light exposition time in the sample	Specific filter fluorescence microscopes of wavelength in confocal microscopes avoid contrast errors, commonly presented by the other ECM fibers.
Non-specific Fluorescence	Background fluorescence due to overstaining and/ or molecules autofluorescence.	Using better blocking agents can avoid unspecific fluorophore bonding; Reduce the fluorophore concentration; Avoid using filters with large wavelength spectrum.	Optimal phloxine addiction avoid high or very low fluorescence; Perform a comparation among filters/ wavelengths also helps to detect autofluorescence of other tissue components.
Fluorophore Degradation	Lower or no light signals over excitation	Store the samples at low temperatures	HE/P do not present fast degradation, since the autofluorescence of elastic fibers and phloxine aggregation avoid this problem.

and their aggregation density avoiding misinterpretation (Wolf-de Jonge et al., 2008).

Studies on the morphogenesis of the outflow tract, a connective tissue-rich cardiac region in mammals (Sinha et al., 2012) and other fish (Gardinal et al., 2019), have demonstrated the development and possible mechanics involved with cardiac physiology associated with the required elastic properties. Also, identification of the concentric lamellae arrangement in the middle layer of large blood vessels is improved with this technique (Sherratt, 2009), making it possible to observe this phenomenon. Also, elastic fibers can be observed in tissue aging processes. The HE/P technique increases the resolution and appearance of the fibers by allowing analysis of elastin loss due to skin aging processes, for example (Alvarez-Román et al., 2004).

4. The elastin fiber staining under histopathological/ experimental conditions

As elastic fibers are important mechanical components of connective tissue, some studies are devoted to describing their degradation, fragmentation, and rupture, or increased deposition due to pathological processes (summarized in Fig. 5). Fragmentation appears different from fiber rupture in some induced experimental models since it is possible to observe trace segments under fluorescence microscopy. In the induction of Peyronie's disease by TGFβ, the HE/P technique it allows identification of the fragmentation of elastic fibers present in the tunica albuginea region (Antoniassi et al., 2020). In skin, relevant processes of fragmentation and mineralization of elastic fibers were well defined in pathological conditions (Fésűs et al., 2021; Makihara et al., 2017). Also, phloxine were useful in two-photon excitation fluorescence and second-harmonic generation to identify elastic system in dermis (Fésűs et al., 2021), demonstrating a high detailed structure also in nonlinear microscopy. Interestingly, without counterstaing, such as HE/P, analysis with these techniques did not demonstrated highlights of elastic disorders (Cocciolone et al., 2019).

Elastic fiber disruption, on the other hand, presents itself in the HE/P technique as continuous fibers with ends, as observed in the stroma of glands of the reproductive system, such as the mammary gland in experimental conditions (Ruiz et al., 2021) and in the prostate during the process of carcinogenesis (Vilamaior et al., 2003), or in the lung parenchyma due to emphysema lesions (Pastor et al., 2006). These fibers lose their tissue continuity, presenting themselves as stromal fibrillar elements more elongated than the elastin fragments mentioned above. It is still interesting to point out that in initial conditions of tumorigenesis in glands, the elastic fibers undergo the process of discontinuity (Ruiz et al., 2021), and later, in more advanced cases (Vilamaior et al., 2003), fragmentation is also observed. Indeed, since the HE/P technique allows the observation of both processes, and stromal remodeling is a crucial condition for the development of neoplasia (Piersma et al., 2020; Winkler et al., 2020), this technique has a great potential to be employed in general histopathological analyses.

A third process, the deposition of these fibers and its arrangement, can also be observed by means of the HE/P technique. The pattern of elastic fibers deposition allows distinguishing pathological processes that depend on these fibers' density. Few examples are dermatofibromas and fibrous tumors (Borucki et al., 2021), idiopathic atrophoderma of Pasini and Pierini (IAPP) lesions (Vieira-Damiani et al., 2017), and in spinal ligaments in patients with diabetes mellitus (Shemesh et al., 2018). By applying the HE/P technique, these studies provided analyses of organization and quantification of these fibers in different tissues. Experimentally, the deposition of elastic fibers in the aorta's elastic lamellae observed in HE/P, makes it possible to identify damage and the elongation of these fibers in the tunica due to the process of vascular calcification (Al-Huseini et al., 2018). Also, irregular or uneven accumulation in parts of the lung stroma can be observed and associated to the aggravation of pulmonary emphysema (Pastor et al., 2006).

The degree of aggregation and organization of the elastic system is also an important factor. The alignment and direction of elastic fibers, associated with fiber density, determines tissue properties in addition to corroborating with several pathologies. In the superficial dermis associated with IAPP lesions (Vieira-Damiani et al., 2017) and scars (Elston et al., 2013), the organization of the elastic system gives the skin different mechanical attributes and topological deformations easily distinguishable in HE/P. In fact, according to Dirk (2002), this technique shows a great difference when compared to stains such as Verhoeff-van Gieson and Weigert's resorcin-fuchsin regarding the morphology of dermal lesions. Not only observed in skin analysis, but in vascular studies (Hays et al., 2008; Hinton et al., 2021), details of elastic system were lost, since the non-aggregation or non-deposition were indistinguishable processes.

5. Conclusions and perspectives

The elastic system presented an important role in different organs which confers a spotlight in analysis of this component of extracellular matrix. HE/P demonstrated after its employment in biological samples

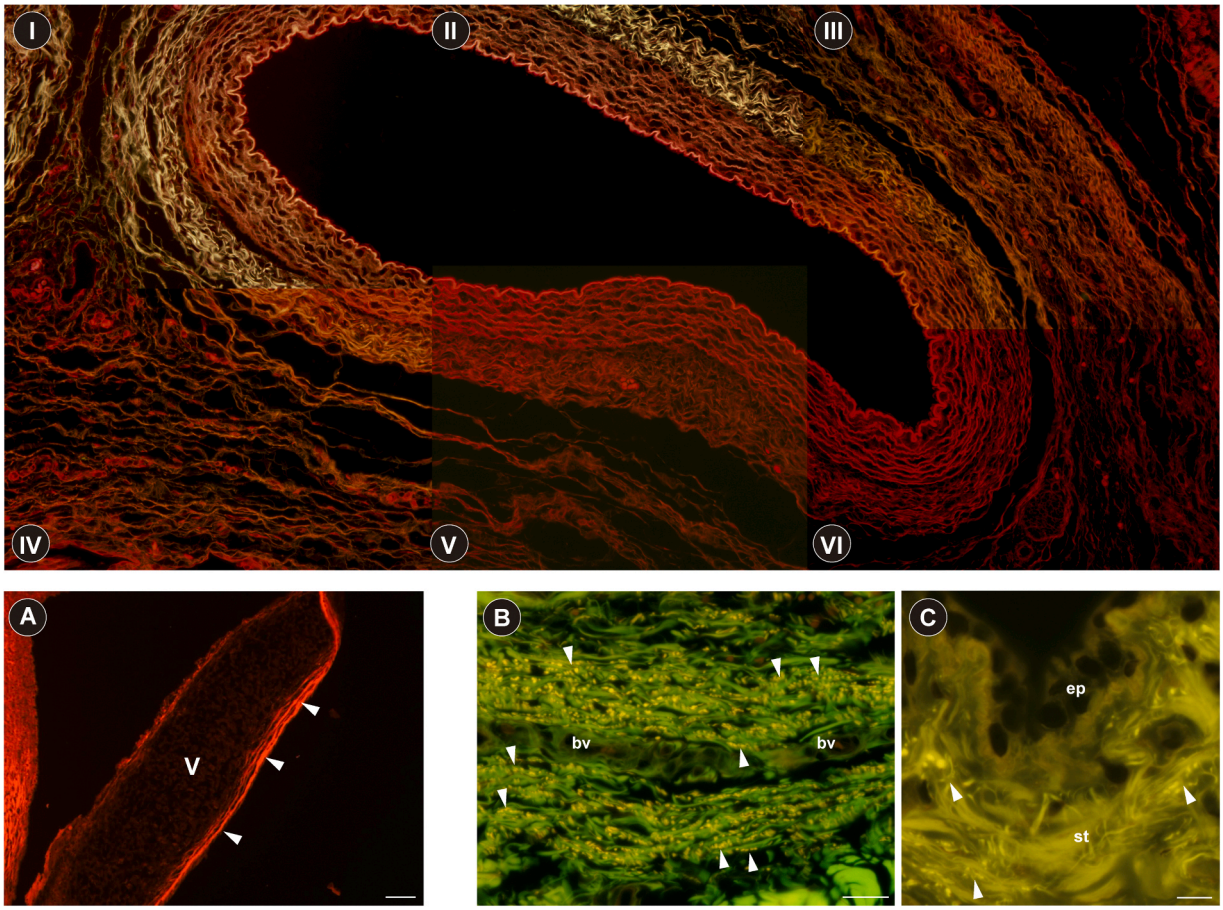

Fig. 5. Normal and pathological morphologies of the jugular artery using HE/P. (I-VI) Fluorescent analysis under different fluorescent wavelengths. (A) Outflow tract: bulbus valve of *Brycon amazonicus* (fish). Note the dense sheet of elastic fibers (white arrowheads) arranged in parallel in the luminal fibrosa of the valve. The elastic sheets contribute to the valves distensibility. (B-C) Histopathological aspects of elastic degradation. (B) Tunica albuginea of experimentally induced Peyronie disease. Elastic fibers are scattered, and fragmentation is present (arrowheads). (C) Mammary stroma under endocrine disruption. Elastic fibers in stroma are under a degradative process induced by rupture. *Scale bars*: 20 μm. *Abbreviations*: v: valve; bv: blood vessel; ep: epithelium; st: stroma.

grater results regarding pathological and normal descriptions. Further, other also simple techniques, such as Verhoeff iron-hematoxylin, Weigert's resorcin-fuchsin, orcein, promoted similar or inferior results to demonstrate details in different organs. We proposed and presented in the present paper the general application of HE/P as a histological technique with a powerful perspective to usage in routine and/or research field.

Future studies could elucidate new perspectives and employment of HE/P technique related to elastogenesis and elastosis in different organs that elastic system appears to be crucial. In fact, the elastic and pre-elastic fibers formation in tissue, such as deposition and pre-formation, should be enlightened in the new generations of microscopy related to confocal and fluorescence microscopy, which have higher resolution and detail power. An interesting fact is that investigations of the intracellular elastin molecule have not yet been discussed or observed. Since the HE/P technique is made possible by the elastin molecule, future studies may determine more precise relationships between these events.

In summary, the HE/P technique has proven effective in demonstrating events in the ECM that delimit modifications in the connective tissue and contribute to the progression of pathological processes, as well as in the morphological constitution of organs. In general, the applications of the technique presented in this paper were excellent for determining functional inferences of the elastic systems, since they presented details regarding the disposition, deposition, and fibrillar structure in processes of degradation, fragmentation, and rupture. New perspectives may be added to this technique in the future, as well as its correlation with other techniques or its improvement. The association between classic staining techniques and new histopathological techniques (such as immunohistochemistry, cytochemistry, and new microscopy generation equipment for 3D analysis) should be addressed to better understand the real correlation between health/disease with elastic system microarchitecture.

Funding

This work was financed by FAPESP (São Paulo Research Foundation) - Grant number: 22/00521-0 to TFRR; CNPq (Brazilian National Council for Scientific and Technological Development) – Contract number: 302938/2020–6 to SRT.

CRediT authorship contribution statement

TFRR, ECRL, SRT: Conceptualization, Investigation, Writing manuscript; LJF, LGS, GEBF: Writing manuscript. TFRR and SRT: Data curation and supervision. Acquisition of the financial support for the project leading to this publication (FAPESP and CNPq). Management and coordination responsibility for the research activity planning and execution.

Declaration of Competing Interest

The authors declare none.

References

Akhtar, R., 2010. Nanoindentation of histological specimens. J. Mater. 24, 638–646. https://doi.org/10.1557/JMR.2009.0130.Nanoindentation.

Al-Gubory, Kaïs H., 2019. Shedding light on fibered confocal fluorescence microscopy: applications in biomedical imaging and therapies. J. Biophotonics 12 (11), 1–12. https://doi.org/10.1002/jbio.201900146.

Al-Huseini, I., Ashida, N., Kimura, T., 2018. Deletion of IjB-Kinase β in smooth muscle cells induces vascular calcification through β-Catenin-Runt-related transcription factor 2 signaling. J. Am. Heart Assoc. 7, 1–15. https://doi.org/10.1161/JAHA.117.007405.

Alvarez-Román, R., Naik, A., Kalia, Y.N., Fessi, H., Guy, R.H., 2004. Visualization of skin penetration using confocal laser scanning microscopy. Eur. J. Pharm. Biopharm. 58, 301–316. https://doi.org/10.1016/j.ejpb.2004.03.027.

Antoniassi, T., Júnior, F.N.F., Spessoto, L.C.F., Guerra, L.H., Campos, S.S., Taboga, S., 2020. Anti-fibrotic effect of mycophenolate mofetil on Peyronie's disease experimentally induced with TGF-β. Int J. Impot Res 32, 201–206. https://doi.org/10.1038/s41443-019-0138-7.

Asadi Kani, Z.F., Nasiri, S., Rafiei, R., Younespour, S., 2014. Evaluation of elastic fibers pattern with orcein staining in differential diagnosis of lichen planopilaris and discoid lupus erythematosus. Acta Med. Iran. 52 (3), 220–227.

Bayguinov, P.O., Oakley, D.M., Shih, C.C., Geanon, D.J., Joens, M.S., Fitzpatrick, J.A.J., 2018. Modern laser scanning confocal microscopy. Curr. Protoc. Cytom. 85. https://doi.org/10.1002/CPCY.39.

Berk, David R., Bentley, Danette D., Bayliss, Susan J., Lind, Anne, Urban, Zsolt, 2012. Cutis laxa: a review. J. Am. Acad. Dermatol. 66 (5), 842.e1–842.e17. https://doi.org/10.1016/j.jaad.2011.01.004.

Borucki, R., Perry, D.M., Lopez-Garcia, D.R., Kazlouskaya, V., Elston, D.M., 2021. Fluorescence microscopy for the evaluation of elastic tissue patterns within fibrous proliferations of the skin on hematoxylin-eosin–stained slides. J. Am. Acad. Dermatol. 85, 419–422. https://doi.org/10.1016/j.jaad.2017.12.073.

Bradbeer, J.N., Riminucci, M., Bianc02, P., 1994. Giemsa as a fluorescent stain for mineralized bone. Joumal Histochem. Cytochem. Brief. Rep.

Carvalho, H.F., Taboga, S.R., 1996. Fluorescence and confocal laser scanning microscopy imaging of elastic fibers in hematoxylin-eosin stained sections. Histochem Cell Biol. 106, 587–592. https://doi.org/10.1007/bf02473274.

Cisne, R., Souza, A.M.T., Pereira-Sampaio, M.A., Babinski, M.A., Gorniak, S.L., Papa, P. C., 2018. Tannic acid solution: a better fixative solution than formalin for elastin and collagen—toxic and morphological assessment. Anat. Rec. 301, 1544–1550. https://doi.org/10.1002/ar.23894.

Cocciolone, A.J., Johnson, E.O., Shao, J.Y., Wagenseil, J.E., 2019. Elastic fiber fragmentation increases transmural hydraulic conductance and solute transport in mouse arteries. J. Biomech. Eng. 141. https://doi.org/10.1115/1.4042173.

Crescenzi, A., Muda, A.O., Origgi, P., Bonfichi, R., Faraggiana, T., 1991. Physico-chemical analysis of resorcin-fuchsin reagents and its relevance to staining quality of elastic fibres. Eur. J. Basic Appl. Histochem 35 (1), 37–44. PMID: 1713788.

Davis, E.C., Li, L., 2017. Histological and Electron Microscope Staining for the Identification of Elastic Fiber Networks. In: Fibrosis. Springer, pp. 385–393. https://doi.org/10.1007/978-1-4939-7113-8_25.

De Carvalho, H.F., Taboga, S.R., 1996. The applicability of hematoxylin-eosin staining plus fluorescence or confocal laser scanning microscopy to the study of elastic fibers in cartilages. C. R. Acad. Sci. III 319, 991–996.

Di Martino, J.S., Akhter, T., Bravo-Cordero, J.J., 2021. Remodeling the ECM: implications for metastasis and tumor dormancy. Cancers. https://doi.org/10.3390/cancers13194916.

Dmitrieva, E.G., Khatsko, S.L., Yakimov, A.A., 2022. Staining method for coronary arteries. Сибирский научный Медицинский Журнал 42 (3), 47–51. https://doi.org/10.18699/SSMJ20220305.

Elliott, Amicia D., 2020. Confocal microscopy: principles and modern practices. Curr. Protoc. Cytom. 92 (1), 1–11. https://doi.org/10.1002/cpcy.68.

Elston, C.A., Kazlouskaya, V., Elston, D.M., 2013. Elastic staining versus fluorescent and polarized microscopy in the diagnosis of alopecia. J. Am. Acad. Dermatol. 69, 288–293. https://doi.org/10.1016/j.jaad.2013.02.030.

Faury, G., 2001. Function–structure relationship of elastic arteries in evolution: from microfibrils to elastin and elastic fibres. Pathol. Biol. 49, 310–325. https://doi.org/10.1016/S0369-8114(01)00147-X.

Fereidouni, F., Todd, A., Li, Y., Chang, C.-W., Luong, K., Rosenberg, A., Lee, Y.-J., Chan, J.W., Borowsky, A., Matsukuma, K., Jen, K.-Y., Levenson, R., 2019. Dual-mode emission and transmission microscopy for virtual histochemistry using hematoxylin- and eosin-stained tissue sections. Biomed. Opt. Express 10, 6516. https://doi.org/10.1364/BOE.10.006516.

Ferraz de Carvalho, C.A., König, B.J., 1982. Light and electron microscopic study on the oxytalan elaunin and elastic fibers in the inferior segment of the human esophagus. Anat. Anz. 152, 141–157.

Fésűs, L., Martin, L., Wikonkál, N., Medvecz, M., Szipőcs, R., 2021. Low concentration phloxine B staining for high chemical contrast, nonlinear microscope mosaic imaging of skin alterations in pseudoxanthoma elasticum. Opt. Info Conf. Pap. 13, 252–261. https://doi.org/10.1364/boe.443507.

Fullmer, H.M., Lillie, R.D., 1958. The oxytalan fiber: a previously undescribed connective tissue fiber. J. Histochem. Cytochem. 6, 425–430. https://doi.org/10.1177/6.6.425.

Gajda, M., Jasztal, A., Banasik, T., Jasek-Gajda, E., Chlopicki, S., 2017. Combined orcein and martius scarlet blue (OMSB) staining for qualitative and quantitative analyses of atherosclerotic plaques in brachiocephalic arteries in apoE/LDLR−/− mice. Histochem. Cell Biol. 147 (6), 671–681. https://doi.org/10.1007/s00418-017-1538-8.

Gardinal, M.V.B., Ruiz, T.F.R., dos Santos, D.D., Vidal, M.R., Moron, S.E., Falleiros Junior, L.R., Taboga, S.R., Franceschini Vicentini, I.B., Vicentini, C.A., 2019. Histochemical characterization and connective fiber distribution of the cardiac outflow tract of pirarucu, Arapaima gigas (Schinz, 1822) (Osteoglossiformes, Arapaimidae). Zoomorphology 138, 525–534. https://doi.org/10.1007/s00435-019-00459-z.

Hays, A.M., Lantz, R.C., Rodgers, L.S., Sollome, J.J., Vaillancourt, R.R., Andrew, A.S., Hamilton, J.W., Camenisch, T.D., 2008. Arsenic-induced decreases in the vascular matrix. Toxicol. Pathol. 36, 805–817. https://doi.org/10.1177/0192623308323919.

Heinz, A., 2021. Elastic fibers during aging and disease. Ageing Res Rev. 66, 101255. https://doi.org/10.1016/j.arr.2021.101255.

Heo, Y.S., Song, H.J., 2011. Characterizing cutaneous elastic fibers by eosin fluorescence detected by fluorescence microscopy. Ann. Dermatol. 23, 44–52. https://doi.org/10.5021/ad.2011.23.1.44.

Hinton, R.B., Juraszek, A.L., Opoka, A.M., Landis, B.J., Smith, J.M., Mecham, R.P., Bove, K.E., 2021. Early aberrant angiogenesis due to elastic fiber fragmentation in aortic valve disease. J. Cardiovasc Dev. Dis. 8. https://doi.org/10.3390/jcdd8070075.

Horobin, R.W., James, N.T., 1970. The staining of elastic fibres with direct blue 152. A general hypothesis for the staining of elastic fibres. Histochemie 22, 324–336. https://doi.org/10.1007/BF00277460.

Humason, G.L., Lushbaugh, C.C., 1960. Selective demonstration of elastin, reticulum and collagen by silver, orcein and aniline blue, 1.

Kajiya, H., Tanaka, N., Inazumi, T., Seyama, Y., Tajima, S., Ishibashi, A., 1997. Cultured human keratinocytes express tropoelastin. J. Invest. Dermatol. 109, 641–644.

Kazlouskaya, V., Malhotra, S., Lambe, J., Idriss, M.H., Elston, D., Andres, C., 2013. The utility of elastic Verhoeff-Van Gieson staining in dermatopathology. J. Cutan. Pathol. https://doi.org/10.1111/cup.12036.

Kielty, C.M., Sherratt, M.J., Shuttleworth, C.A., 2002. Elastic fibres. J. Cell Sci. 115, 2817–2828.

Kim, Y., Ko, H., Kwon, I.K., Shin, K., 2016. Extracellular matrix revisited: roles in tissue engineering. Int Neurourol. J. 20, S23–S29. https://doi.org/10.5213/inj.1632600.318.

Kozel, B.A., Rongish, B.J., Czirok, A., Zach, J., Little, C.D., Davis, E.C., Mecham, R.P., 2005. Elastic fiber formation: a dynamic view of extracellular matrix assembly using timer reporters. J. Cell. Physiol. 207 (1), 87–96. https://doi.org/10.1002/jcp.20546.

Liem, K.F., 1988. Form and function of lungs: the evolution of air breathing mechanisms. Am. Zool. 28, 739–759.

Lillie, R.D., Gutiérrez, A., Madden, D., Henderson, R., 1968. Acid orcein-iron and acid orcein-copper stains for elastin. Stain Technol. 43, 203–206. https://doi.org/10.3109/10520296809115069.

Lockhart-Cairns, Michael P., Newandee, Helena, Thomson, Jennifer, Weiss, Anthony S., Baldock, Clair, Tarakanova, Anna, 2020. Transglutaminase-mediated cross-linking of tropoelastin to fibrillin stabilises the elastin precursor prior to elastic fibre assembly. J. Mol. Biol. 432 (21), 5736–5751. https://doi.org/10.1016/j.jmb.2020.08.023.

Makihara, H., Hidaka, M., Sakai, Y., Horie, Y., Mitsui, H., Ohashi, K., Goshima, Y., Akase, T., 2017. Reduction and fragmentation of elastic fibers in the skin of obese mice is associated with altered mRNA expression levels of fibrillin-1 and neprilysin. Connect Tissue Res 58, 479–486. https://doi.org/10.1080/03008207.2016.1255205.

Mecham, R.P., 2018. Elastin in lung development and disease pathogenesis. Matrix Biol. 73, 6–20. https://doi.org/10.1016/j.matbio.2018.01.005.

Mecham, R.P., Levy, B.D., Morris, S.L., Madaras, J.G., Wrenn, D.S., 1985. Increased cyclic GMP levels lead to a stimulation of elastin production in ligament fibroblasts that is reversed by cyclic AMP. J. Biol. Chem. 260, 3255–3258.

Menke, Laura, Sperber, Hannah S., Aji, Amit Koikkarah, Chiantia, Salvatore, Schwarzer, Roland, Sieben, Christian, 2023. Advances in fluorescence microscopy for orthohantavirus research. Microscopy 72 (3), 191–203. https://doi.org/10.1093/jmicro/dfac075.

Narayanan, A.S., Sandberg, L.B., Ross, R., Layman, D.L., 1976. The smooth muscle cell. III. Elastin synthesis in arterial smooth muscle cell culture. J. Cell Biol. 68, 411–419.

Newton, A.V., 1966. Collagen, elastic tissue and resorcin fuchsin. Nature 209, 1146–1147.

Ouni, E., Bouzin, C., Dolmans, M.M., Marbaix, E., Pyr dit Ruys, S., Vertommen, D., Amorim, C.A., 2020. Spatiotemporal changes in mechanical matrisome components of the human ovary from prepuberty to menopause. Hum. Reprod. 35, 1391–1410. https://doi.org/10.1093/humrep/deaa100.

Ozsvar, Jazmin, Yang, Chengeng, Cain, Stuart A., Baldock, Clair, Tarakanova, Anna, Weiss, Anthony S., 2021. Tropoelastin and Elastin Assembly. Front. Bioeng. Biotechnol. 9 (February), 1–11. https://doi.org/10.3389/fbioe.2021.643110.

Paddock, S.W., Eliceiri, K.W., 2014. Laser scanning confocal microscopy: history, applications, and related optical sectioning techniques. Methods Mol. Biol. 1075 9–47. https://doi.org/10.1007/978-1-60761-847-8_2.

Parasassi, T., Yu, W., Durbin, D., Kuriashkina, L., Gratton, E., Maeda, N., Ursini, F., 2000. Two-photon microscopy of aorta fibers shows proteolysis induced by LDL hydroperoxides. Free Radic. Biol. Med 28, 1589–1597. https://doi.org/10.1016/S0891-5849(00)00275-6.

Pastor, L.M., Sánchez-Gascón, F., Girona, J.C., Bernal-Mañas, C.M., Morales, E., Beltrán-Frutos, E., Canteras, M., 2006. Morphogenesis of rat experimental pulmonary emphysema induced by intratracheally administered papain: changes in elastic fibres. Histol. Histopathol. 21, 1309–1319. https://doi.org/10.14670/HH-21.1309.

Percival, K.R., Radi, Z.A., 2016. A modified Verhoeff's elastin histochemical stain to enable pulmonary arterial hypertension model characterization. Eur. J. Histochem 60, 2588. https://doi.org/10.4081/ejh.2016.2588.

Percival, K., Radi, Z., 2017. Comparison of five elastin histochemical stains to identify pulmonary small vasculature. *. J. Histotechnol. 40, 73–78. https://doi.org/10.1080/01478885.2017.1337678.

Piersma, B., Hayward, M.K., Weaver, V.M., 2020. Fibrosis and cancer: a strained relationship. Biochim Biophys. Acta Rev. Cancer 1873. https://doi.org/10.1016/j.bbcan.2020.188356.

Puchtler, H., Sweat, F., Bates, R., Brown, J.H., 1961. On the mechanism of resorcin-fuchsin staining. J. Histochem Cytochem 9, 553–559. https://doi.org/10.1177/9.5.553.

Puchtler, H., Sweat, F., 1964. Histochemical specifity of staining methods for connective tissue fibers: Resorcin-fuchsin and van Gieson's picro-fuchsin. Histochemie 4, 24–34. https://doi.org/10.1007/BF00304175/METRICS.

Puchtler, H., Waldrop, F.S., 1979. On the mechanism of Verhoeff's elastica stain: a convenient stain for myelin sheaths. Histochemistry 62, 233–247. https://doi.org/10.1007/BF00508352.

Putlyaeva, Lidia V., Lukyanov, Konstantin A., 2021. Studying Sars-Cov-2 with Fluorescence Microscopy. Int. J. Mol. Sci. 22 (12). https://doi.org/10.3390/ijms22126558.

Ronchetti, I.P., Alessandrini, A., Baccarani Contri, M., Fornieri, C., Mori, G., Quaglino, D., Valdrè, U., 1998. Study of elastic fiber organization by scanning force microscopy. Matrix Biol. 17, 75–83. https://doi.org/10.1016/S0945-053X(98)90126-3.

Rosenbloom, J., Abrams, W.R., Mecham, R., 1993. Extracellular matrix 4: the elastic fiber. FASEB J. 7, 1208–1218. https://doi.org/10.1096/fasebj.7.13.8405806.

Ross, M., Pawlina, W., 2016. Histology - Text and atlas.

Ruiz, T.F.R., Colleta, S.J., Leonel, E.C.R., Taboga, S.R., 2021. Mammary carcinoma in aged gerbil mothers after endocrine disruption in pregnancy and lactation. Endocr. Relat. Cancer. https://doi.org/10.1530/ERC-21-0198.

Sambani, Kyriaki, Kontomaris, Stylianos Vasileios, Yova, Dido, 2023. Atomic force microscopy imaging of elastin nanofibers self-assembly. Materials 16 (12), 1–18. https://doi.org/10.3390/ma16124313.

Sawada, T., Sugawara, Y., Asai, T., Aida, N., Yanagisawa, T., Ohta, K., Inoue, S., 2006. Immunohistochemical characterization of elastic system fibers in rat molar periodontal ligament. J. Histochem Cytochem 54, 1095–1103. https://doi.org/10.1369/jhc.5A6905.2006.

Schmelzer, C.E.H., Duca, L., 2022. Elastic fibers: formation, function, and fate during aging and disease. FEBS J. 289, 3704–3730. https://doi.org/10.1111/febs.15899.

Schmelzer, C.E.H., Hedtke, T., Heinz, A., 2020. Unique molecular networks: formation and role of elastin cross-links. IUBMB Life 72, 842–854. https://doi.org/10.1002/iub.2213.

Shemesh, S., Sidon, E., Kaisler, E., Sheinis, D., Velkes, S., Ohana, N., Benayahu, D., 2018. Diabetes mellitus is associated with increased elastin fiber loss in ligamentum flavum of patients with lumbar spinal canal stenosis: results of a pilot histological study. Eur. Spine J. 27, 1614–1622. https://doi.org/10.1007/s00586-017-5315-0.

Shen, Guihua, Dong, Jiyan, Xiang, Zheng, Liu, Li, Yang, Lin, 2022. Double staining of elastic fibre and immunohistochemistry is helpful to differentiate pleural invasion of lung cancer. J. Clin. Pathol. 75 (3), 215–216. https://doi.org/10.1136/jclinpath-2021-207779.

Sherratt, M.J., 2009. Tissue elasticity and the ageing elastic fibre. Age (Omaha) 31, 305–325. https://doi.org/10.1007/s11357-009-9103-6.

Sinha, T., Wang, B., Evans, S., Wynshaw-Boris, A., Wang, J., 2012. Disheveled mediated planar cell polarity signaling is required in the second heart field lineage for outflow tract morphogenesis. Dev. Biol. 370, 135–144. https://doi.org/10.1016/j.ydbio.2012.07.023.

Titford, M., 2005. The long history of hematoxylin. Biotech. Histochem. 80, 73–78. https://doi.org/10.1080/10520290500138372.

Torre, Â.F., de Almeida Junior, H.L., Jorge, V.M., de Almeida, A.L., 2021. Transmission and scanning electron microscopy of cutis rhomboidalis. Bras. Dermatol.

Uitto, Jouni, Li, Qiaoli, Urban, Zsolt, 2013. The complexity of elastic fibre biogenesis in the skin - a perspective to the clinical heterogeneity of cutis Laxa. Exp. Dermatol. 22 (2), 88–92. https://doi.org/10.1111/exd.12025.

Ushiki, T., 2002. Collagen fibers, reticular fibers and elastic fibers. a comprehensive understanding from a morphological viewpoint. Arch. Histol. Cytol. 65, 109–126. https://doi.org/10.1679/aohc.65.109.

Verhoeff, F.H., 1908. Some new staining methods of wide applicability: including a rapid differential stain for elastic tissue. J. Am. Med. Assoc. L (11), 876–877. https://doi.org/10.1001/jama.1908.25310370042004a.

Vieira-Damiani, G., Lage, D., Christofoletti Daldon, P.É., Tibúrcio Alves, C.R., Cintra, M. L., Metze, K., Adur, J., Pelegati, V.B., Carvalho, H.F., Cesar, C.L., 2017. Idiopathic atrophoderma of Pasini and Pierini: a case study of collagen and elastin texture by multiphoton microscopy. J. Am. Acad. Dermatol. 77, 930–937. https://doi.org/10.1016/j.jaad.2017.02.044.

Vilamaior, P., Suzigan, S., Carvalho, H., Taboga, S., 2003. Structural characterization and distribution of elastic system fibers in the human prostate and some prostatic lesions. Braz J. Morphol. Sci. 20, 101–107.

Weiss, J., 1954. The nature of the reaction between orcein and elastin. J. Histochem Cytochem 2, 21–28. https://doi.org/10.1177/2.1.21.

Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K.J., Werb, Z., 2020. Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nat. Commun. 11, 1–19. https://doi.org/10.1038/s41467-020-18794-x.

Wise, Steven G., Mithieux, Suzanne M., Weiss, Anthony S., 2009. Engineered tropoelastin and elastin-based biomaterials. In: Advances in protein chemistry and structural biology, First edition, 78. Elsevier. https://doi.org/10.1016/s1876-1623(08)78001-5.

Wolf-de Jonge, I.C., Heger, M., van Marle, J., Balm, R., Beek, J.F., 2008. Suture-free laser-assisted vessel repair using CO2 laser and liquid albumin solder. J. Biomed. Opt. 13, 1. https://doi.org/10.1117/1.2953531.

Wulff, S., Hafer, L., Cheles, M., Couture, R., Holliday, J.M., Smith, S., Stanforth, D.A., 2004. Guide to Special Stains. Dako Cytomatior 1–95.

Yanagisawa, Hiromi, Davis, Elaine C., 2010. Unraveling the mechanism of elastic fiber assembly: the roles of short fibulins. Int. J. Biochem. Cell Biol. 42 (7), 1084–1093. https://doi.org/10.1016/j.biocel.2010.03.009.

Yanagisawa, Hiromi, Wagenseil, Jessica, 2020. Elastic fibers and biomechanics of the aorta: insights from mouse studies. Matrix Biol. 85–86 (xxxx), 160–172. https://doi.org/10.1016/j.matbio.2019.03.001.