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CASE REPORT

Application of fluorescence microscopy on hematoxylin and eosin-stained sections of healthy and diseased teeth and supporting structures

Andiara De Rossi, Lenaldo B. Rocha, Marcos A. Rossi

Department of Pathology, Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Destruction of dental tissue and supporting structures is usually microscopically assessed by routine hematoxylin and eosin (HE)-stained sections. This short communication is concerned with the potential role of fluorescence microscopy of HE-stained sections to study morphological aspects of intact and pathological teeth in dental research. This methodology improves the visualization of the anatomical structures of the intact teeth, especially anatomical features and periodontal ligament spatial distribution. This technique also improves the visualization of the root and bone resorption and the delineation of the periapical lesion extension. The fluorescence microscopy technique of HE-stained sections is an easy, reliable and inexpensive method that seems to be a useful tool for evaluating morphological aspects of intact and pathological teeth in dental research.

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Case report

Inflammatory diseases involving dental supporting tissues often lead to extensive tissue destruction. The components that are first destroyed are periodontal ligament fibers followed by alveolar bone, cementum, and dentin. The evaluation of these changes in tissue samples is usually performed on hematoxylin and eosin (HE)-stained sections. Eosin stains the acidophilic tissular components, cell cytoplasm and most proteins of the extracellular matrix in shades of pink. This short communication is concerned with the potential role of fluorescence microscopy of HE-stained sections to study morphological aspects of intact and pathological teeth in dental research.

Eosin is a known xanthene dye obtained by halogenization of fluorescein (1). Although eosin is usually not regarded as a fluorochrome, high-fluorescence emission has been described for this dye (2). Fluorescence of standard HE-stained sections can be evaluated using any epi-illumination-equipped microscope. The absorption and emission maxima of eosin in alcoholic solution is, respectively, 527 and 550 nm, with both peaks laying in the green light range; however, in the fluorescent microscope, we achieved a highly satisfactory emission illuminating tooth sections with blue light (450–490 nm) using a commercially available long-pass fluorescence filter for fluorescein. The main advantage of using the fluorescent properties of eosin from routinely HE-stained tissues are: (a) the rapid and distinct assessment of tissues without further sectioning and any special staining; (b) prolonged observation with minimal bleaching; and (c) easy re-evaluation of archive sections as the fluorescence persists for many years.

Fluorescence microscopy of HE-stained sections has been sporadically reported in medical research for the assessment of myocardial infarction (3), liver biopsy studies (4), visualization of elastic fibers (5), and morphometric evaluation of human or animal spleen (6). However, the usefulness of employing fluorescence microscopy to evaluate distinctive insights about the condition of teeth and periodontal structures has not yet been explored in dental research.

There are two major findings in this study. First, fluorescence analysis of the anatomical aspects of intact

Correspondence: Andiara De Rossi, Department of Pathology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, 14049-900 Ribeirão Preto, São Paulo, Brazil. Tel./fax: +55 16 3602 3130,
E-mail: andiaraderossi@bol.com.br
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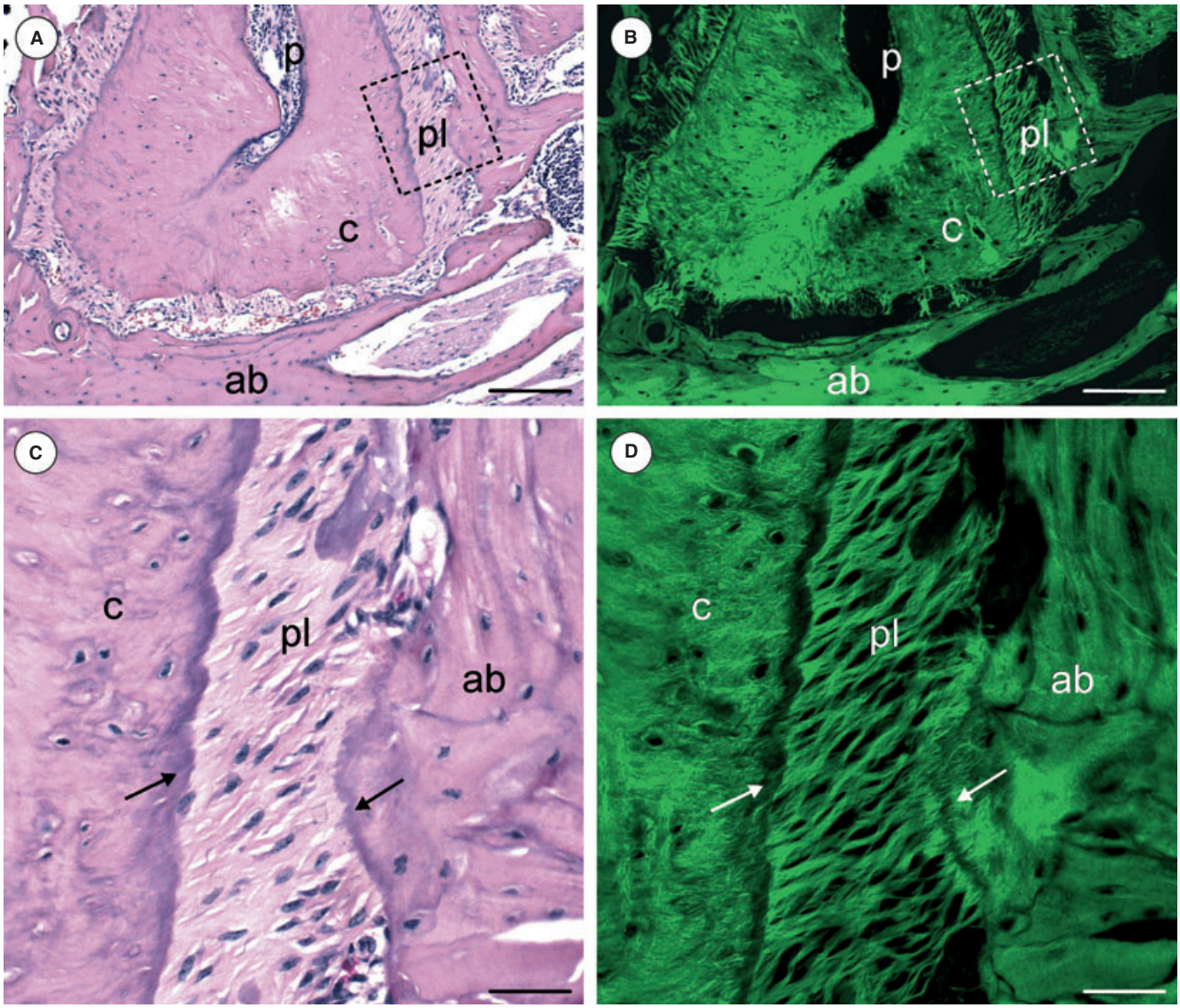

Figure 1 (A,C) Conventional light microscopy of intact mouse first molar with vital pulp tissue (p) and normal periapical region. The periodontal ligament (pl) is mostly composed of fibroblasts, interspersed with collagen fibers and numerous vascular and neural elements. (B,D) The same areas observed in fluorescence mode reveals the complex architecture of the periodontal ligament (pl), the varying sizes of fiber bundles, and the specific spatial alignment of collagen fibers. Arrows point to the origin and insertion of periodontal ligament fibers perpendicular to teeth surface and inserting in the alveolar bone (ab). Cell-occupied spaces appear as black dots in the cellular cementum (c), alveolar bone and amongst the collagen fibers in the periodontal ligament. Scale bars: 80 μm (A,B) and 20 μm (C,D).

teeth improves periodontal ligament imaging, resulting in a clear picture of intact collagen fiber structure and distribution. Collagen fibers appear highly fluorescent against a dark background. This distinct feature of the fluorescent image generated a highly contrasted impression of the specific spatial fiber arrangement and structural details. This includes the thickness and depth of Sharpey fibers inserting into the cement and bone. In addition, details of extracellular matrix organization in inflammatory periapical disease, such as extension of ligament destruction and organization of the periapical granulation tissue, especially the fibrillar framework, can be easily observed (Fig. 1). Second, the identification and visualization of the perimeter of periapical lesions is also improved along with areas of bone and tooth resorption. The root apex surface is corrugated with lacunae indicating cementum resorption. Bone surface resorption areas (Howship's lacunae) are evident, similar to the cementum surface. Furthermore, fluorescence microscopy shows that the lesion consists of a loose connective tissue. The periodontal ligament appears completely destroyed at the bone side, whereas ligament fibers still remain attached to the cementum (Fig. 2).

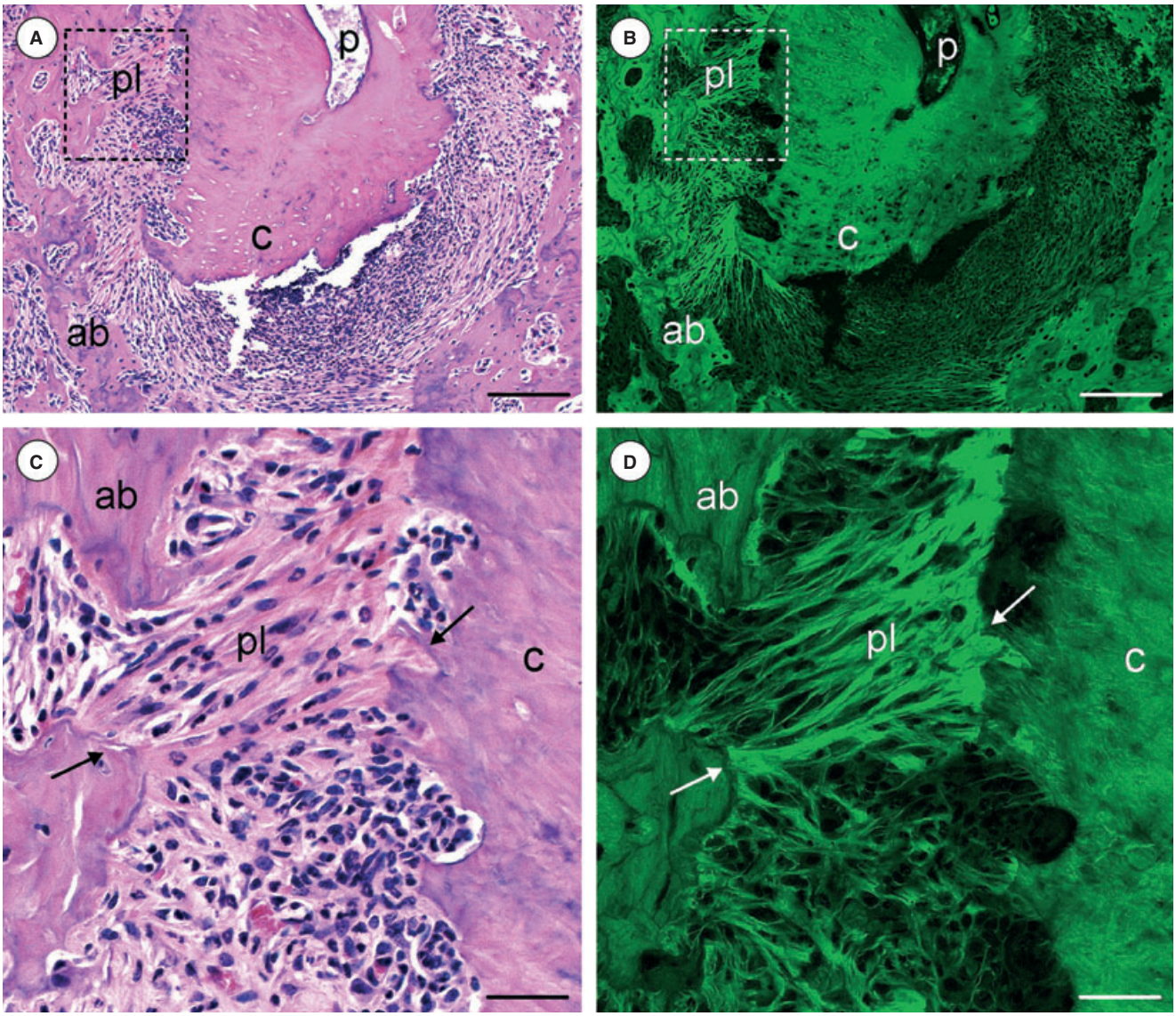

Figure 2 (A,C) Conventional light microscopy of a mouse first molar with chronic periapical lesion. The periapical region is capped by extensive and dense chronic inflammatory infiltrate. The surface of the root apex is corrugated with large lacunae indicating advanced cementum (c) resorption. The limits between intact and resorbed cementum and alveolar bone (ab) are not clear within the lesion. (B,D) Same areas as observed in (A) and (C) in fluorescence mode. Intact periodontal ligament fibers (pl) show strong green fluorescence. Arrows point to the origin and insertion of remaining periodontal ligament fibers. Fluorescence microscopy clearly shown the intact and resorbed structures. The periapical lesions are easily delineated when compared with the same image observed in brightfield mode. Scale bars: 80 μm (A,B) and 20 μm (C,D).

Extracellular matrix organization has been analyzed by a series of histochemical techniques, such as the widely used picrosirius red staining that requires transmitted polarized light (7). Anisotropy of the collagen allows the visualization of its structures as bright yellow-red fibers against a dark field. Despite its usefulness, we found that the structures observed using the fluorescence of eosin showed finer details of tissue organization (Fig. 3). The fluorescence technique is superior to light microscopy with transmitted light due to optimal signal ratio obtained with fluorescence and due to the fact that this technique allows higher resolution than conventional light microscopy, because fluorescent structures act as independent light emitters and resolution is not limited to 0.2 μm as is the case for structures in the light microscope. The wide use of HE in dental diagnosis and research opens the possibility that HE-stained tissue sections plus fluorescence microscopy technique would be an easy, reliable, and inexpensive useful tool for evaluating morphological aspects of normal and pathological teeth.

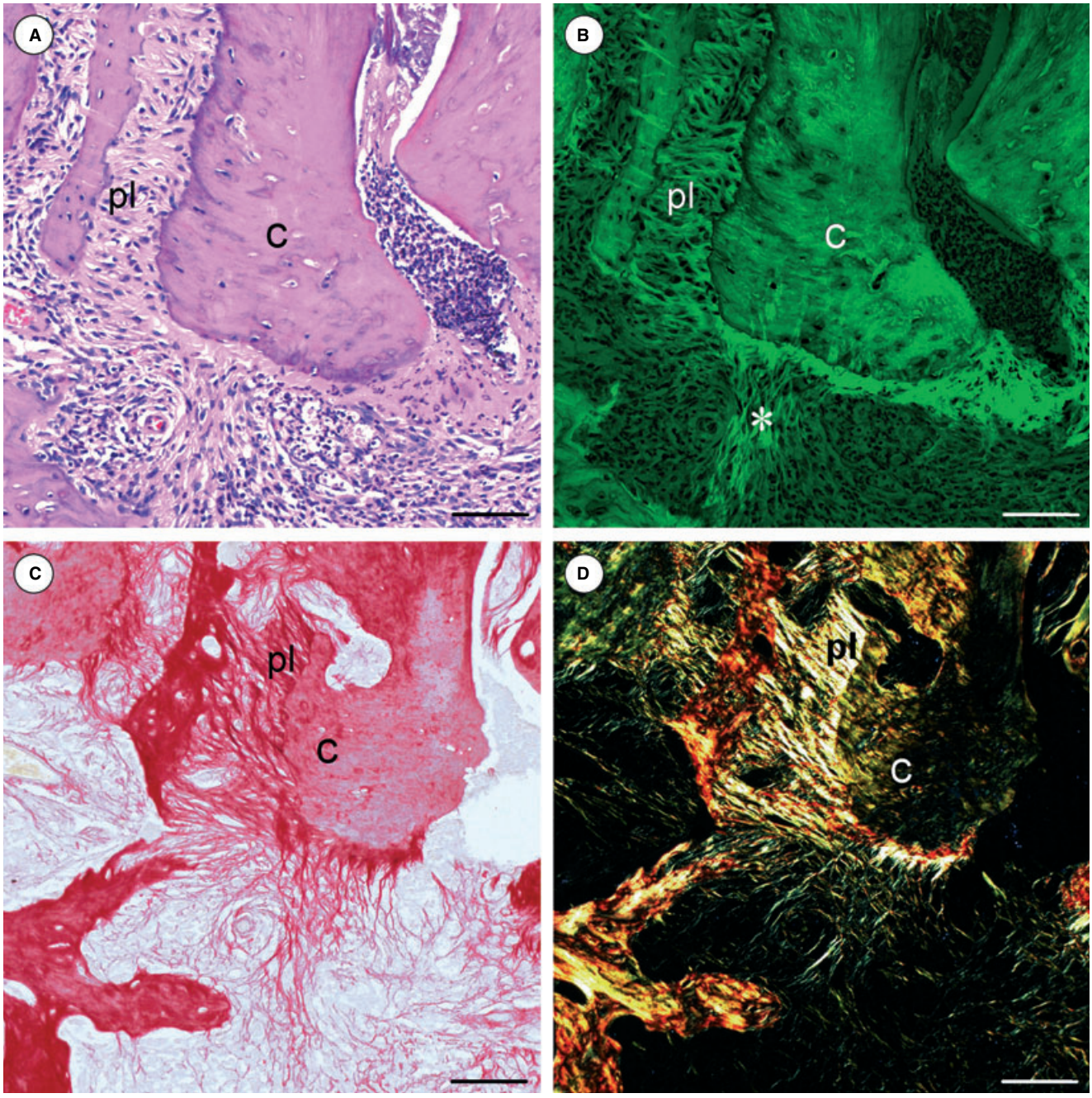

Figure 3 Serial sections of a mouse tooth is shown stained by HE and viewed with transmitted light (A) and epifluorescence illumination (B), and stained with picrosirius red and examined with transmitted light (C) and polarized light (D). Fluorescent eosin reveals richer details of periapical tissue notably the periodontal ligament (pl) including remnants within periapical granulation tissue. In contrast, picrosirius red, especially using polarized light, offers a poor view of the extracellular matrix other than the periodontal ligament. Scale bars: 40 μm.

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