Trichrome (Gomori Protocol) Staining for Bone and Cartilage Tissues

(Yi Zhu @ 01/08/2024; commented by TCH)

BACKGROUND:

Masson's trichrome is a classical method of visualization with numerous variants. The nuclear dye can be Masson's hemalum or several hematoxylin solutions. The cytoplasmic dye is fuchsine. An aniline-derived dye allows the differentiation of collagen fibers. The nuclei are brownish blue; the cytoplasm is red; secretions are red or green; and the muscles and collagen fibers are green-stained if fast green or sulfo green (in Masson–Goldner's variant) is used and blue if aniline blue is used. This staining method yields good results for morphological studies because the different parts of the tissue are very well contrasted.

REAGENTS:

Trichrome, Gomori One-Step, Aniline Blue Stain Kit (Newcomer Supply; Part# 9176A; Thermo Fisher Cat# NC9485545). The kit includes:

Solution A: Bouin Fluid
Solution B: Ferric Chloride, Acidified
Solution C: Hematoxylin 1%, Alcoholic
Solution D: Trichrome Stain, Gomori One-Step, Aniline Blue
Solution E: Acetic Acid 0.5%, Aqueous

STAINING PROCEDURE

1. Deparaffinize and hydrate sections. (Refer to H & E Staining Protocol) In the meantime preheat Solution A in 56-60°C water bath.
2. Mordant sections in preheated Solution A for 1 hour at 56-60°C water bath. Cool at room temperature for 10 mins.
3. Wash well in running tap water, and rinse in distilled water.
4. Mix 20ml Solution B with 20ml Solution C. (Can be recycled for up to 2 weeks)
5. Stain in B&C mix for 10 mins.
6. Wash in running tap water for 10 mins, and rinse in distilled water.
7. Stain with Solution D for 20 mins.
8. Differentiate in Solution E for 2 mins.
9. Rinse quickly in distilled water.
10. Dehydrate, clear in xylene, add Permount oil, and cover slips (*press firmly for 20''-30'' to make sure the tissue section is leveled on the same focal plane*). (Refer to H & E Protocol)

COMMENTS

Masson's Trichrome Staining (MTS) is **a useful tool for analyzing fibrosis in a plethora of disease pathologies by differential staining of tissue components**. It is used to identify collagen fibers in different tissues like heart, lung, skin, and muscles. The recipes evolved from Claude L. Pierre Masson's (1880–1959) original formulation have different specific applications, but all are suited for distinguishing cells from surrounding connective tissue.

Most recipes produce red keratin and muscle fibers, blue or green collagen and bone, light red or pink cytoplasm, and dark brown to black cell nuclei.

The trichrome is applied by immersion of the fixated sample into Weigert's iron hematoxylin, and then three different solutions, labeled A, B, and C:

- Weigert's hematoxylin is a sequence of three solutions: ferric chloride in diluted hydrochloric acid, hematoxylin in 95% ethanol, and potassium ferricyanide solution alkalized by sodium borate. It is used to stain the nuclei.
- Solution A, also called **plasma stain**, contains acid fuchsin, Xylidine Ponceau, glacial acetic acid, and distilled water. Other red acid dyes can be used, e.g. the Biebrich scarlet in Lillie's trichrome.
- Solution B contains phosphomolybdic acid in distilled water.
- Solution C, also called **fibre stain**, contains Light Green SF yellowish, or alternatively Fast Green FCF. It is used to stain collagen. If blue is preferred to green, methyl blue or water blue can be substituted.

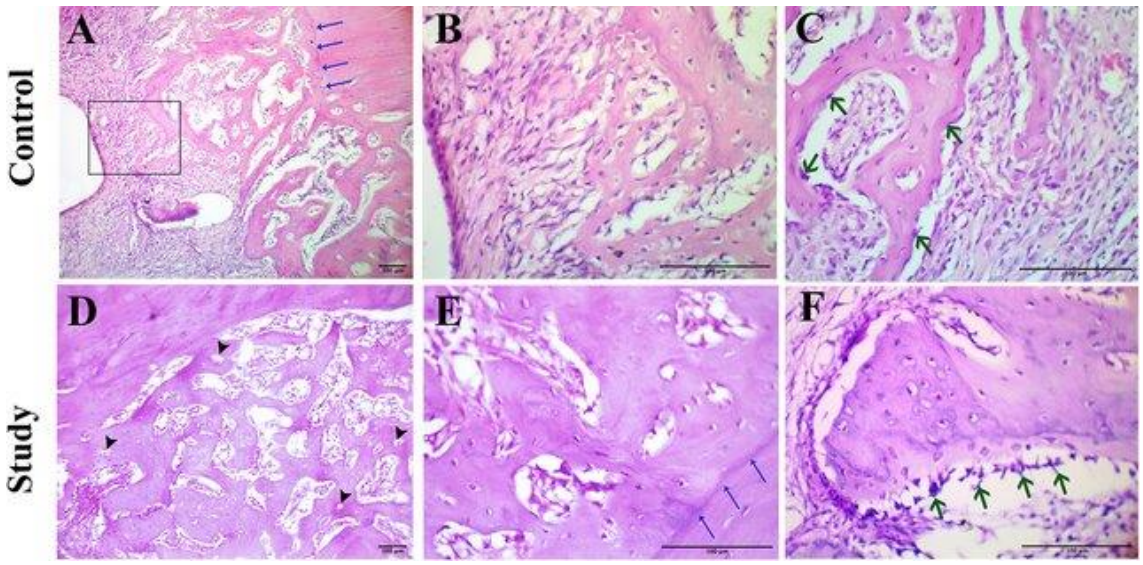
Standard applications: Masson's trichrome staining is widely used to study muscular pathologies (muscular dystrophy), cardiac pathologies (infarct), hepatic pathologies (cirrhosis) or kidney pathologies (glomerular fibrosis). It can also be used to detect and analyze tumors on hepatic and kidney biopsies

Gomori trichrome was developed by George Gomori in 1950. This staining is intended to identify an increase in collagen fibers in the connective tissue or to differentiate between collagen and smooth muscle fibers. The principle of Gomori trichrome staining is **based on single-step staining**. One of the oldest single-step approaches to trichrome staining is **van Gieson's** method, which stains muscle and cytoplasm yellow, and collagen red. Another is the **Gömöri trichrome stain,** which closely mimics **Masson's trichrome**.

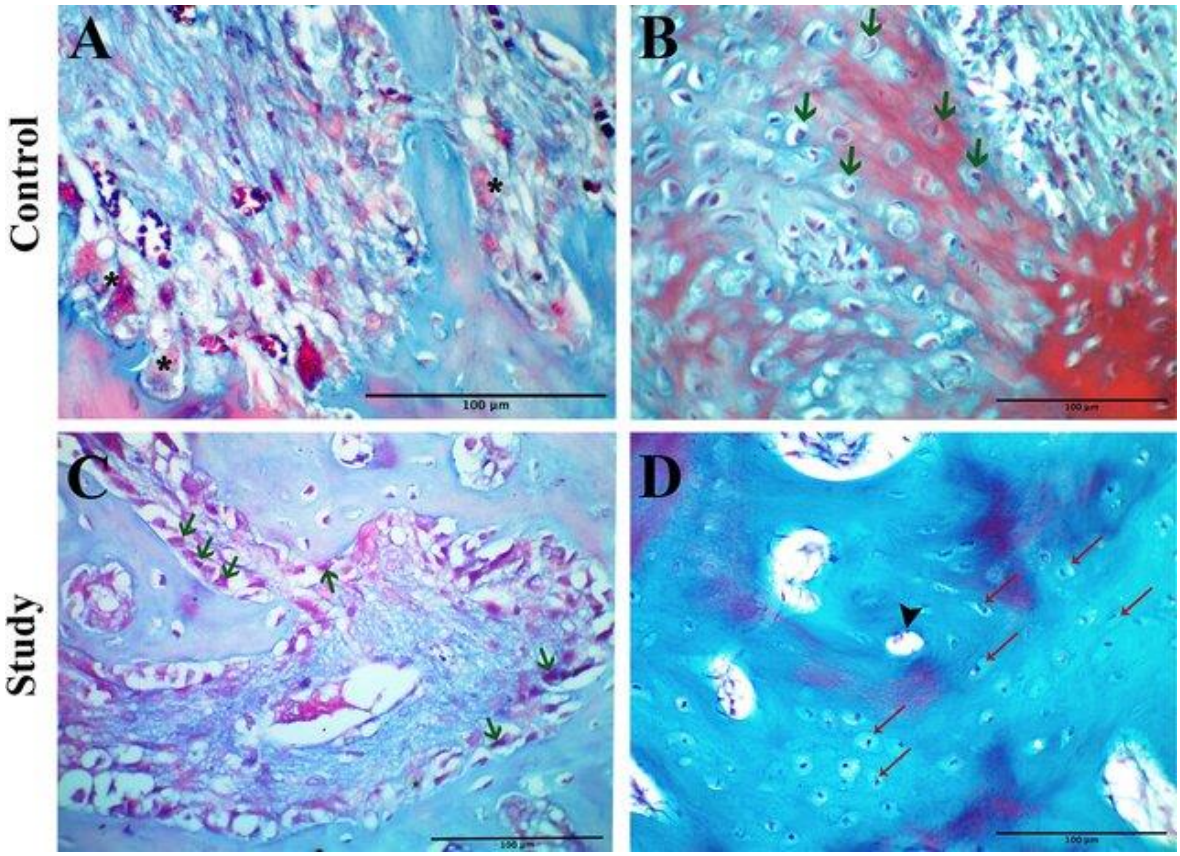
One-step Trichrome Staining Variants: **Gomori**, Papanicolaou, Cason, Engel & Cunningham, Kostowiecki, Ladewig, McFarlane, Papanicolaou, Pollak, Sweat, Meloan, Puchtler, Wallart & Honette

Multi-step Trichrome Staining Variants: **Goldner**, Lillie, Masson type, Masson-A, Masson-B, Masson – C, Masson 44/41, Weiss, Slidders OFG, Picro-Mallory long, Picro-Mallory short, Patay, Obadiah, Möllendorf, Milligan, Brillmeyer, Bensley, Crossman, Haythorne, Heidenhain's Azan, Hollande, Klatskin, Koneff, Kricheski, Laidlaw, Lee-Brown, Lendrum & McFarlane, Lendrum Slidders & Fraser, Lewis & Miller

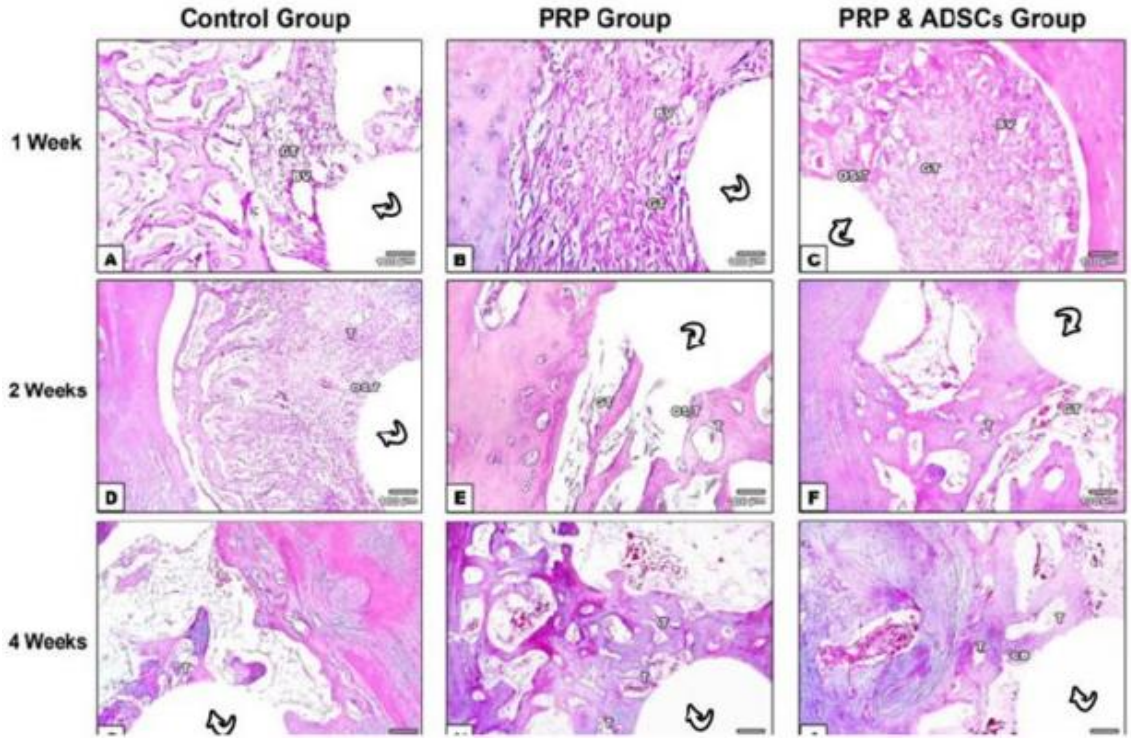
GOMORI TRICHROME STAINING SAMPLES



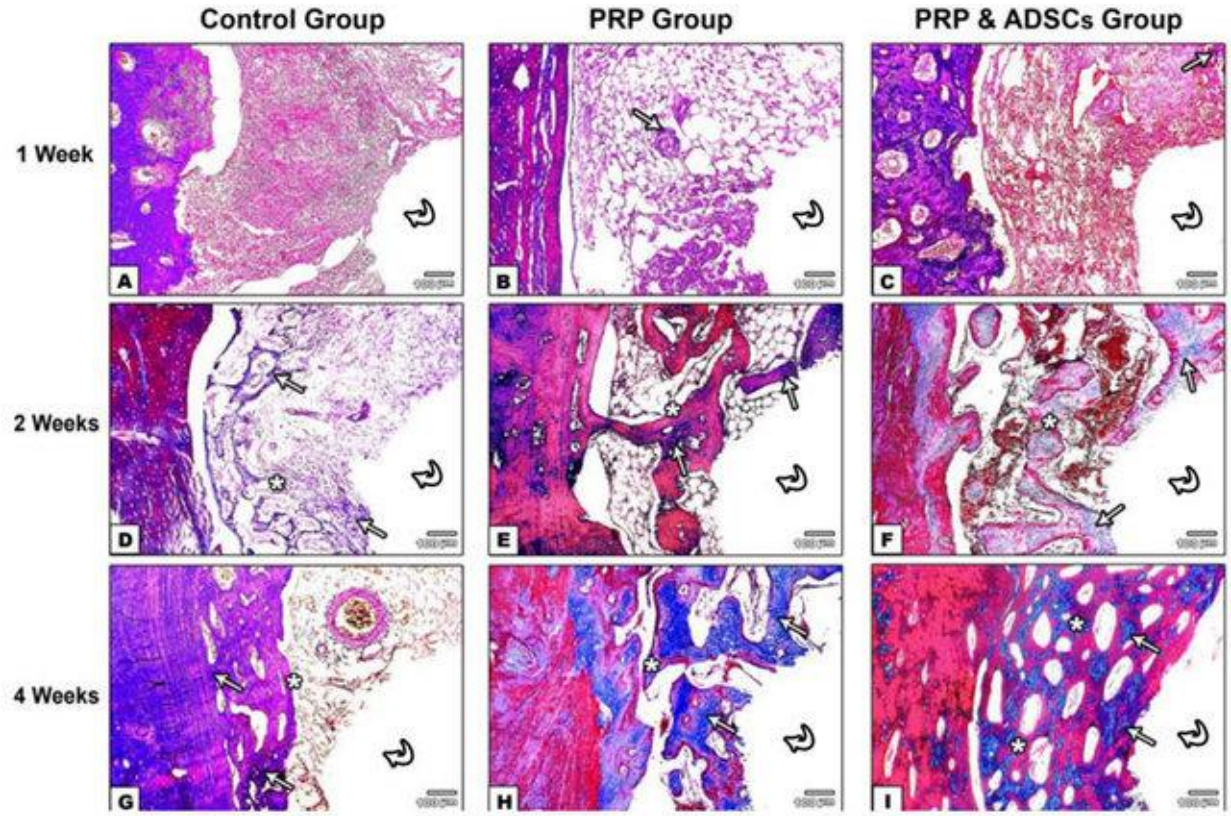
Light micrographs (LM) of H&E stained decalcified sections. A and D ×100 magnification. B, C, E, F ×400 magnification. A showing irregular trabeculation of the newly formed bone. The demarcation line between the native and the new bone (blue arrows). B higher magnification of the inset in the previous micrograph showing the extension of immature bone formation toward the defect center. C showing the discontinuous layer of osteoblasts (green arrows). D showing thick and well-organized bone trabeculae with primary osteon formation (arrowheads). E showing the interconnectivity and thickness of the trabeculae and their osteointegration with the native bone (blue arrows). F showing high activity of osteoblasts (green arrows).



LM of decalcified sections stained with Gomori trichrome stain, ×400 magnification. A showing collagen mapping the forming bone trabeculae. Osteoclasts on the surface of the defect margin and the new trabeculae (asterisks). B showing bone formation with early trapping of osteoblasts (green arrows). C showing an active area of bone formation consisting of collagen and voluminous osteoblasts (green arrows). D showing an osteon formation in the regenerated bone (arrowhead) and entrapped osteocytes (red arrows).



Hematoxylin and eosin staining of femur bone with or without PRP or (PRP with ADSCs) after 1, 2 and 4 weeks after implantation. Scale bar is 100 mm. (A-C) There were difference between control group (A,D,G), PRP (B,E,H) and PRP&ADSCs (C,F,I) in terms of new formation of granulation tissue (GT), widening in blood vessels (BV), osteoid tissue (OS.T) formation, and trabeculae (T) around mini-implant in the one, two and four week specimen. (curved arrow was the site of the implant).



Masson's trichrome staining of femur bone with or without PRP or (PRP and ADSCs) after 1, 2 and 4 weeks after implantation. Scale bar is 100 mm. Photomicrograph showing appearance of collagen fibres (blue stain, labelled arrow) and newly grown bone (red stain, labelled star) after 1, 2, and 4 weeks. (curved arrow was the site of the implant) All data were analysed by ANOVA and Bonferroni post-hoc. Data points are mean±SEM $P \geq 0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared with control group, differences not statically significant was labelled as (ns).