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Short communication

Surgical techniques influence local environment of injured spinal cord and cause various grafted cell survival and integration

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HIGHLIGHTS

- Surgical technique of microaspiration in SCI results in severe scarring and huge cavities.
- Neural tissue bridging rarely occurs after grafting embryonic brainstem cells into the microaspirated lesion.
- Crush injury prevents severe cavitation, and facilities grafted cell survival and integration.

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ABSTRACT

Background: Cellular transplantation to repair a complete spinal cord injury (SCI) is tremendously challenging due to the adverse local milieu for graft survival and growth. Results from cell transplantation studies yield great variability, which may possibly be due to the surgical techniques employed to induce an SCI. In order to delineate the influence of surgery on such inconsistency, we compared lesion morphology and graft survival as well as integration from different lesion methodologies of SCI.
New method: Surgical techniques, including a traditional approach cut + microaspiration, and two new approaches, cut alone as well as crush, were employed to produce a complete SCI, respectively. Approximately half of the rats in each group received injury only, whereas the other half received grafts of fetal brainstem cells into the lesion gap.
Results: Eight weeks after injury with or without graft, histological analysis showed that the cut + microaspiration surgery resulted in larger lesion cavities and severe fibrotic scars surrounding the cavity, and cellular transplants rarely formed a tissue bridge to penetrate the barrier. In contrast, the majority of cases treated with cut alone or crush exhibited smaller cavities and less scarring; the grafts expanded and blended extensively with the host tissue, which often built continuous tissue bridging the rostral and caudal cords.
Comparison with existing methods: Scarring and cavitation were significantly reduced when microaspiration was avoided in SCI surgery, facilitating graft/host tissue fusion for signal transmission.
Conclusion: The result suggests that microaspiration frequently causes severe scars and cavities, thus impeding graft survival and integration.

1. Introduction

Cell transplantation is a promising approach to improve functional recovery following spinal cord injury (SCI). The concept of transplanting early stage neurons is attributed to their capacity for differentiation and axonal growth under neuron-intrinsic mechanisms in the host (Reier, 2004; Gaillard et al., 2007). This holds the potential to reconstitute supraspinal control of denervated caudal spinal neurons and relay signals across the lesion gap. To date, this therapeutic approach has achieved considerable positive feats by implanting fetal neurons or neural restricted precursors into a small lesion created by a contusion or incomplete transection of the rat spinal cord, whereby grafted cells expand and fill the cavity (Jakeman and Reier, 1991; Lepore and Fischer, 2005). However, unanticipated complications occurred when the cell transplantation was applied to a completely-transected spinal cord lesion (Lu et al., 2012; Sharp et al., 2014).

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A SCI model with complete transection is considered the most rigorous to study axonal regeneration, as it eliminates false evaluation resulting from spared axons and their sprouting (Blesch and Tuszynski, 2003). Recently, Lu et al. transplanted embryonic spinal cord-derived neural stem cells (NSCs)/progenitors into a completely transected rat thoracic spinal cord. They reported remarkable graft-host integration, axonal growth, and locomotor functional recovery (Lu et al., 2012). Yet, a follow-up replication study did not fully duplicate these outcomes even though the same researcher conducted the surgical procedure (Sharp et al., 2014). In addition, the replication revealed severe non-neuronal partitions and large cavities at lesion/graft site in most cases. Accordingly, this caused a controversy in the original study. Though a following response from the researchers discussed the possible reasons for this discrepancy (Tuszynski et al., 2014), the real source of variability has not been ascertained. As the first step to produce an injury model, surgical techniques are critical to establish a harsh environment for cell grafting. Based on our observation and experience, we posit that variations in SCI surgical techniques affect local conditions of injury, which in turn, determine the success of subsequent cell transplantation.

To test this hypothesis, in the present study, three different surgical techniques were used to produce a complete SCI, including 1) cut + microaspiration, 2) cut only, and 3) crush. Unlike previous two-week delayed transplantation, we have here grafted mechanically-dissociated fetal brainstem cells into the lesion gap immediately after injury. Donor tissues were treated and implanted as small chunks instead of single cell solutions in order to maximally preserve the embryonic extracellular matrixes (ECM). Therefore, this study was not aimed to verify reproducibility, but rather to discover the reason behind the enormous variability in cell transplantation for SCI. The findings may help indicate a more suitable injury model to optimize cell transplantation strategies for better restoration of function following SCI.

2. Materials and methods

2.1. Animals

A total of 44 adult female Fischer 344 rats weighing 150–200 g were used. Institutional Animal Care and Use Committee and Society for Neuroscience guidelines on animal care were strictly followed to minimize the number of animals used and potential suffering. Animals were anesthetized with 2% isoflurane before spinal cord surgery and cell grafting. Animals were divided into 2 cohorts: injury alone and injury plus cell graft. Based on different surgical techniques, the cohort of injury alone included 1) cut + microaspiration (n = 7), 2) cut only (n = 11), and 3) crush (n = 5); whereas the cohort of injury plus graft, which consisted of embryonic day 14 (E14) brainstem-derived neural stem cells (BS-NSCs)/progenitors, included: 4) cut + microaspiration plus cells (n = 6), 5) cut only plus cells (n = 10), and 6) crush plus cells (n = 5). Microaspiration was only applied in dura-opened surgery, whereas it was not done in crush injury (Groups 3 and 6) because the dura was not opened. In comparison to animals with cut only (Groups 2 and 5), those receiving cut + microaspiration (Groups 1 and 4) provided adequate information about the effect of microaspiration.

2.2. Surgery of SCI

All animals underwent a T3 dorsal laminectomy. In the 1st group of rats, the dura was cut longitudinally in the midline. Then the spinal cord at T4 level was completely cut using a combination of iridectomy microscissors (Fig. 1**Aa**) and subsequently aspirated with self-made aspirators. In each rat, all three aspirators were used from large to small in diameter (Fig. 1**Ab–d**), to remove spared tissue piece by piece, which was often present in the ventral and lateral edges. The 2nd group of rats received a similar dura lesion, however only a cut to the spinal cord without aspiration was performed. After infiltrated blood and fluid were absorbed with a fine tip cotton swab, visual verification ensured the completed transection ventrally and laterally. In the 3rd group of rats, the dura was kept intact and the spinal cord was completely crushed at T4 level for a total of 10 s with Dumont 5/45 forceps (Fig. 1**Ae**). For those receiving injury only, the overlying musculature and skin was immediately closed after hemostasis. Animals were administered with Lactated Ringer's solution (Baxter Healthcare), cefazolin (10 mg/kg), and buprenex (0.1 mg/kg) post-operatively. Bladders were manually expressed at least twice daily until sacrifice.

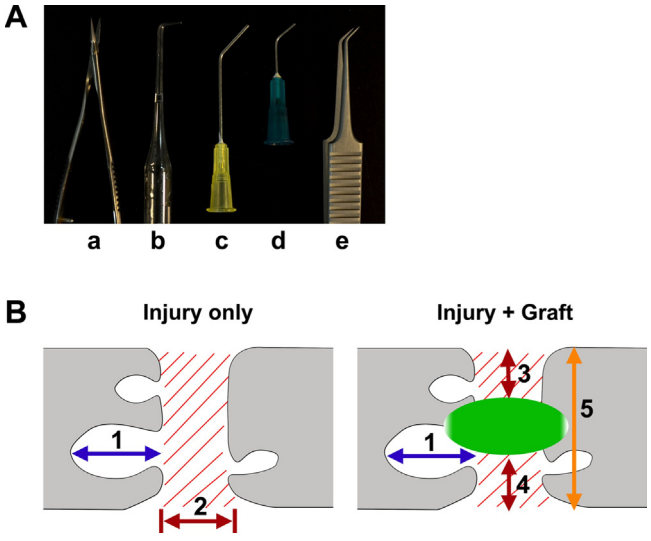

Fig. 1. Surgical tools (**A**) to create a complete SCI include (**a**) iridectomy microscissors, (**b–d**) three self-made aspirators with different diameters in the tip, and (**e**) fine forceps for crush. Schematic diagrams (**B**) illustrate how the sizes of cavities (**1**) and fibrotic scars (**2** or **3**) were measured. The size of cavity is referred to the rostro-caudal distance (**1**) of the biggest one in each section. The rostro-caudal distance of fibrotic scarring (**2**) in lesion is defined in the spinal cord with injury only, while the vertical depth of fibrotic scars (**3** + **4**) is scaled and divided by the width of the section (**5**) in grafted cords.

2.3. Cell preparation and grafting

E14 brainstem from Fischer 344 transgenic rats expressing enhanced green fluorescent protein (EGFP) under the ubiquitin C promoter provided donor tissue for grafting (Rat Resource and Research Center, University of Missouri). At this stage, the grafts are composed of a mixture of NSCs, neuronal restricted precursors, and glial restricted precursors. GFP-expressing E14 brainstem was freshly dissected and mechanically dissociated into small chunks. Then cells were resuspended in a fibrin matrix (25 mg/ml fibrinogen, 25 U/ml thrombin) containing growth factors to support graft survival as described previously (Lu et al., 2012; Hou et al., 2013). Immediately after SCI, the dura was sutured in the model with an opened dura before cell injection in order to retain cells within the lesion. E14 cells were injected into the lesion through the dura with a 10 ul Hamilton syringe in all types of injury models. A total of 8–10 μl of cells (1.0×10^6/μl) were microinjected into the lesion site per rat. Musculature and skin were immediately closed. Animals were treated as described above and survived for an additional 8 weeks.