REVIEW

Olfactory ensheathing cells and spinal cord repair

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Abstract. The olfactory ensheathing cell is a specialized glial cell that assists in growth of the axons of the olfactory sensory neurons as they are generated and regenerated throughout adult life. There is increasing evidence in animal models that transplantation of olfactory ensheathing cell promotes recovery after transplantation into the injured spinal cord. Olfactory ensheathing cell transplants have promoted regrowth of axons across the injury site and led to recovery of functional behaviours including climbing, walking, reaching, and breathing. Most evidence comes from olfactory ensheathing cells derived from the olfactory bulb. This is an impractical site for human biopsy compared to the easy accessibility of olfactory ensheathing cells from the olfactory mucosa in the nose. Our experiments demonstrated that nasal olfactory ensheathing cells led to functional improvement after complete spinal cord transaction in rat. After devising methods to grow human olfactory ensheathing cells from nasal biopsy we recently initiated a Phase I clinical trial of transplantation into the human paraplegic spinal cord. (Keio J Med 54 (1): 8–14, March 2005)

Key words: spinal cord injury, cell therapy, paraplegia, glia

Introduction

The adult olfactory epithelium is a site of continuing neurogenesis throughout life.[1] Replacement of olfactory sensory neurons is a highly regulated via multiple growth factor pathways.[2] The stem cell source of new sensory neurons is unknown but thought to be one of the basal cells of the olfactory epithelium.[3,4] This regenerative capacity of the olfactory epithelium provides the context in which operate the specialized glia of the olfactory nerve, the olfactory ensheathing cells.

It is 20 years since the first paper was published on the biology of olfactory ensheathing cells.[5] In the following 10 years there were 13 research papers on the biology of olfactory ensheathing cells and 3 reviews. In 1994 Ramon Cueto and Nieto-Sampedro[6] published the first paper demonstrating that olfactory ensheathing cells assisted sensory axons to regrow into the spinal cord after dorsal rhizotomy. This was followed by a paper from Doucette's lab describing the survival of olfactory ensheathing cells after transplantation into the brain[7] and a paper from Raisman's group demonstrating functional recovery from corticospinal tract lesions after transplantation of olfactory ensheathing cells.[8] In the 7 years since 1997 there have been 50 papers published describing olfactory enheathing cell transplantation, 51 describing the biology of olfactory ensheathing cells and 31 review articles featuring olfactory ensheathing cells, mostly in reference to nervous system repair. Clearly olfactory ensheathing cells have aroused the interest of the scientific community, especially those interested in repair of the spinal cord. Table 1 summarises the studies of olfactory ensheathing cells in spinal cord repair. What are olfactory ensheathing cells? What makes them of interest in spinal cord repair? What can they do to repair the injured spinal cord?

The olfactory ensheathing cell is a specialized glial cell that associates with the olfactory nerve, surrounding the sensory axons as they leave the olfactory epithelium and accompanying them from the nose to the brain where they synapse with the mitral cells of the olfactory bulb.[9] The olfactory ensheathing cell has properties of both Schwann cells and astrocytes, with a phenotype closer to the Schwann cell.[10] There are two properties of the olfactory ensheathing cell that make it unique. The first is that it exists both outside the central nervous system (like Schwann cells) and inside the central nervous system (like astrocytes). The second

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Table 1 Transplantation of Olfactory Ensheathing Cells into Injured Spinal Cord

Transplant model (see Notes)	Outcome	Reference
Lesioned corticospinal tract	Regeneration, recovery of function	8
Lesioned corticospinal tract	Regeneration	18
Demyelinated spinal cord	Remyelination	19
Demyelinated spinal cord	Remyelination	20
Demyelinated spinal cord, primate	Remyelination	21
Demyelinated spinal cord	Remyelination	51
Demyelinated spinal cord, cryopreserved cells	Remyelination	53
Photochemical lesion of spinal cord	Axon regeneration/sparing, recovery of function	57
Photochemical lesion of spinal cord	Regeneration, recovery of function	58
Transected spinal cord	Regeneration, recovery of function	13
Transected spinal cord, pig OEC	Axon regrowth	22
Transected spinal cord, nasal OECs	Regeneration, recovery of function	24
Transected spinal cord, delayed transplantation, nasal OECs	Regeneration, recovery of function	25
Transected spinal cord	Axon conduction	50
Transected spinal cord	Regeneration, recovery of function	52
Transected spinal cord, OECs genetically engineered to produce GDNF	Axon regeneration, recovery of function	48
Transected spinal cord, cryopreserved cells	Regeneration, recovery of function	59
Hemisection of spinal cord	Regeneration, recovery of function	14
Hemisection of spinal cord, delayed transplantation	Regeneration, recovery of function	15
Hemisectioned spinal cord	Regeneration, recovery of function	17
Hemisectioned spinal cord, nasal OECs	Scar and cavity reduction	28
Dorsal hemisection of spinal cord, OECs genetically engineered to produce BDNF	Regeneration, recovery of function	47
Spinal cord injury, OEC cell line	Regeneration, recovery of function	55
Contused spinal cord	*No regeneration at 6 weeks*	29
Contused spinal cord	Axon regeneration/sparing	54
Contused spinal cord	Axon regeneration/sparing	56

1. All experiments done on rodent models except where noted ''primate''. 2. All transplantations were done at time of spinal cord injury except where noted ''delayed transplantation''. 3. All olfactory ensheathing cells were derived from olfactory bulb except where noted ''nasal OECs''. 4. ''Pig OECs'' indicates OECs from olfactory bulb of genetically engineered pig. 5. ''OECs genetically engineered'' indicate olfactory bulb cells engineered to produce BDNF or GDNF, where noted. 6. ''Cryo-preserved cells'' indicates OECs that were grown *in vitro*, frozen, thawed and then transplanted.

special property arises from the fact that there is a continual neurogenesis within the olfactory epithelium that produces new sensory neurons throughout adult life, including human.[11] This neurogenesis is a highly regulated process in which olfactory ensheathing cells assist, although the specific mechanisms of that assistance remain undescribed. The olfactory ensheathing cell is therefore ''Schwann cell-like'' in assisting axon growth, but ''astrocyte-like'' in being able to live within the central nervous system. It was this combination of properties that made it a candidate for nervous system repair.[6–8,12] It remains a strong candidate with the vast majority of the 53 published transplant studies reporting positive outcomes.

Olfactory ensheathing cells have been used in a variety of spinal cord repair models including complete transection, hemisection, tract lesion, contusion, and demyelination. They have also been transplanted in other nerve repair models including dorsal rhizotomy (Table 2), peripheral nerve transection, and optic nerve repair (Table 3).

Table 2 Transplantation of Olfactory Ensheathing Cells after Dorsal Rhizotomy

Level of rhizotomy	Sensory axon regeneration?	Reference
Lower thoracic	Yes	6
L3-L6	Yes	60
L6-L2	Yes	16
C3-T3	*No*	61
L4	Yes	62
cervical and lumbar	*No*	63
cervical and lumbar	*No*	64

The most exciting results of transplantation of olfactory ensheathing cells into the injured spinal cord has been the functional recovery of paw preference, after corticospinal tract lesions,[8] the recovery of coordinated walking after complete spinal cord transection[13] and recovery of paw use and climbing after spinal cord hemisection.[14,15] These and other reports illustrate the long sought for goal of stimulating recovery of locomo-

Table 3 Transplantation of Olfactory Ensheathing Cells into Brain and Peripheral Nerves

Transplant model	Outcome	Reference
Fimbria-fornix transection	Regrowth of axons across lesion	7
Fimbria-fornix transection	Regrowth of axons across lesion	65
Abducens tract lesion	*No regeneration of tract*	66
Optic nerve transection	Regrowth of axons	67
Optic nerve transaction	Regrowth of axons	33
Parkinsonian rat, co-transplantation with dopaminerigic neurons	Regeneration, functional recovery	68

tion in human paraplegics and quadriplegics (Table 1). Despite the excitement generated by these findings it should not be forgotten that advances in aspects other than locomotion would also improve the management and quality of life of these persons. For example, bowel and bladder control remain among the most sought after improvements and recent results indicate that olfactory ensheathing cell transplants can assist recovery of bladder function.[16] Similarly, olfactory ensheathing cells show promise in assisting repair of descending pathways regulating phrenic nerve activity,[14,17] a result which, if repeated in human has the potential to take many quadriplegics off assisted respiration. Olfactory ensheathing cells do not normally myelinate the olfactory nerve but when transplanted into the spinal cord they appear to myelinate the regrowing axons in the region of injury after corticospinal tract lesions[18] and they remyelinate axons in several demyelinating models.[12,19–21]

Olfactory ensheathing cells are present in the olfactory bulb, the region of termination of the olfactory sensory axons within the central nervous system. Initial transplantation studies used these cells isolated from the newborn olfactory bulb.[6,8,18,19] For human therapy, the olfactory bulb was initially suggested as a potential source for these cells, either from cadavers or from persons having skull-base surgery or from pigs.[21,22] These sources are unlikely to gain wide acceptance for human therapy because of ethical concerns, as well as the immunological and other technical issues to overcome. In any case these non-autologous sources are not necessary because olfactory ensheathing cells are theoretically available within the olfactory mucosa, an accessible site for biopsy in all individuals.[23] Several years ago we set out to determine whether olfactory ensheathing cells from the adult nose would assist recovery from spinal cord injury in the same way that olfactory ensheathing cells from the newborn olfactory bulb. If so this would make feasible a human clinical trial.

Repair of the Spinal Cord with Olfactory Ensheathing Cells from the Nose

Within the olfactory mucosa, the olfactory ensheathing cells lie in the lamina propria, beneath the olfactory epithelium, and towards the caudal part of the nasal cavity the lamina propria is dominated by the presence of the olfactory nerve bundles as they course posteriorly and dorsally to enter the skull at the cribriform plate. In an initial series of experiments we transplanted pieces of olfactory lamina propria into the gap formed when the spinal cord was sectioned at the mid-thoracic level in the rat.[24] The control animals received pieces of respiratory lamina propria, a tissue containing many of the same non-olfactory cell types such as fibroblasts, endothelium, and peripheral nerve Schwann cells. Ten weeks later the animal receiving olfactory lamina propria showed movement of the hind limbs, behaviour that was dependent on intact descending spinal cord tracts. These transplants also improved reflex inhibition of spinal motor circuits, which suggests that descending pathways have regenerated. Cells from the transplants migrated into the spinal cord, both rostrally and caudally, and axons could be seen within the transplants. Retrograde tracing demonstrated that axons from brainstem serotonergic nuclei had penetrated the lesion and grown into the spinal cord caudal to the lesion and transplant. Immunochemistry verified the presence of serotonergic fibres caudal to the transplant. None of these changes were seen after transplants of respiratory lamina propria although similar recovery was seen after transplantation of dissociated olfactory ensheathing cells that were grown *in vitro* from the adult lamina propria.[24] These experiments demonstrated that adult olfactory ensheathing cells could assist recovery when transplanted at the time of the spinal cord injury.

Our next experiments addressed the question of whether olfactory tissue transplants could assist recovery if transplantation was delayed. It was expected that any human trial of these cells would be delayed until after the acute phase when the patient's condition had stabilized. The olfactory lamina propria was transplanted into the region of the lesion four weeks after complete spinal cord transection of the spinal cord in the rat.[25] Ten weeks later, animals receiving olfactory lamina propria transplants were able to move their hindlimbs and they showed histological evidence for regrowth of brainstem serotonergic neurons across the site of lesion and transplantation. This was not observed in animals receiving transplants of respiratory lamina propria.[25]

These experiments established the feasibility of using nasal olfactory ensheathing cells for spinal cord repair.[26] Recently they were shown to inhibit cavity and scar formation when transplanted into the injured spi-

nal cord,[27] and to promote angiogenesis and extensive sprouting of sensory and motor axons into and through the lesion site.[28] As previously, they also promoted regrowth of brainstem serotonergic axons through the lesion site as well as regrowth of brainstem tyrosine hydroxylase-positive axons.[28] Olfactory ensheathing cells from the adult olfactory bulb were not successful when transplanted into the contused spinal cord.[29]

The mechanisms by which olfactory ensheathing cells permit or promote recovery after spinal cord injury are not known. This provides the stimulus for many investigations into the biology of olfactory ensheathing cells and into their interactions within the spinal cord after transplantation. The growth factors secreted by olfactory ensheathing cells are also being investigated.[2] *In vitro* olfactory ensheathing cells provide both soluble and contact signals to promote sprouting of neurites.[30–34] Similarly, experiments indicate that both soluble and contact signals are important for inducing axonal sprouting within the spinal cord. When transplanted into the spinal cord in porous capsules olfactory ensheathing cells stimulated axonal sprouting of corticospinal tract axons, although not to the same extent as olfactory ensheathing cells injected directly into the spinal cord.[35]

The interaction of olfactory ensheathing cells with other glial cells seems to contribute to their efficacy in the spinal cord. In mixed cultures olfactory ensheathing cells migrate over both Schwann cells and astrocytes but tend to associate with astrocytes, rather than Schwann cells and unlike Schwann cells they do not promote hypertrophy of astrocytes.[36] This may help explain why olfactory ensheathing cells can associate with astrocytes within the central nervous system, in contrast to Schwann cells. Paradoxically there is evidence that transplantation of olfactory ensheathing cells into the spinal cord promotes migration into the injured region of host Schwann cells which may participate in the regenerative process.[37] Other cells are also proposed to be involved, with evidence that meningeal cells also migrate into the injured region, either from the host or from the less-than-pure cultures of olfactory ensheathing cells used in transplantation. The conditions used to culture olfactory ensheathing cells have also been an area of active investigation. Like Schwann cells, but unlike astrocytes, olfactory ensheathing cells express the low affinity nerve growth factor receptor, $p75^{NTR}$.[38] This allows it to be immuno-separated from the astrocytes of the olfactory bulb. This is now the most commonly used method for obtaining a starting population of olfactory ensheathing cells, although O4 expression is also used[39] and a recent method describes a method of differential adhesion.[40] In addition to olfactory ensheathing cells, the olfactory lamina propria contains a population of Schwann cells associated with the trigeminal nerve.[41] This makes immuno-purification problematic because of the shared $p75^{NTR}$ and O4 epitopes.[10,42] We developed a method of purifying olfactory ensheathing cells from human olfactory lamina propria using differential adhesion followed by culture in a serum-free medium containing the neurotrophins.[43] Olfactory ensheathing cells express NGF and BDNF[44] and their high affinity neurotrophin receptors, TrkA and TrkB.[43] They also express TrkC but not its ligand NT3.[43] Of the neurotrophins, NT3 was the most effective in stimulating olfactory ensheathing cell proliferation and survival *in vitro*.[43] A serum-free medium containing this growth factor was effective in sustaining only olfactory ensheathing cells and allowing us to generate many millions over a few weeks (Fig. 1). These cultures did not contain Schwann cells as judged by expression of HNK1, a molecule expressed by trigeminal Schwann cells but not olfactory ensheathing cells.[39] These methods establish another milestone in the path to using these cells autologously in human trials, providing the means to grow millions of cells from single biopsies. Olfactory biopsies do not affect the sense of smell.[45,46]

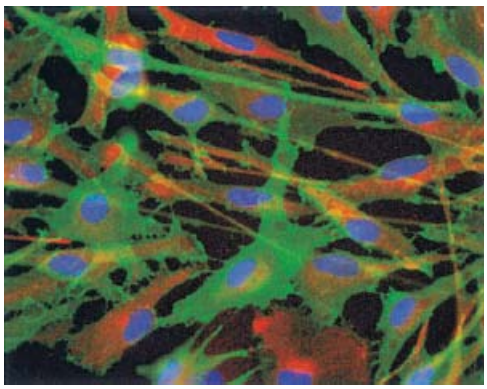

Fig. 1 Olfactory ensheathing cells from the olfactory lamina propria grown *in vitro* expressing GFAP (red) and $p75^{NTR}$ (green). The nuclei are stained with bisbenzimide (blue).

Repairing the Spinal Cord in Humans

There are several outstanding question relating to the use of olfactory ensheathing cells in future therapies. Certainly the optimal time for transplantation is yet to be established, although it would seem that avoiding the early acute inflammatory is advisable. Other surgical aspects need to be optimized such as the site and route of administration and the optimal number of cells delivered. Rather than intraspinally it may be advantageous to inject cells into the cystic cavity or into the cerebrospinal fluid. Future therapies may also

use olfactory ensheathing cells genetically engineered to produce growth factors that may assist their survival or promote regenerative mechanisms within the host tissue.[47,48] It is even possible that co-transplantation of olfactory ensheathing cells with other cells such as hematopoetic stem cells, neural stem cells, or embryonic stem cell-derived cells may become the therapy of choice.[49] The first step is to demonstrate the safety of olfactory ensheathing cell transplantation into the human spinal cord. To this end we commenced a Phase I clinical trial in which autologous olfactory ensheathing cells are transplanted into the injured spinal cord of paraplegic humans with complete injuries at T4-T10. The primary aim of the trial is to test the safety of the transplant procedure designed to assess adverse outcomes across a broad range of medical, neurological and psychosocial examinations. A secondary aim was to assess positive outcomes comparing a transplanted group and in a non-surgical control group over a period of three years post-surgery.

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