acta histochemica 113 (2011) 231–233

Contents lists available at ScienceDirect

acta histochemica

journal homepage: www.elsevier.de/acthis

Editorial

# Picrosirius red staining revisited

Picric acid has been credited as the only chemical used in histopathology that has served as both a fixative and a dye. It was introduced as the first synthetic dye for silk in 1771 (Puchtler et al., 1988). In 1897, Pol André Bouin used picric acid as one of the main components of his fixative, which was a great favorite of pathologists, who could be identified by the unavoidable yellow staining of their fingers caused by the picric acid. Bouin's fixative solution has now largely been replaced by buffered formaldehyde fixatives, though it is still commonly used for fixation of testes, where nuclear preservation is superior. In 1889, Ira van Gieson introduced a staining technique that combined picric acid with acid fuchsin, which proved to be very popular for staining connective tissue and has even been described as possibly the most successful single histological technique ever devised. It was widely used for staining connective tissue fibers of muscle a strong red color. Unfortunately the red color of the stained connective tissue fades fairly rapidly after mounting and within a few months the red color completely disappears so if you wanted to preserve an image, it was essential to photograph the freshly stained preparations.

Sirius red F3BA (also known as F3B or Direct Red 80) in combination with picric acid was first described in 1964 as a selective histological stain for connective tissue (Sweat et al., 1964). The staining technique was originally introduced to determine sites of amyloid, but it was soon apparent that it also stained collagen. Picrosirius red staining is probably the last of the histological stains to reach any degree of popularity. A brief check of any recipe book of histological techniques will show that apart from minor modifications of existing methods, the latest entries for diagnostic histological staining of tissues come to a sudden stop in the 1960s. Since then there have been no new histological stains of any note, which is somewhat surprising. This can possibly be ascribed to the introduction of newer diagnostic and analytical procedures, in particular the use of immunohistochemical methods and advanced microscopy techniques. There also may be a connection with the loss of prestige and demise of the classical histologist willing to experiment with stains, dyes and dye chemistry.

Picrosirius red staining has proved to be an increasingly useful and popular histological staining technique owing to the simplicity of the procedure and its consistent successful outcome. The staining is permanent and almost foolproof. The chemicals needed are relatively cheap and last a long time, the stock solutions are simple to prepare and last indefinitely and all the procedures are done at room temperature. Picrosirius red staining can be quite spectacular and colorful providing results that are much more informative than routine H & E staining and which can result in images and information on a par with those of more complex trichrome staining methods.

The main uses of picrosirius red staining have been to stain collagen and differentiate between differing forms of collagen fibers (Weatherford, 1972). Picrosirius red staining works very well on decalcified skeletal tissues and is an excellent method for demonstrating bone canaliculi, replacing other classical methods such as Schmorl's technique (Figs. 1A–C). In primary (woven bone) or in bone undergoing repair after injury, the fibers appear red (without polarization optics), whereas in lamellae of secondary bone the orderly arranged collagen fibers appear green (Fig. 1B).

If picrosirius red stained sections are viewed using polarization optics with crossed polars, the collagen fibers showing birefringence (anisotropy) (Constantine and Mowry, 1968; Junqueira et al., 1979) (Fig. 1D). The polarization birefringence colors appear to be a measure of the thickness of the collagen fibers, the density of their packing and spatial arrangement (Dayan et al., 1989). Whereas it was once thought that the polarization colors were a measure of the collagen type, this is now known to be untrue (Piérard, 1989) and specific collagen types (numbering more than 20) can only be determined using immunohistochemical techniques with specific antibodies.

## Uses of picrosirius red staining in histopathology

Histopathologists regularly use picrosirius red staining as a simple technique to evaluate degrees of fibrosis, such as in the liver. Interstitial myocardial fibrosis and scar tissue developing after cardiac infarct is differentially stained with the picrosirius red method (Whittaker et al., 1994). The staining is also useful in the differential diagnosis of neoplasms involving fibrosis, such as thyroid follicular neoplasms, where there is capsular invasion (Koren et al., 2001) and also in some salivary gland tumors. It is also a useful diagnostic tool to study asbestoid degeneration in hyaline cartilage (Line et al., 1988). The marked staining of the collagen makes it very easy to apply quantitative image analysis to determine the degree of fibrosis.

Picrosirius red staining can be very useful to determine developmental and repair processes in bone and dental tissues (Figs. 2A and B) and can be used to follow healing processes after tooth extraction (Artzi et al., 2001; Roush et al., 1988). It is also useful to evaluate the osteointegration of

0065-1281/$ - see front matter 
doi:10.1016/j.acthis.2010.02.002

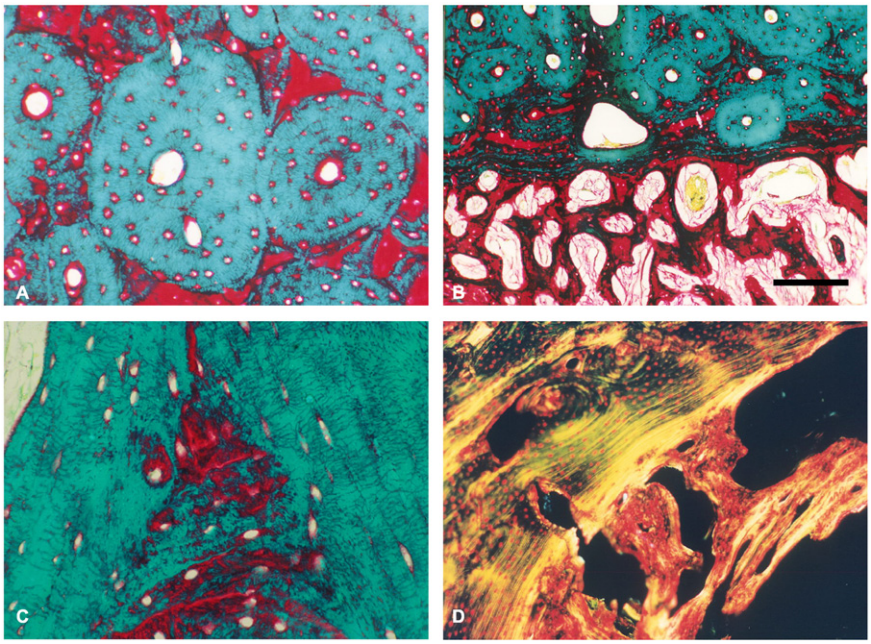


**Fig. 1.** Picrosirius red staining of dog jawbone. (A) Osteons (Haversian systems) seen in transverse section. The lamellae with their orderly arranged collagen are stained a greenish color. Scale bar, 0.1 mm; (B) lamella of compact bone stain a greenish color, in contrast to the lamellae of the spongy bone, which are stained a red color. Scale bar, 0.2 mm; (C) immature (woven) bone is stained red, whereas in the mature bone (lamellar bone) the lamellae are stained a green. The bone canaliculi are well demarcated with this staining technique; (D) compact and woven bone showing different birefringent colors. Scale bar 0.2 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

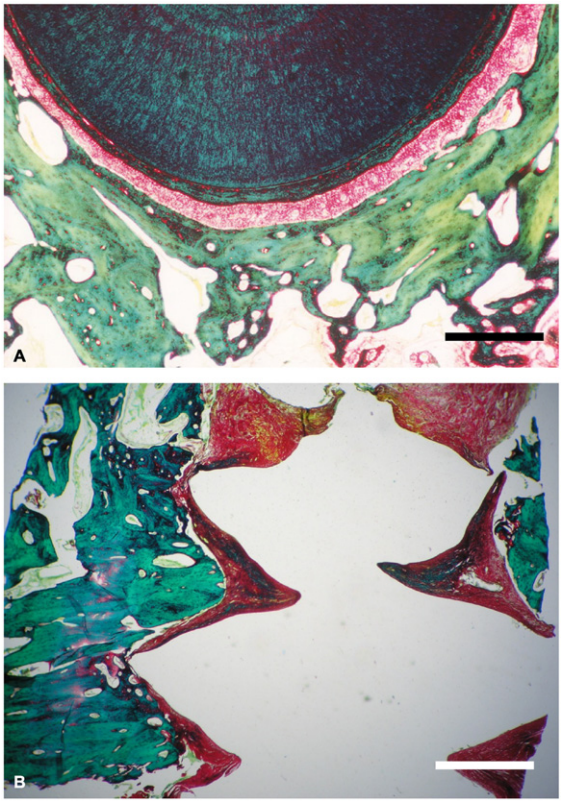


**Fig. 2.** Picrosirius red staining. (A) The region of insertion of a dog tooth into its socket. Dentine-cementum and bone show different staining patterns. Scale bar, 0.5 mm. (B) The site where a metallic screw of a dental implant was removed and which shows good osteointegration. The interface surface shows a layer of newly formed collagen (in red), whereas the older bone has a green color. Scale bar, 1 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

metal screws of dental implants in jaws of experimental animals (Fig. 2B).

## Dangers of picric acid

Picric acid (2,4,6-trinitrophenol) is scary stuff as it can be explosive if dry or in contact with metals. Despite its widespread use in histology laboratories for over a century, institutional health and safety officers tend to view the chemical as extremely hazardous owing to its potential explosive properties and they would prefer us not to use it at all or even have it in the laboratory. Picric acid is supplied by manufacturers as a 25–35% hydrated powder, but this can become dangerous if allowed to dry out. You must ensure that your picric acid powder container is tightly sealed and does not become dehydrated. You should also ensure that the picric acid waste is not disposed of in the drains, but is collected for chemical waste disposal.

## Staining protocols

Although there are several modifications of the picrosirius red staining technique, the protocol used regularly in my laboratory is as follows:

1. Dewax sections in xylene and hydrate via graded ethanols to water.
2. Stain for 10 min in a solution of 1% picric acid with 0.1% fast green.
3. Wash 3 times in 1% acetic acid.
4. Stain for 20 min (or up to 1 h) in a solution of 0.2% sirius red F3B with 0.2% picric acid.
5. Wash in 1% acetic acid.
6. Air-dry at room temperature (several hours or overnight).
7. Add DPX mountant and coverslip.

## References


Artzi Z, Tal H, Dayan D. Porous bovine bone mineral healing of human extraction sockets: 2. Histochemical observations at 9 months. J Periodontol 2001;72:152–9.

Constantine VS, Mowry RW. Selective staining of human dermal collagen. II. The use of picrosirius red F3BA with polarization microscopy. J Invest Dermatol 1968;50:419–23.

Dayan D, Hiss Y, Hirshberg A, Bubis JJ, Wolman M. Are the polarization colors of picrosirius red-stained collagen determined only by the diameter of the fibers? Histochemistry 1989;93:27–9.

Junqueira LCU, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem J 1979;11:447–55.

Koren R, Yaniv E, Kristt D, Shvero J, Veltman V, Grushko I, et al. Capsular collagen staining of follicular thyroid neoplasms by picrosirius red: role in differential diagnosis. Acta Histochem 2001;103:151–7.

Line SRP, Torloni H, Montes GS, Junqueira LCU. A note on the histochemical and morphological characterization of the asbestoid degeneration of cartilage. Histochemistry 1988;88:411–3.

Piérard GE. Sirius red polarization method is useful to visualize the organization of connective tissue but not the molecular composition of their fibrous polymers. Matrix 1989;9:68–71.

Puchtler H, Meloan SN, Waldrop FS. Are picro-dye reactions for collagens quantitative? Chemical and histochemical considerations Histochemistry 1988;88:243–56.

Roush JK, Breur GJ, Wilson JW. Picrosirius red staining of dental structures. Stain Technol 1988;63:363–7.

Sweat F, Puchtler H, Rosenthal SI. Sirius red F3BA as a stain for connective tissue. Arch Pathol 1964;78:69–72.

Weatherford TW. Staining of collagenous and non-collagen structures with picrosirius red F3BA. J Med Sci 1972;9:383–8.

Whittaker P, Kloner RA, Boughner DR, Pickering JG. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. Basic Res Cardiol 1994;89:397–410.



*Editor-in-Chief, Acta histochemica*
Raymond Coleman
*Department of Anatomy and Cell Biology, Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, P.O. Box 9649, Haifa 31096, Israel*
*E-mail address:* coleman@tx.technion.ac.il