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Safranin-O and Type II Collagen Stains for *In Vitro* Chondrogenesis

Materials

- 10% Formalin, neutral buffered – VWR catalog #VW3239-4 or equivalent
- 100% Ethanol
- Xylene or xylene substitute
- Eosin stain
- Paraffin
- Weigert's iron hematoxylin
- Fast Green FCF
- 1% acetic acid
- 0.1% aqueous Safranin-O
- Proteinase K – DAKO code # S3020 or equivalent
- Immunohistochemical staining kit – DAKO code # K4006 or equivalent
- Type II Collagen specific primary antibody
- Harris hematoxylin
- Chondrogenic pellet cultures

Procedure

Chondrogenic Pellet Processing

- Fix each chondrogenic cell pellet in 10% neutral buffered formalin for 1-24 hours at room temperature
- Transfer each pellet to the corner of a small histology transfer bag, fold the bag and place it into a small histology cassette, labeled in pencil.
- Transfer the cassette to 70% ethanol. The pellets remain in the cassettes throughout processing until embedded
- Dehydrate pellets in successive ethanol washes of 70%, 70%, 80%, 80%, 95% and 95%, for 15 minutes each
- Stain pellets with Eosin by dipping briefly in Eosin, followed by three brief rinses in 100% ethanol. This stain serves only to visualize the pellets and aid in their handling. The pellets will stain orange
- Incubate pellets in two changes of 100% ethanol, 20 minutes each
- Transfer to xylene or xylene substitute, two changes, 20 minutes each
- Transfer to three changes of 58°C paraffin, 20 minutes each
- Remove each pellet from its histology cassette and bag and embed in a paraffin block as per standard embedding procedures
- Cut ribbons of 5 μm sections of each pellet and transfer to a 40°C water bath
- Transfer 4-6 sections of each pellet onto positively charged glass microscope slides
- Incubate the slides at 60°C for one hour, then cool for 10 minutes
- Stain the pellet sections or store the slides at room temperature for future staining

Safranin-O Staining for Glycosaminoglycans

- Deparaffinize pellet sections in xylene (3 x 3 min), 100% ethanol (2 x 3 min), 95% ethanol (2 x 3 min) then 70% ethanol (1 x 3 min)
- Stain in Weigert's iron hematoxylin for four minutes, then destain in fresh acid alcohol (1 ml concentrated HCl in 100 ml 70% ethanol) and rinse in tap water
- Stain in 0.02% aqueous fast green FCF for three minutes, then wash in 1% acetic acid for 30 seconds
- Stain in 0.1% aqueous Safranin-O for five minutes

- Dehydrate in 95% ethanol (2 x 5 min), 100% ethanol (3 x 5 min), then xylene (3 x 5 min)
- Mount sections in synthetic resin
- Proteoglycans will stain red, cytoplasm will stain gray green and nuclei will stain black

Immunostaining for Type II Collagen

- Deparaffinize pellet sections in xylene (3 x 3 min), 100% ethanol (2 x 3 min), 95% ethanol (2 x 3 min), 70% ethanol (1 x 3 min) then deionized water for 5 minutes
- Quench endogenous peroxidase activity by incubating the pellet sections with DAKO Peroxidase Block (included in kit) or 0.3% hydrogen peroxide in methanol for five minutes, followed by washes in deionized water and wash buffer (Tris buffered saline, 0.05% Tween)
- Treat pellet sections with proteinase K for five minutes, followed by washes in deionized water and wash buffer
- Incubate sections with a serum-free protein block (DAKO # X0909) for five minutes, then wash with buffer
- Incubate sections with an appropriately characterized primary antibody to Type II Collagen for 30 minutes, then wash with buffer
- Incubate sections with the appropriate peroxidase labeled secondary reagents and substrate-chromogen (peroxide-DAB) as per kit instructions
- Counterstain with hematoxylin and appropriate bluing agent
- Mount section in synthetic resin
- Type II Collagen will stain brown (with DAB) and nuclei will stain blue

References:

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