Stem cell-based in utero therapies for spina bifida: implications for neural regeneration

The history: Myelomeningocele – also known as spina bifida – is a devastating congenital anomaly of the central nervous system that is caused by the malformation of the spinal cord and vertebral column during embryogenesis. Depending on the location of the spina bifida lesion on the spine, patients suffer from neurological dysfunction ranging from paraparesis and incontinence to complete paralysis. The current standard of care for spina bifida is in utero surgical repair of the defect, which has been shown to minimize the secondary deficits associated with this disorder (Adzick et al., 2011). Despite these successes, this approach does not reliably improve neurologic function of affected children. Several groups, including our own, have performed studies aimed at augmenting the in utero surgical repair of spina bifida by applying principles of stem cell and tissue engineering to provide an enhanced protection of the exposed neural elements (Saadai et al., 2011, 2013; Wang et al., 2015; Brown et al., 2016). The ultimate goal of these studies is to improve the neurologic function in patients while maintaining the benefits of the existing fetal surgical treatment.

The pathogenesis of spina bifida has been well studied since the early 1950s, and a broad consensus now exists among researchers and clinicians that there is a “two-hit” mechanism that is largely responsible for the observed neurological problems associated with the disorder (Meuli and Moehrlein, 2014). The first “hit” is the initial failure in neurulation of the spinal cord during development, while the second “hit” occurs when the exposed neural elements are progressively damaged due to chemical exposure to the amniotic fluid and mechanical trauma to the unprotected cord tissue during the remainder of the pregnancy. The ideal treatment for spina bifida would be to prevent the first hit and ensure proper neurulation to begin with. This was partially accomplished with the advent of folic acid supplementation, and it is currently estimated that proper dosages of prenatal vitamins can reduce the risk of neural tube defects such as spina bifida and anencephaly by 50% or more. However, despite the widespread adoption of prenatal vitamins, spina bifida remains the most common cause of childhood paralysis. In contrast to the preventative therapy of prenatal vitamins, in utero surgery aims to prevent the damage incurred in the “second hit” phase: after neurulation has failed but before chemical insult and mechanical trauma have irreversibly destroyed nervous tissue. In this sense, the use of stem cell and tissue engineering to enhance surgical repair of the spina bifida defect is intended to exert protection and stimulate regeneration of the fetal spinal cord.

Therapeutic strategies: Stem cell-based strategies for spina bifida are similar in design to other proposed modes of stem cell therapy and can be grouped into two general therapy types: (1) Therapies capable of replacing damaged/lost cells and tissues; (2) Therapies capable of generating a microenvironment conducive to protection, regeneration, or enhanced healing of native tissues.

Lessons from preclinical and basic science research into parallel mechanisms of repair in adult spinal cord injury are particularly relevant here. A major distinction is that in utero spina bifida repair has the unique advantages of being diagnosable before irreversible nerve damage has occurred and being treatable during gestation when the fetus is still rapidly developing. While it has been theorized that the fetal environment may provide the additional receptivity to bioengineered tissue and cell grafts that is necessary for a successful Type 1 therapy (Fauza et al., 2008; Saadai et al., 2013), long term engraftment of neural tissue may not be required if a Type 2 therapy can prevent irreversible damage from occurring. These characteristics may make spina bifida particularly amenable to stem cell therapies, and working to advance the treatment of nerve damage in spina bifida has the potential to directly benefit children suffering from the disease as well as to generate knowledge, techniques, and technology that could eventually be extrapolated to other neurologic disorders.

Type 1 therapies: Replacement of damaged spinal cord tissues has long been a goal of researchers and clinicians hoping to restore function to those who are afflicted with severe spinal cord injury. In recent years, a heavy focus has been placed on the delivery of a stem/progenitor cell therapy that can replace or regrow destroyed neural tissues (Manley et al., 2017; Rosenzweig et al., 2018). Ideally, the transplanted stem/progenitor cells would engraft into the host tissues and form new neural connections, thus completely restoring lost neurologic function. Despite the obvious value of this type of therapy, promising preclinical experiments have shown lackluster results in human clinical trials (Kim et al., 2017). Additionally, this approach is likely more critical to patients suffering from an acquired spinal cord injury than those fetuses or newborns afflicted with developmental birth defects such as spina bifida. Acquired spinal cord injury – by definition – begins when physical trauma disrupts the function of neurons in the spinal cord. Spina bifida is characterized by spinal cord injury caused by the specific gestational environment secondary to incomplete neural tube formation/development. Though Type 1 therapies may be valuable to children diagnosed with spina bifida after birth, or adults who have been living with spina bifida their entire lives, the potential exists to limit mechanical injury with in utero interventions before irreversible damage to the susceptible fetal neural tissue occurs. Thus, it may be unnecessary to replace damaged nervous tissue if sufficient native tissue can be protected from being damaged before birth.

Type 2 therapies: Therapies that are capable of minimizing the damage to the developing spinal cord are in many ways more promising for the in utero treatment of spina bifida than those aimed at replacing or rebuilding new functional neural tissues and connections. The developmental pathogenesis of the disorder and the ability for early clinical diagnosis by ultrasound makes it more amenable to a tissue engineering or stem cell therapy approach designed to salvage or regrow native tissues. Several research groups are currently pursuing Type 2 treatment therapies for spina bifida in utero, and the strategies they employ are varied but often contain similar engineering elements (Saadai et al., 2011; Wang et al., 2015; Brown et al., 2016). Two key elements are scaffold systems and stem cells. Scaffolds may be composed of synthetic materials (e.g., polymers), natural materials such as collagen or other extracellular matrix molecules, or created by decellularizing tissues of deceased animals or humans. These may then be used to provide secondary structural support to enhance
the standard of care that currently involves a simple closure of the defect with the patient’s skin. The improved mechanical stability provided by an engineered scaffold can be further modified by the addition of cytokines, neurotrophic factors or growth factors, such as brain-derived neurotrophic factor or hepatocyte growth factor which are known to be important for neural development and protection, within or on the surface of the scaffold or hydrogel. In this way, these factors can be localized to the site of the defect and directly influence the developing tissues, potentially even protecting them from additional damage. The most sophisticated use of these scaffolds is as a delivery system for stem cells that are intended to further modulate the local microenvironment. This use of stem cells is distinct from those described in Type 1 therapies in that the cells delivered are intended solely to favorably modify endogenous biological processes rather than engage into the patient as new tissue. In the case of spina bifida, an ideal Type 2 therapy would provide enhanced protection to the fragile neural tissues while simultaneously limiting inflammation and preventing further death of neurons.

Mesenchymal stromal cells appear well suited for this purpose. These cells are widely studied from several tissue sources across numerous species. They are characteristically capable of plastic adherence, trilineage multipotency, have a well-described immunophenotype (Dominici et al., 2006), and are fully compatible with delivery scaffolds. More importantly, even though mesenchymal stromal cells typically appear to be incapable of engraftment when transplanted in vivo, they can be modified while the environment through secretion of growth factors, cytokines and extracellular vesicles (Spees et al., 2016). In many ways, mesenchymal stromal cells are the ideal candidate cell type for a Type 2 therapy. Indeed, our lab’s existing preclinical work in applying human placental-derived mesenchymal stromal cells in a large animal model of spina bifida suggests that this approach may be sufficient to restore ambulation to a fetus that would have otherwise been born paralyzed (Wang et al., 2015).

**Conclusions:** The pathogenesis of spina bifida makes it a logical candidate for treatment with stem cell based therapies. Spina bifida is diagnosable before irreversible nerve damage has occurred and can be treated while the fetus is still developing and receptive to bioengineered tissue. These characteristics likely make spina bifida uniquely amenable to both Type 1 and Type 2 therapies, and breakthrough made in the unique developmental environment of spina bifida have the potential to help spina bifida patients and lay the groundwork for neuroregenerative or neuroprotective therapies in other neuromuscular diseases. Though the ability to fully replace nervous tissue and allow paralyzed patients to walk again remains the elusive Holy Grail of regenerative medicine, effective therapies with the potential to dramatically improve a spina bifida patient’s quality of life may not require the ability to generate new nervous tissue. Through the creative use of existing technologies including in utero surgery, bioengineered scaffolds, and wound healing associated growth factors and stem cells, clinicians and scientists are on the cusp of developing a new era of treatments for those affected with spina bifida.

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