THE USE OF SIRIUS RED FOR PLASTIC HISTOLOGY OF THE INTERVERTBRAL DISC

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Statement of Purpose: Sheep and goats have become a convenient, practical and economical animal for orthopaedic research. For spine surgery, they have been useful for investigating fusion devices, growth factors and their carriers, instrumentation methods, vertebroplasty and kyphoplasty methods, disc replacement and vertebral body corpectomy. Surgical models allowing for a comprehensive evaluation of novel motion preserving therapeutic approaches to the degenerating and aging spine are becoming increasingly more important. Animal models can provide basic science data that support biological plausibility as well as temporality, specificity, and dose-response relationships. Recently disc replacement, nucleus pulposus (NP) replacement / augmentation, and anulus fibrosus (AF) repair, as well as cell-based treatment strategies targeting the intervertebral disc have been evaluated in various animal models. Animal models are needed to effectively show how therapeutic strategies ameliorate, resolve or prevent disc degeneration. Finally, animal models play a critical role in preclinical safety trials for new devices and therapies emerging in the field of soft non-fusion spine. The anatomy and morphology of the bovine, ovine, caprine, canine and non-human primate spine have been extensively studied. Their biomechanical behavior has been validated as possible models to study the degenerating disc. While there is abundant literature of *in vitro* models and their comparison to the human spine, there still is a paucity of study data when it comes to surgical models addressing new intervertebral disc technologies (1). Our expertise in "womb-tomb" preclinical trials addressing device safety and efficacy issues targets the design and execution of preclinical trials in relevant large animal models. Here we report about our extensive experience in intervertebral disc histology using plastic embedding techniques for hard tissue processing. The novel stain has been developed as a method for morphological identification of collagen and glycosylated aminoglycans (GAGs) in non-decalcified IVD specimen embedded in methyl methacrylate for sectioning. Sections with and without biomaterial implants have been used. The stain is a variation of some older procedures with changes added to enhance staining of plastic sections at 6 to 8 microns in thickness.

Methods: The staining sequence was: The staining procedure for methyl methacrylate sections did not require removal of the MMA. A procedure for removing MMA has been used and did not show any advantage in the final staining protocol as it allowed sections to float off the slide more easily. MMA sections dried onto silane charged slides (Thermo-Shandon Microscope Slides Colorfrost Plus) were used with a minimum of 48 hours drying time. Slides were individually covered with Kisol-Folie and bibulous paper cut to slide size, stacked and clamped for drying at 50ºC. Slides were removed and cooled for staining. If a nuclear stain was required, the standard format for Weigert's Iron Hematoxylin was used. Then slides were placed in 0.2% Direct Red 80/Picro Solution for 1 hour at room temperature, rinsed in 2 changes of 0.01N HCL Solution for 1 minute each. Then dehydrated, cleared, mounted and coverslipped.

Results/Discussion: A similar staining protocol for paraffin histology of the IVD was previously reported by Gruber et al (2). We have adapted some of these concepts for plastic histology for large intact *en bloc* discs of our large animal IVD models. Using this staining protocol, nuclei stained blue/black, collagen stained red, sulfated and carboxylated acid mucosubstances stained blue and connective tissue components stained yellow. This staining technique also allowed for a more qualitative and semi-quantitative differentiation of collagen density and regional shifts in intra-discal matrix concentration. Various degrees of proteoglycan accumulation could be readily detected. Regional intra-discal matrix changes or accumulations, which occur at various stages of disc degeneration, also became quite evident using this new staining technique. While this stain does not replace immunohistochemistry, it clearly enhances the interpretative power of tissue morphology in the IVD. This stain was used for histopathology on various discs harvested and processed *en bloc* from sheep, calves, mini-cattle and dogs enrolled in various preclinical trials evaluating novel motion preserving technologies of the spine. In certain cases, depending on the physico-chemical properties of the test device, this novel stain was able to distinctly stain the test device. This greatly aided in the histopathological evaluation of peri-discal tissue, including regional lymphnodes for device related fragmentation and migrating debris.

Conclusions: The new staining technique described here is another addition to the armamentarium of any histology laboratory specializing in plastic embedding and non-decalcified tissue histology. There is an increasing need of regulatory preclinical safety and efficacy trials evaluating new biomaterials and tissue engineering concepts in animal models. Due to anatomical and test article scale ratios, large animal models are often required. Processing discs of large animals *en bloc* is challenging and plastic embedding greatly preserves tissue morphology. This staining technique has now been validated for plastic histology.

References: (1) Lotz, JC. Animal Models of Intervertebral Disc Degeneration: Lessons Learned. *Spine 2004, Volume 29, Issue 23.* (2) Gruber HE et al. An Improved Staining Method for Intervertebral Disc Tissue. *J Biotechnic & Histochemistry 2002, 77(2):81-83*

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