Nerves and blood vessels in degenerated intervertebral discs are confined to physically disrupted tissue

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FIGURE 1

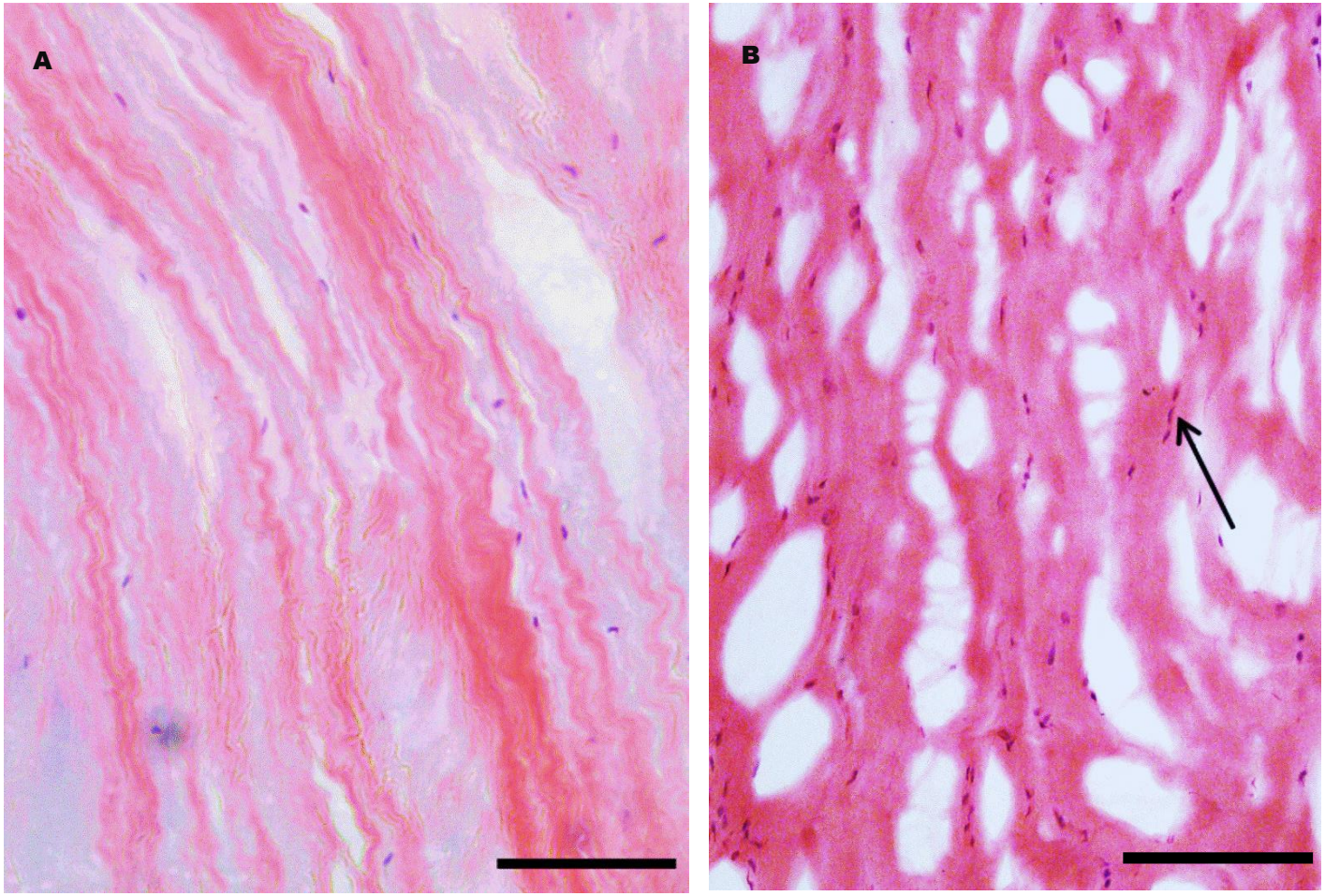

Figure 1: Outer annulus fibrosus (OAF) tissue stained with H&E. A) Non-degenerated scoliotic disc (scale bar 200 µm). B) Degenerated spondylolisthesis disc (scale bar 100 µm). Note the elongated fibroblast-like cells (arrow) aligned with the crimped collagen fibres, and the numerous microscopic tears and splits.

FIGURE 2

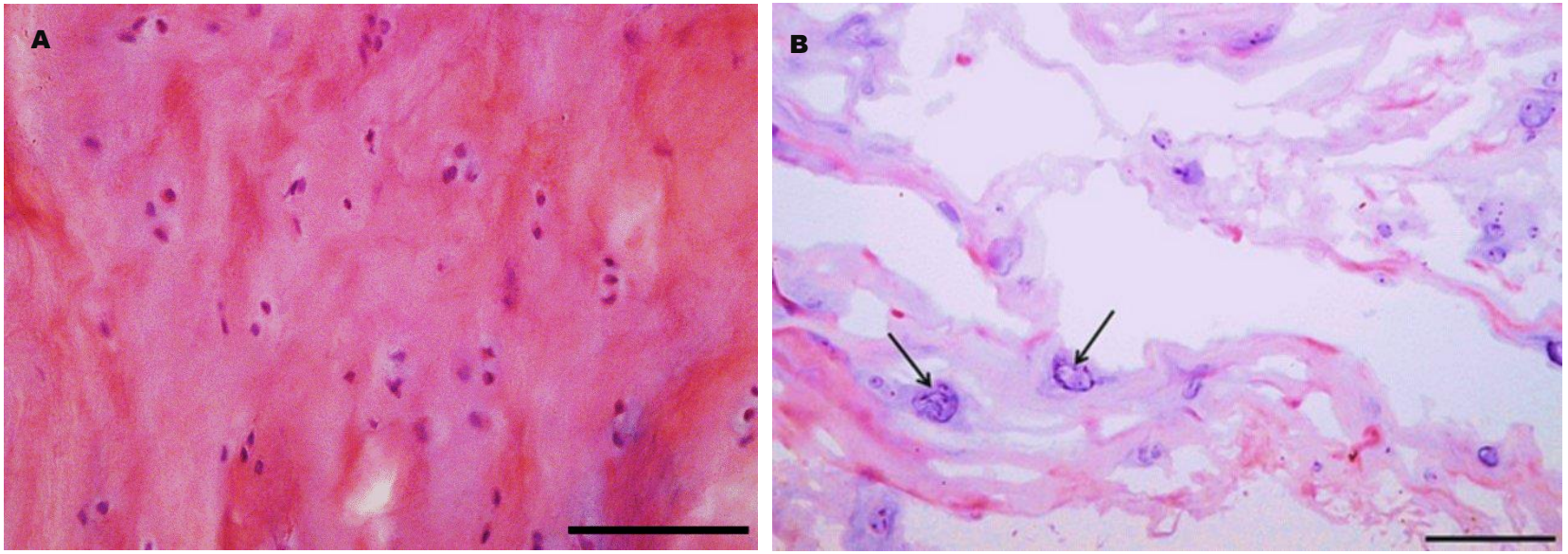

Figure 2: Inner annulus fibrosus (IAF) tissue stained with H&E. A) Non-degenerated (Pfirrmann grade 2) spondylolisthesis disc (scale bar 50 µm). B) Herniated disc (scale bar 100 µm). Note the rounded cell morphology, and the small cell clusters (arrows).

FIGURE 3

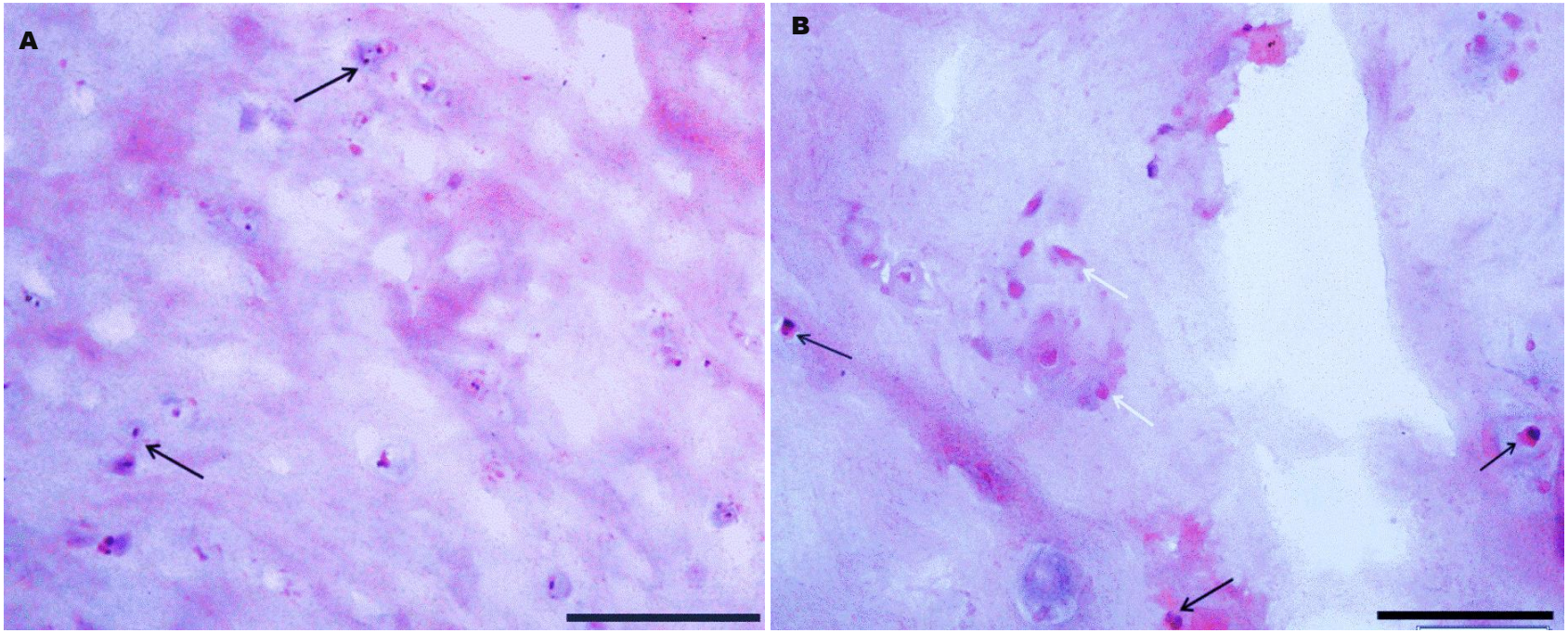

Figure 3: Nucleus pulposus (NP) tissue stained with H&E. A) Non-degenerated disc (scale bar 30μm). B) Degenerated disc with cell clusters around a matrix fissure (scale bar 100μm). Note the amorphous matrix in both images, and the larger cell clusters in B. White arrows indicate cells with disrupted cell membranes and some loss in nuclear stain, in contrast to cells with distinct nuclear and cytoplasmic staining (black arrows).

FIGURE 4

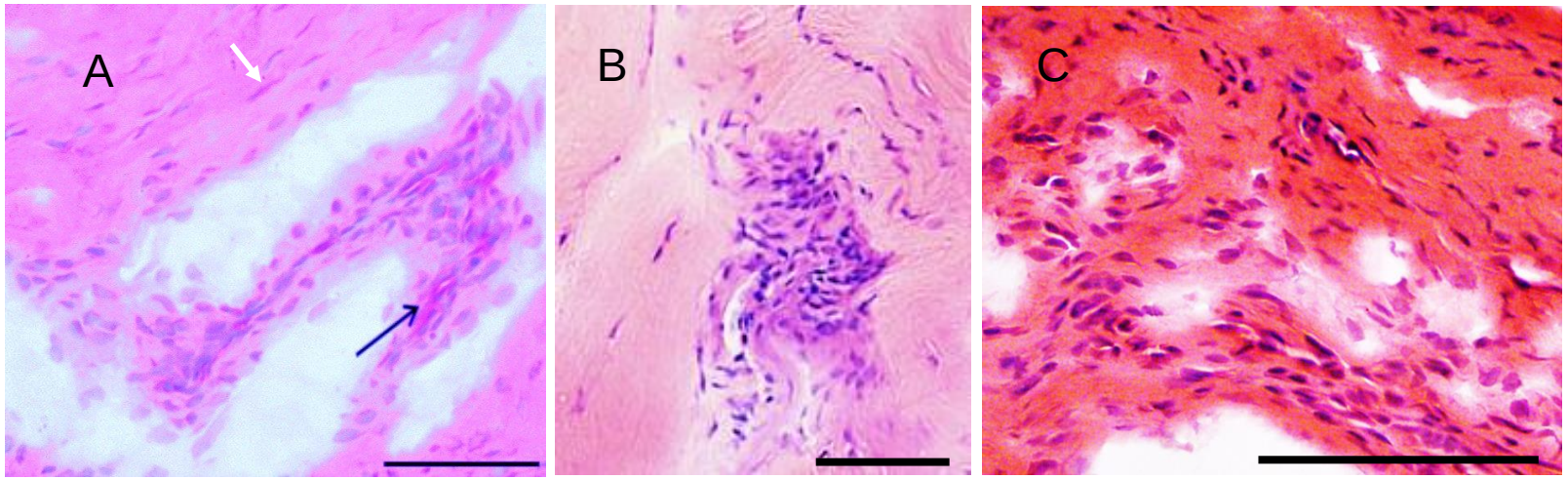

Figure 4: Inflammatory cells in herniated annulus fibrosus. H&E stain, scale bar = 50μm. A) Outer annulus fibrosus (OAF) tissue from a herniated disc with sciatica. Note the blood vessels in oblique transverse section (black arrow) together with inflammatory cells, and fibroblasts (white arrow). B) Inner annulus fibrosus (IAF) tissue from a herniated disc without sciatica. Note the disorganised infolding lamellae. C) Outer annulus fibrosus (OAF) tissue from a herniated disc with sciatica.

FIGURE 5

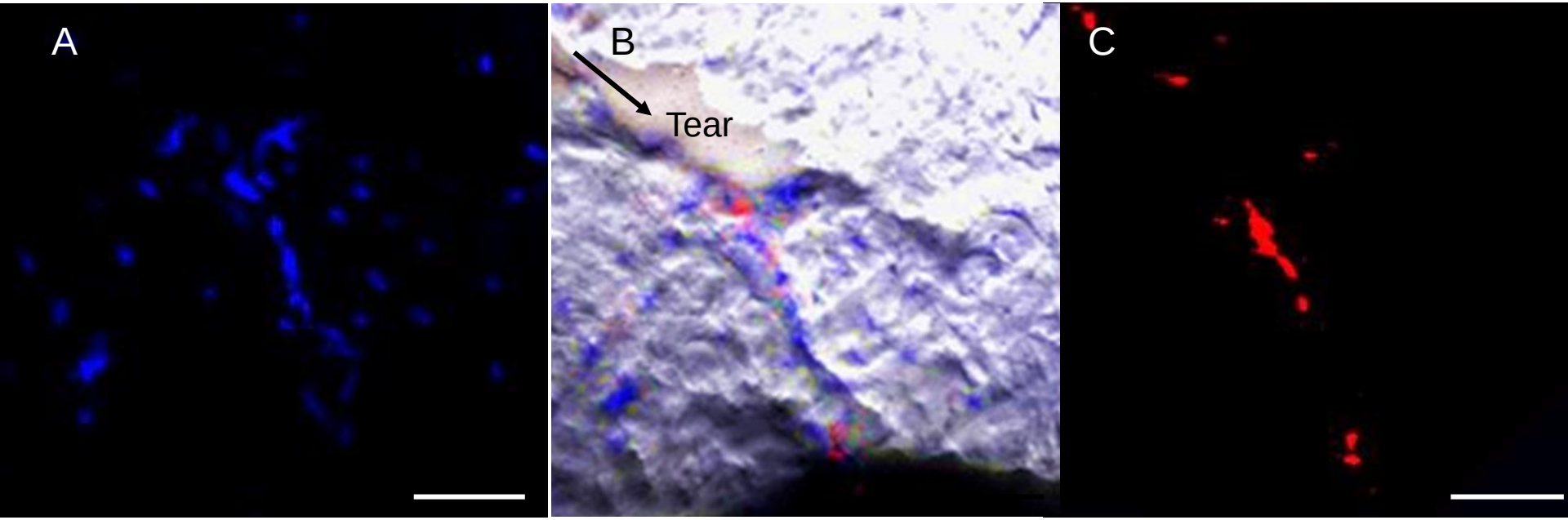

Figure 5: Co-localisation of blood vessels and matrix defects in degenerated inner annulus fibrosus (IAF) tissue. A) Cell nuclei stained blue with DAPI suggest blood vessels. C) Blood vessel immunopositive to CD-31 shown in red. B). Composite image showing blood vessels co-locallised with matrix features as indicated by phase contrast imaging. Scale bar = 50μm.

FIGURE 6

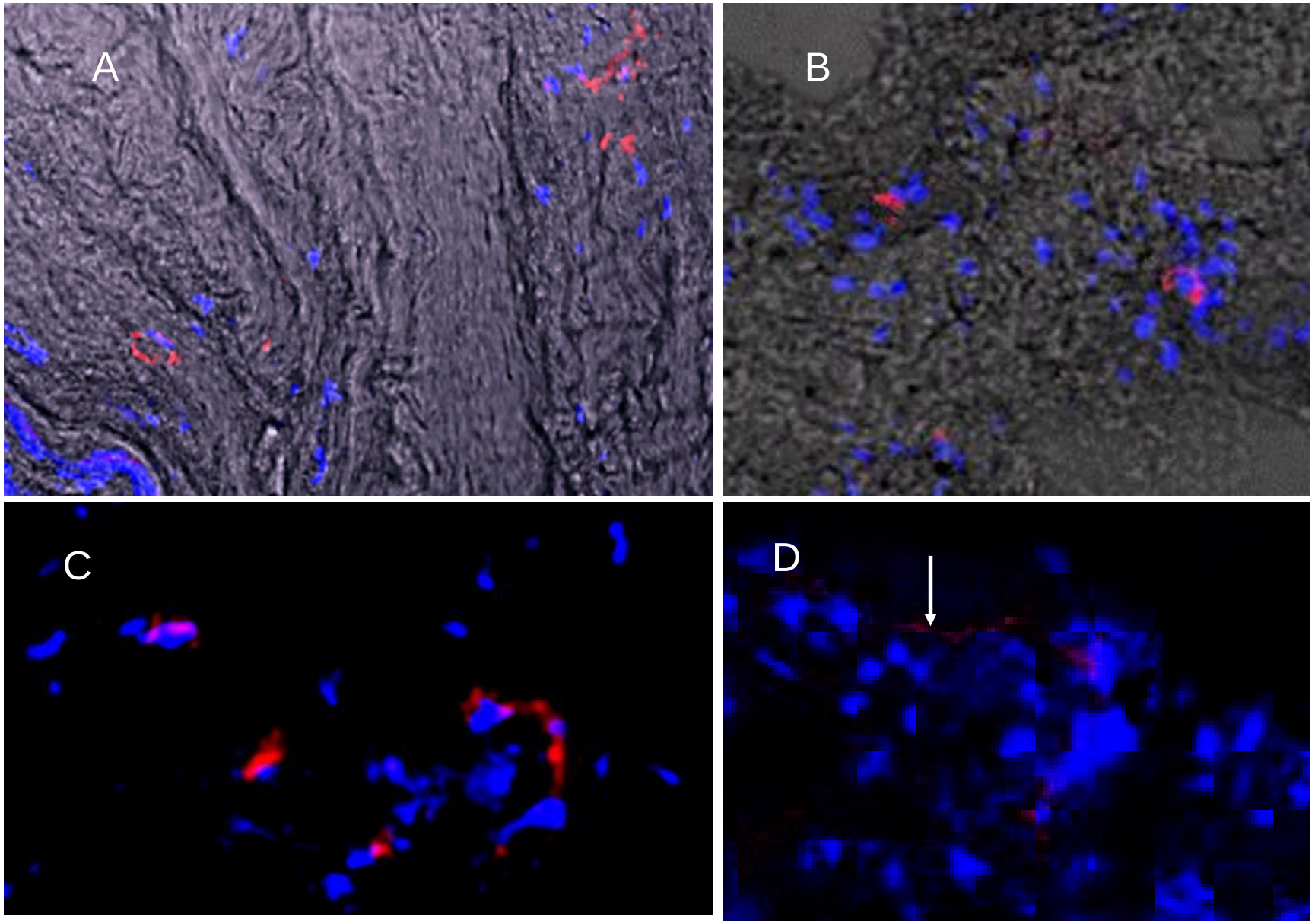

Figure 6: Nerves in annulus tissues of herniated discs. Cell nuclei are stained blue with DAPI. Scale bar = 50μm. A) Fine nerves stained red for Substance P. Phase contrast imaging suggests matrix features. B) Nerves stained red for PGP 9.5. C) Nerves stained red for PGP 9.5. D) Fine peripheral nerve (white arrow) stained red for Substance P.

FIGURE 7

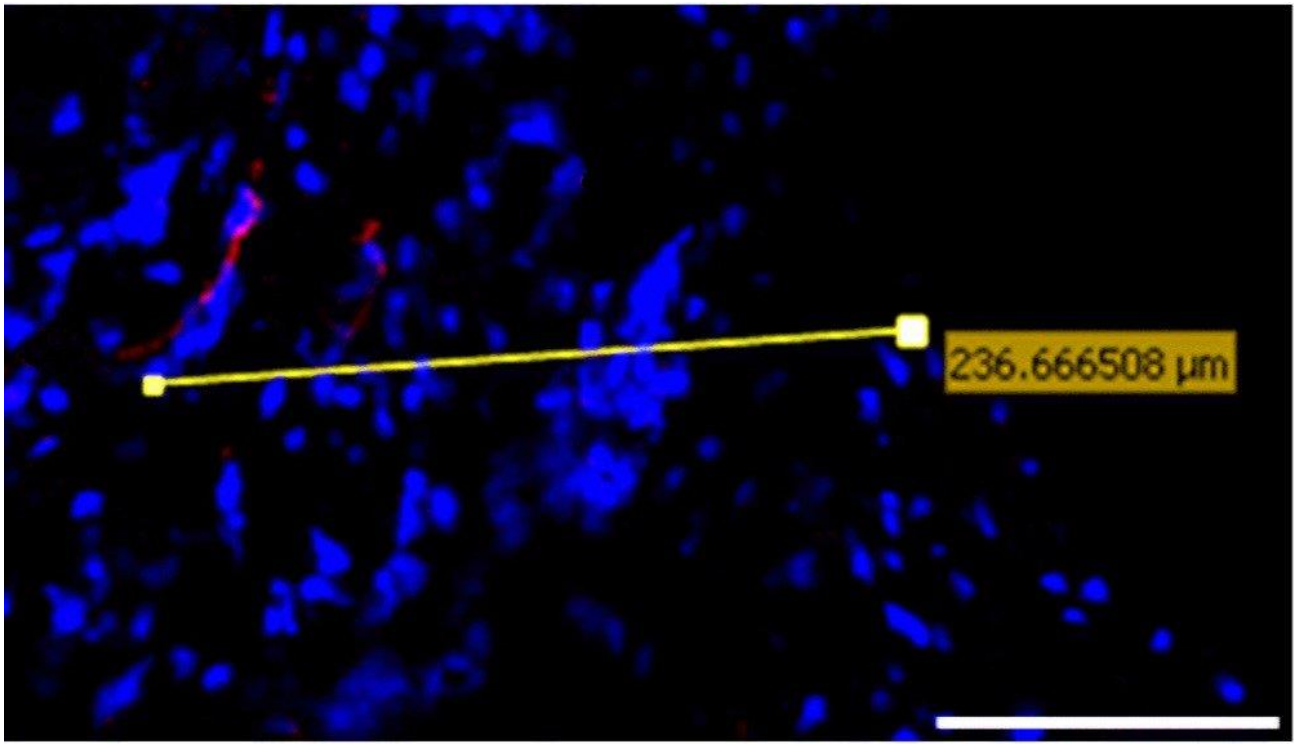

Figure 7: 'Volocity' image analysis software was used to measure the furthest distance of each stained object (in this case a nerve immunostained for PGP 9.5) from the nearest free surface. Scale bar = 100 µm.

FIGURE 8

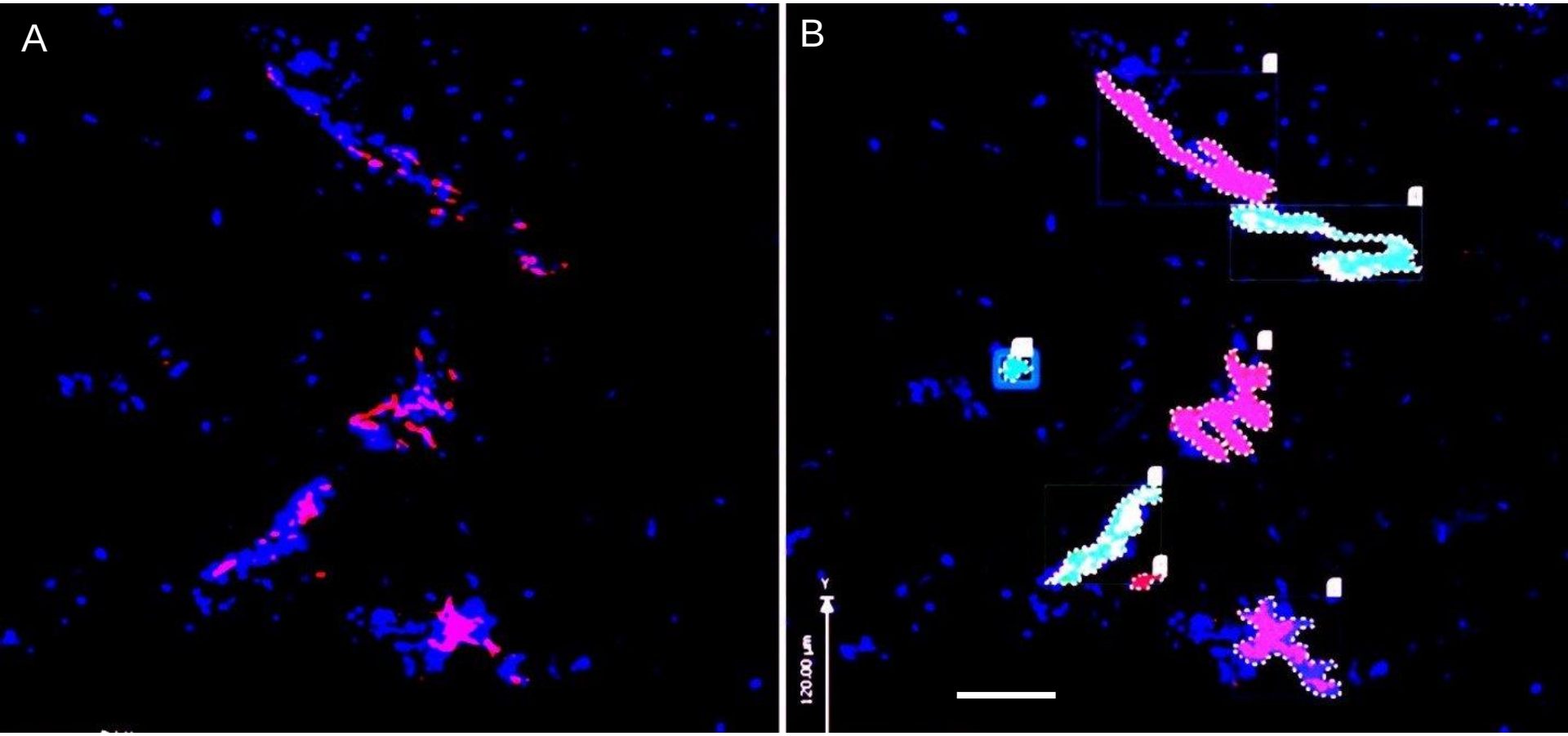

Figure 8: Quantification of blood vessels in inner annulus fibrosus tissue (IAF) of a herniated disc using confocal microscopy. A) Blood vessel(s) immunostained stained red for CD-31. Cell nuclei stained blue by DAPI. Scale bar 100 µm. B) 'Volocity' image analysis software was used to identify stained objects, count them, colour code them, and measure the area occupied by each.