**Perspective: Emerging topics in life sciences**

**Stem Cell Sprays for Neurological Injuries: A Perspective.**

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**Abstract**

Injuries to the brain and spinal cord have major clinical consequences with high costs for healthcare systems. Neural cell transplantation therapies have significant translational potential to promote regeneration post-injury with clinical trials commencing for various pathologies. However, there are challenges associated with current clinical approaches used for systemic or direct delivery of transplant cells to neural tissue in regenerative applications. These include risks associated with surgical microinjection into neural tissue (e.g. haemorrhage, cell clumping) and high cell loss due to systemic clearance or with cell passage through fine gauge needles into densely packed neural tissue. This article presents lines of evidence supporting the concept that cell spray delivery technology can offer significant translational benefits for neural transplantation therapy, versus current cell delivery methods. Potential benefits include rapid/homogenous cell delivery, release over large surface areas, minimal invasiveness, compatibility with neurosurgical procedures in acute injury, no predictable clinical complications and the capacity to combine cell therapies with drug/biomolecule delivery. Accordingly, we consider that development of cell spray delivery technology represents a key goal to develop advanced cell therapies for regenerative neurology.

Injury to the central nervous system (CNS) results in cell loss/damage, glial scarring,

and induction of inflammatory mechanisms which contribute to an environment inhibitory to repair (1,2). The limited regeneration post-injury leads to devastating long-term neurological sequelae for patients such as memory loss, paresis, paralysis or epilepsy (3–5). Novel therapies are urgently required to improve patient outcomes and reduce financial burdens on healthcare systems. Stem cell transplantation as a regenerative therapy (for cell/trophic factor replacement) has been tested for several neurological conditions with proven functional benefit (6–11).

However, key problems associated with current modes of cell delivery represent a barrier to clinical translation. Venous or arterial delivery results in cell loss during passage through the lungs, liver or spleen with the remaining cells having a limited ability to cross the blood-brain barrier (12,13). Bypass of the blood-brain-barrier has been achieved with intranasal stem cell delivery. Transplanted cells reach distant brain parenchyma by migrating along the olfactory neural pathway from the nasal mucosa through the cribriform plate into the brain and the cerebrospinal fluid where cortical surfaces are reached (14). Its use has shown *in vivo* benefits in several rodent models of neurological pathology. For example, intranasal inhalation of mesenchymal stem cells significantly reduced grey and white matter loss and increased peri-lesional neurons resulting in improved sensorimotor functioning in a rat model of subarachnoid hemorrhage (15). This route of delivery has not yet been trialled in human pathologies, to our knowledge. Advantages of such an approach include non-invasive delivery of transplants without the need for surgery. However, we speculate that in pathologies with widespread damage, the extent of tissue loss may exceed the 'pathotropic' (migration towards pathology sites) capacity of intranasally delivered stem cells, with cell attrition.

Alternatively, neural cell therapies can be delivered directly into the CNS parenchyma through fine bore stereotactic cannulae, with multiple injection foci possible. For example, in a recent clinical trial, bone marrow stromal cells HUNS001–01, were injected through a cannula (outer bore diameter of 2.1mm) (9), with up to three target sites. Invasive delivery methods impose risks of infection, mechanical trauma and haemorrhage (16). From a cell efficacy perspective, mechanical pressures on cells injected through fine needles into densely packed neural tissue may inflict cellular damage and reduce viability (16,17). Such factors may contribute to the observations that neural cell transplant efficiency/survival is less than 5% *in vivo* (18). This is an important consideration for regeneration, as functional recovery correlates directly with graft survival. Physical blockages and cell compaction may occur within cannulae during transplantation; neurosurgeons can choose to inject at higher pressures to overcome blockages or change cannulae, exposing the CNS to additional injection tracts. Clumping may occur especially given high cell densities used for *in vivo* application; a recent clinical trial used a density of 20 million neural stem cells in 400 µL in HypoThermosol (5 x107 cells/mL) for injection into the putamen of stroke patients (19). Accordingly, there is a critical need to develop novel

transplant delivery strategies for cell therapy in regenerative neurology.

![Diagram

Description automatically generated]()

Figure 1. Schematic diagram summarising the potential clinical and regenerative advantages offered by a transplant cell spray delivery approach for neuro-regenerative applications.

Stem cell spray technology for delivery of neural transplant populations could provide a transformative solution (Figure 1). This is a minimally-invasive, technically simple and rapid delivery method, with the advantages of limiting infection and haemorrhage risks. Aerosolization of cells can permit delivery over large surface areas with homogenous distributions. Such a delivery approach could potentially achieve widespread cell distribution when coupled with the inherent pathotropic capacities of some transplant populations facilitating deeper penetration of tissues (20,21). The high water content and flexible/soft mechanical properties of neural tissues, for example 3.2 -3.4 kPa for human brain and spinal cord (22,23), means these could represent a desirable target for spray delivery due to a cushioning effect minimising physical impact. Spray use would be particularly advantageous for direct cell delivery onto injury sites during neurosurgical procedures in acute injury, for example, during penetrative traumatic brain/spinal cord injury debridement, during craniotomy for haemorrhagic strokes or removal of cortical arteriovenous malformations. This minimises risks of repeated surgeries/anaesthesia often required with injectable cell therapies. Administering cell sprays prior to dural closure has the potential to promote early CNS regeneration and ultimately improve neurological recovery. Finally, spray technology could enable early administration of cell therapies following traumatic neural injuries which is critical in achieving the best outcomes (11).

During the process of aerosolisation, cells immersed in droplets are directed at a target surface achieving widespread, rapid and homogenous coverage. We speculate that offers a significant advantage versus simply delivering droplets of cell transplant populations onto the neural tissue surface (e.g. via a dropper format) which may provide unreliable and inconsistent coverage. Such a delivery approach may also be less suitable for repair in extensive areas of pathology if transplants were to filter away from target tissues, due to the effects of gravity, prior to cell adhesion.

The feasibility of stem cell spray technology has been trialled for a limited number of pathologies with promising results. Autologous skin graft cell sprays to treat partial thickness burns achieve rapid epithelisation utilising smaller donor sites compared to traditional skin grafts (24). Successful orthopaedic clinical application has been demonstrated, using an airbrush spray to deliver chondrocytes for cartilage repair in osteoarthritis. This approach demonstrated surgical feasibility without a reduction in the matrix-producing capacity of transplanted cells (25). *However, to our knowledge, spray delivery technology has never been tested for direct delivery of stem cells into sites of CNS pathology.*

Experimental studies from our group (unpublished data) support the concept that developing neural cell transplant sprays is feasible and safe. Several major transplant populations have been tested including neural stem cells, oligodendrocyte precursor cells, olfactory ensheathing cells and astrocytes. Post spray viability has been uniformly high, with sprayed cells retaining cellular properties that underpin their therapeutic potential such as proliferation/differentiation capacity.

Our studies also indicate that optimised spray delivery to improve graft survival/integration and tissue regeneration, must account for physical spray parameters including droplet size and viscosity, spray velocity and nozzle bore size (26,27). Developing bespoke, tuneable spray devices in collaboration with the pharmaceutical industry is needed to achieve optimal cell delivery formats. Devices must be tailored to increase droplet size /impact cushioning, using lower droplet viscosity solutions and increased nozzle bore diameter (27). Shear force damage during spraying could be reduced by cell shrinkage using hyperosmolar sugar solutions as previously shown for macrophage (immune cells sprays) delivered to the respiratory tract (28), but assessment of neural cell viability would be required.

Further refinements to the spray delivery concept seem feasible. Dual-chamber devices (29,30) e.g. for the application of dural sealants, provide a pre-existing platform for advanced spray formats in neurosurgical procedures offering the capacity for ‘combinatorial delivery’ of multiple cell types, growth factors or pharmaceutical agents such as antibiotics/immunotherapies (to limit bacterial or fungal infections, for example). Advances in cell transport polymer technology (31) incorporated into spray devices can additionally offer improved cell transit to locations remote from cell manufacture sites, without specialised cold chain transport requirements for both research and clinical applications.

Based on these considerations, an optimised transplant spray could offer a novel and versatile cell delivery approach- of high relevance potentially in military or resource poor environments with limited infrastructure for the manufacture/delivery of cell therapy.

**Declaration of interests**

The authors have no competing interests to declare.

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**Author contributions**

WW, DE, NT and DMC contributed to research and writing the manuscript, AM contributed to research and graphic production. CA and DMC reviewed and edited the manuscript.

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