Neonatal Mice Intervertebral Discs Restore Function Following Herniation Injury

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Introduction: Endogenous healing of the adult intervertebral disc (IVD) is limited due to its avascular microenvironment [1] as well as its complex biomechanical demands [2]. To date, there are still no regenerative strategies available to improve IVD healing and restore its function. One limitation toward establishing effective strategies for repair is the paucity of available models for IVD regeneration. Inspired by a few studies in heart [3], cochlear hair cells [4], and tendon [5] showing that neonatal mice retain regenerative healing capacity up to 7 days after birth, we recently established and validated a puncture injury method for neonatal mouse IVD and demonstrated improved AF healing compared to adults at early stages [6]. However, long-term healing and functional outcomes were not investigated. Therefore, the objectives of the current study were to determine the structural and biomechanical properties of neonatal and adult mouse IVDs after long-term healing.

Methods: Severe needle puncture injuries were induced in vivo using a dorsolateral approach in caudal IVDs of neonatal (postnatal day 5, n=7) and adult (4-5 month, n=6) ScxGFP mice with a syringe needle tip of 80% IVD height (neonates: 31G, adults: 26G) [7] to a depth of 50% of dorsal-ventral width and labeled with India Ink. All procedures were approved and guided by the IACUC. ScxGFP was observed in AF cells [8] and used in this study to locate the IVD pre-injury and to distinguish AF cell phenotype after injury, and picrosirius red/alcian blue (PR/AB) staining was performed to determine changes in collagen structure and composition. Disc height index was assessed from anterior-posterior tail radiographs using digital x-ray images (UltraFocus, Faxitron) and ImageJ [9]. Mechanical behaviors of injured and control motion segments were assessed using axial (ELF3200, TA Instruments) and torsion (AR2000ex, TA Instruments) test systems with custom grips. Injured and control IVDs from neonates and adults were subjected to 20 cycles of axial tension and compression at ±0.5N at 0.1 mm/sec, followed by 5 min equilibration of 0.1 MPa axial load and 20 cycles of torsion at ±20 degrees at 0.5 Hz. Torsional stiffness and torque range were determined from the cyclic of testing. Statistical analyses were performed using paired Student’s t-tests for disc height index and unpaired Student’s t-tests for normalized tensile stiffness, torsional stiffness and torque range. All measurements were taken at day 56 (d56) post-injury, with selected observations at intermediate times.

Results: AB staining for the proteoglycan-rich nucleus pulposus (NP) at d28 confirmed sustained loss of NP following injury of all animals, indicating successful induction of puncture (Fig. 1). For neonates, co-staining with PR revealed a collagen-rich matrix occupying the repair region within the puncture track, although staining intensity was reduced compared to adjacent AF tissue and lamellar organization was not restored (Fig. 1). While AF cells adjacent to the injury site retained ScxGFP expression at d28, the presence of non-ScxGFP (DAPI+) cells in the repair region suggested possible infiltration of new fibroblasts (Fig. 1). Consistent with previous findings [7], the disc height index of punctured adult IVDs was significantly impaired relative to uninjured internal controls at d56 (Fig. 2). However, there was no difference detected in the neonatal injury group (Fig. 2). Similarly, normalized torsional stiffness, torque range, and axial tensile stiffness were lower than control IVDs in adults (62%, p=0.038, 53%, p=0.019, and 48%, p=0.015, respectively), while neonatal values remained normal (Fig. 3, axial data not shown). These results indicate that neonatal healing restored critical functional features in contrast to adult healing, suggesting partial structural regeneration.

Discussion: The neonatal AF has remarkable capacity to restore biomechanical function following severe injury. This highlights the promise of this model for investigating mechanisms of improved healing. We showed functional IVD healing in neonatal mice at d56, while the adult IVD remained impaired. Complete AF rupture and sustained loss of NP was characteristic of both neonatal and adult injury responses highlighting the severity of this injury, yet the neonatal AF healing response restored biomechanical function. The cellular mechanisms regulating this improved AF healing in the neonate are not known, and understanding these processes will point towards potential strategies to improve functional healing of adult IVDs following injury. In contrast to our previous studies in transected neonatal tendon, which healed via recruitment and tenogenic differentiation of ScxGFP tenocytes [5], our findings suggest that neonatal AF healing is regulated by an undiscovered population of ScxGFP-negative cells occupying the repair site. Although tendon and AF share similar embryonic origins and cell markers [10], differences in the local microenvironment, mitotic potential, structure, and loading are likely factors driving distinct biological responses post-injury. Ongoing studies will elucidate the cellular mechanisms underlying neonatal IVD healing, such as the identity of these ScxGFP-negative repair cells and their source.

Significance: Current strategies to repair AF injuries have failed due to high re-heritance rates of implants, which could maintain or worsen clinical symptoms, meritting the investigation of strategies to promote endogenous repair. This novel neonatal mouse model of AF injury has the potential to identify cell populations and molecular factors responsible for improved healing that may inform therapies to promote repair in adult IVDs.

Figures:

Figure 1: PR/AB and ScxGFP post-injury in neonates at d28. PR/AB staining demonstrated the puncture track was filled with collagenous ECM. ScxGFP expression was retained following injury in AF cells adjacent to injury site. A population of non-ScxGFP expressing cells was observed in the repair region (red outline). Yellow outline: AF, blue outline: NP, magnification=20X.

Figure 2: X-rays of injured (yellow boxes) and adjacent uninjured IVD caudal (Co) levels at d56 in adults and neonates. Disc height index was normal in neonates but impaired in adults (n=5-7, *p<0.001).

Figure 3: Torsional stiffness and torque range were lower in adults but normal in neonates at d56 compared to internal, uninjured controls (n=5-7, *p<0.05).

References:

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