Nano Neuro Knitting: using nanotechnology to repair the brain and spinal cord

Rutledge G. Ellis-Behnke*,**, Yu-Xiang Liang**, Si-Wei You***, David K. C. Tay**, Shuguang Zhang*, Wutian Wu**, Kwok-Fai So** and Gerald E. Schneider*

*Massachusetts Institute of Technology, 77 Mass. Ave. Cambridge, MA USA 02139
rutledg@mit.edu, shuguang@mit.edu, jerry@mit.edu
**University of Hong Kong, Faculty of Medicine, 21 Sassoon Rd, Hong Kong SAR, China
rutledge@hku.hk, yxliang@hku.hk, dkctay@hku.hk, wtwu@hku.hk, hrmaskf@hku.hk
***Fourth Military Medical Hospital, 17 Changlexi Street, Xi'an 710032, Shaanxi, China
yousiwei@fmmu.edu.cn

ABSTRACT

After traumatic brain and spinal cord injury several formidable barriers must be overcome, such as scar tissue formation after injury and the failure of many adult neurons to initiate axonal extension. Here we show that a more permissive environment can be created using regeneration-enhancing factors (REF), such as scar-reducing materials or chemical growth factors, delivered in liquid form or through cells. Our results show both restoration of function after spinal cord injury in rats as well as a more rapid functional return of vision in the brain of adult hamsters, indicating that SAPNS provides a true three dimensional (3D) environment for the migration of living cells.

Keywords: regeneration, self-assembly, central nervous system, neural stem cell, spinal cord, traumatic brain injury

1 INTRODUCTION

The determining factor in functional recovery was once thought to be the amount of re-innervation. However, it is now known that the level of recovery is determined not only by the number of axons but also the local density of their connections as well as the rehabilitation work done to drive reinnervation and refinement through training.

Traumatic spinal cord injury results in a loss of nervous tissue, characterized by the formation of large cystic cavities and resulting in a partial or complete loss of neurological functions [1-6]. For the restoration of function following spinal cord injury, damaged axons need to regenerate across and beyond the site of injury. One of the major efforts to repair spinal cord injury has been to construct a bridge through the injured area over which the axons can grow. To do that, many potential strategies have been applied, including the transplantation of 1) fetal spinal cord tissue [7]; 2) peripheral nerves [8]; 3) Schwann cells [9]; 4) olfactory ensheathing cells [10]; 5) embryonic stem cells [11]; 6) marrow stromal cells [12]; 7) neural stem cells [13]; and 8) genetically modified cells [14, 15].

Neurons in the brain of adult mammalian central nervous system (CNS) have limited capability to regenerate their axons after injury. It is generally accepted that this limited capacity of CNS axons to regenerate is partly due to the local environment of the injured CNS axons. Traumatic injury in CNS is often followed by robust glial reaction, the failure of CNS axons to regenerate is partly attributed to the inhibitory surface of the glial scar and extracellular matrix (ECM) produced by oligodendrocytes and astrocytes. Furthermore, a tissue gap formed after traumatic injury would completely block the re-innervation of the CNS axons.

We recently demonstrated that a self-assembled nanofiber scaffold (SAPNS) could repair the injured optical pathway and restore visual function by creating a permissive environment [16]. We hypothesized that by adding REFs we could create a more permissive environment, thus allowing return of function after spinal cord injury and a faster rate of functional return in the brain.

2 MATERIALS AND METHOD

2.1 Spinal cord

Materials used were Schwann cells (SC) described previously [17, 18], embryonic NSC cultures as described previously [19, 20], 1% RADA16-I (SAPNS) [16].

30 adult female wild type Sprague-Dawley rats (220g-250g) and 3 adult female green fluorescent protein (GFP) transgenic Sprague-Dawley rats (220g-250g) were used in this project; the rats were divided to 5 groups: 1) saline control group, 2) untreated SAPNS group, 3) pre-treated SAPNS alone group, 4) SAPNS seeded with NSCs group and 5) SAPNS seeded with SCs group. Each group has 6 wild type rats and 3 GFP rats were added to the pre-cultured SAPNS alone group.

A dorsal laminectomy on the sixth and seventh cervical vertebra was performed. Assisted by a microscope, 5 μl of pre-cultured SAPNS scaffold, or equal volume of the cultured mixture of SAPNS and SCs or NSCs, respectively, was transferred from the culture dish into the lesion cavity.

2.2 Brain

Materials used were 1% RADA16-I (SAPNS) and Chondroitinase-ABC.

The brachium of the superior colliculus was completely transected in a group of 22 adult golden hamsters and 1% SAPNS and/or 2.5 units/ml (final concentration) Chondroitinase ABC were injected into the lesion site. The progression of axonal regeneration and the re-innervations of the superior colliculus were monitored at 4, 6, and 12 weeks following the lesion by intravitreal injection of CTB-FITC.

3 RESULTS

3.1 Functional recovery in the spinal cord

The spinal cord injury created a large cavity in the injury site, and multitudinous macrophages (ED1 positive cells) surrounded the cavity. The SAPNS that was used alone, when directly transplanted to the injured spinal cord, did not integrate well with the host and caused serious inflammation. There were obvious gaps and cysts between the implanted and host tissue (Fig 1b).

In contrast, when the SAPNS was pre-treated with a culture medium before transplantation it was able to properly bridge the lesion area (Fig 1c).

SAPNS implants cultured with either the SCs or NSCs showed more axons than the implants of SAPNS alone (Fig 1d, e).

The motor functions of both the transplanted and control rats were assessed by footprint patterns weekly after transplantation. Among the transplanted groups, the results of the footprint patterns were significantly improved in the groups of SAPNS plus SCs and SAPNS plus NSCs.

3.2 Faster functional recovery in the brain

SAPNS, used in combination with Chondrointinase-ABC, facilitates the retinal fibers to regenerate across the lesion site. The re-innervations of the superior colliculus were observed in the combination group as early as four weeks after the transection while controls showed no reinnervation at any time point. In the behavioral study the adult hamsters showed a functional return of vision in the SAPNS/Chondroitinase-ABC treated cases beginning at 6 weeks post surgery, sooner than those treated with either SAPNS or Chondroitinase-ABC alone.

Each animal was tested every other day for 12 weeks by presenting stimulus that consisted of a seed and small cork. This was supported by a long wire that allowed the presentation of the seed from the back of the animal. The animals would respond to the stimulus by turning toward the seed stimulus. All trials were videotaped and later analyzed for responses. We found that 5 of the animals responded well to the stimulus. Figure 3 shows an animal turning when a stimulus is presented.

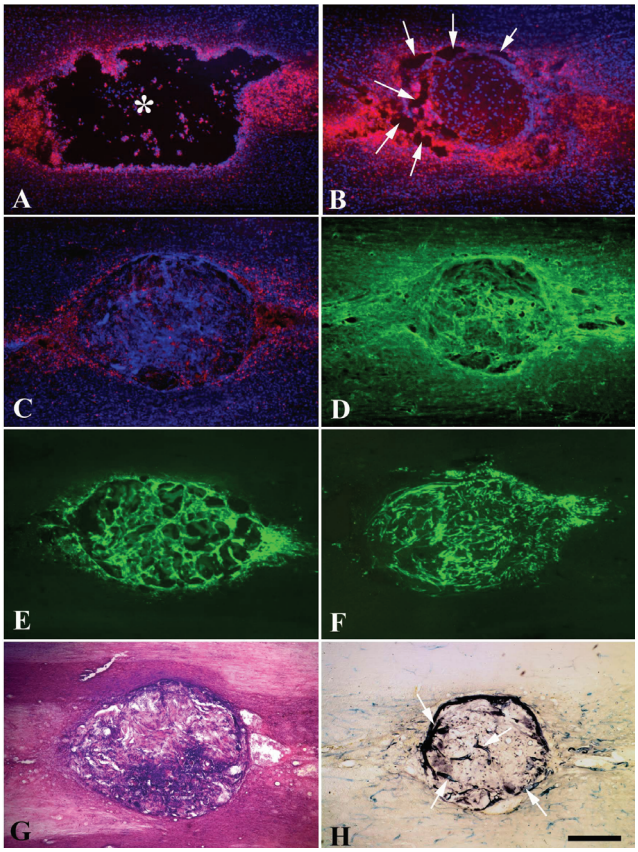

Fig 1 Integration of implants within the injured spinal cord. (a) Control. Lesion cavity filled only with saline during surgery. 6 weeks after transection the injury site shows inflammation and many ED1 positive cells (macrophages, red) surrounding the big cavity (star). Nuclei (blue) are labeled with DAPI. **(b) Uncultured SAPNS.** Direct transplantation into the injured spinal cord caused serious inflammation showing gaps and cysts (arrows) between the implant and host tissue and many ED1 positive cells (red) in the lesion area. Nuclei (blue) are labeled with DAPI. **(c-g) Pretreated with culture medium before transplantation**. **(c)** SAPNS implants integrated very well with host tissue, with no obvious cavities and only slight inflammation, compared with the uncultured SAPNS. Many host cells migrated into the implants, shown by DAPI staining. **(d)** In pre-cultured SAPNS, transplanted into the injured spinal cord of GFP transgenic rats without either Schwann cells (SC) or neural stem cells (NSC), many green fluorescent protein (GFP) positive cells migrated into the implant. **(e)** In wild type rats, where SAPNS with either GFP positive NSCs or **(f)** GFP positive SCs were transplanted into the spinal cord, many engrafted cells (green) survived, some migrating into the host tissue. **(g)** HE staining showed a high level of integration between the implants and host. **(h)** AP histochemistry staining showed (arrows) that blood vessels grew into the implants. (Scale bar = 500 μm)

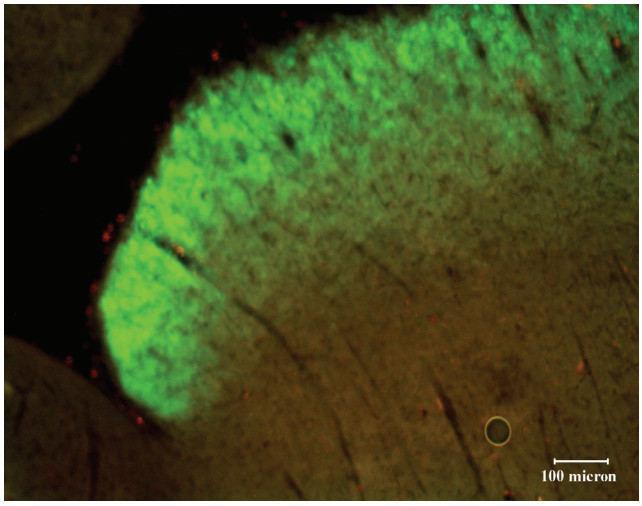

Fig 2 On the left is a dark-field photo of a parasagittal section from brain of 8-week post surgery and treated hamster with SAPNS and ABC Chondroitinase at the time of surgery in the lesion site. The axons have grown through the site of lesion and are reinnervating the superior colliculus (SC).

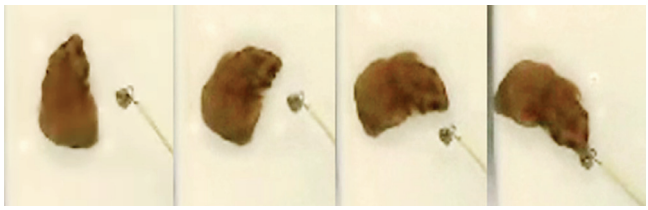

Fig 3 This adult animal turns toward the stimulus in the affected left visual field in small steps, prolonged here by movements of the stimulus away from him. Each frame is taken from a single turning movement, at times 0.00, 0.27, 0.53, and 0.80 sec from movement initiation. The animal reached the stimulus just after the last frame. This is about 0.20 sec slower than most turns by a normal animal.

4 DISCUSSION

Using SAPNS in combination with regeneration-enhancing factors (REF) such as SCs and NSCs for spinal repair, or Chondroitinase-ABC for brain repair, creates a more permissive environment.

We found good evidence of spinal cord regeneration: two-way cell migration; axon regeneration across lesion cavity sites; large blood vessels growing into the implants and improved locomotor activity in the hindlegs of rats.

In the brain we demonstrated a more rapid functional return of vision in adult hamsters. We found that SAPNS and Chondroitinase-ABC used together, facilitated retinal ganglion cell axons, to regenerate across the lesion site. The combination of SAPNS and Chondroitinase-ABC had a synergistic effect evidenced by the larger area of reinnervation than either the SAPNS or Chondroitinase-ABC alone (Table 1). This effect was observed in all treatment groups as early as four weeks after the transection, while saline controls showed no reinnervation at any time point. An example of the higher percentage of reinnervation in the SC is shown in Figure 3.

This indicates that SAPNS, in combination with the REFs, provided a true 3D environment for the migration of living cells.

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Results / Group / Survival time	Saline only	SAPNS only	Chon-ABC only	SAPNS + Chon-ABC
4 weeks	N=0/3	*(N=1/1)	*(N=2/2)	*(N=1/2)
6 weeks	N=0/3	**(N=2/2)	*(N=1/2)	**(N=1/2)
12 weeks	N=0/4	***(N=7/7)	*(N=1/3)	****(N=5/7)

Table 1 Anatomical results of all groups (semi-qualitative, the average proportion of SC re-innervation). Only in the SAPNS-treated cases did the cut heal. In the Chondroitinase-ABC cases the axons grew around the bottom of the cut. * Sparse; ** about 10%; ***about 50%; ****about 70%