CHAPTER 10

Combining Stem Cells and Materials for Nerve Tissue Regeneration

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STEM CELL THERAPY FOR NEURONAL REGENERATION

One of the earliest translational efforts in bringing regenerative cells to clinical application has been in the damaged or diseased nervous system. This includes both central and peripheral nervous systems and the neural retina. Historically, this was driven by a simple concept that if the indication involved pathology in a particular neural population, then isolating a primary tissue with a suitable cell population could deliver a suitable therapy. Hence, the 1970s and 1980s saw the rapid development all the way to the clinic in small anecdotal trials of transplantation of cells directly derived with minimal manipulation from anatomically or phenotypically suitable tissues, usually of foetal origin (e.g., Barker et al., 2015 regarding the treatment of Parkinson's disease). Although the use of these cells can provide a proof of concept for a cell-based therapy, donated primary cells cannot provide a long-term solution to the rollout of a viable therapy, because of limited tissue supply and variable consistency and quality of cells derived from primary tissues.

STEM CELLS AND THEIR MECHANISMS OF ACTION

Beginning in the late 1990s, with the development of multipotent stem cells derived from human foetal and adult brain and the isolation of pluripotent cells from the early developing embryo (hESCs, human embryonic stem cells), the focus shifted to the possibility of more reproducible and expandable cells and cell lines that could retain the characteristics of the primary cells but could be expanded more economically to serve a much greater patient need and not be dependent on the availability of precious primary tissues. However, the clinical translation of multipotent stem cells derived from neural stem cells (NSCs), originally from the developing brain, or from pluripotent cells differentiated by exposure to a range of growth factors and cytokines in culture, has been slow but is expected to start delivering controlled clinical data over the next few years. The key activities of these cells involve a level of continued survival and staged differentiation into therapeutically relevant cell types as well as a local paracrine delivery of factors including growth factors, cytokines, enzymes, etc., possibly at least partly mediated

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by exosomes (Holm et al., 2018; Jing et al., 2018). The development of induced pluripotent stem cells (iPSCs) (Maherali and Hochedlinger, 2008; Yamanaka, 2012) has opened further possibilities for therapeutic cells, including autologous treatments using the patient's own cells. The first clinical trial using iPS-derived retinal pigmental epithelial (RPE) cells in age-related macular degeneration has tentatively started.

However, the predominant cell types currently used in clinical trials for a range of indications, mostly characterised by significant inflammation, are mesenchymal stem cells (MSCs) or their variants. The key distinction between MSCs and cells derived from pluripotent or multipotent tissue sources is that MSCs are not typically engrafting; they rely primarily on their paracrine chemical and exosome release or mitochondrial or other organelle transfer activities (Gao et al., 2016). MSCs are typically delivered peripherally rather than locally as their activities are not specific to the damaged organ. For example, Athersys Inc (Cleveland, OH, USA) is developing an MSC variant, multipotent adult progenitor cells (MAPCs) cell therapy product, for acute ischaemic stroke. From preclinical data, the mechanism of action is not directly to the cerebrovascular system or the brain itself, but rather to the peripheral immune organs, notably the spleen. There is evidence that the MAPCs prevent the outflow of splenocytes that occurs in the 48 h after stroke, hence partially protecting vulnerable neurons (Bing et al., 2017).

Unlike MSCs, (Gage, 2000) NSCs, be they derived from foetal brain tissue donations or differentiated from pluripotent cells, are in general required to be administered locally in the brain parenchyma to exert beneficial effects in animal models of neurologic dysfunction (Smith et al., 2012). The cells are capable of limited (up to 10% of cells implanted) engraftment and some level of circuit reformation in animal lesion models but also induce host-derived immune cell changes as well as neurogenesis, vasculogenesis and other regenerative changes (Ottoboni et al., 2017; Pollock et al., 2006; Sinden et al., 2017). The NSC line (CTX0E03) is delivered by means of a specialised small volume delivery cannula under stereotaxic control to live tissue adjacent to the infarct. CTX0E03 has been evaluated for safety and dose also with changes in neurologic function in two open label clinical trials of patients with chronic disability after ischaemic stroke (Sinden et al., 2017; Kalladka et al., 2016) and will progress to a randomied, surgically controlled Phase IIB pivotal trial in 2018. These studies indicate the feasibility and safety of naked stem cell therapy when appropriately targeted in patients with an unmet medical need, with the prospect of clinical efficacy data within reach.

APPLICATIONS OF MATERIALS TO STEM CELL THERAPY

An alternative approach to stem cell-based therapies is to apply the cells in a material format where the biological activities of the cells can be better exploited or controlled.

The use of a biocompatible material as a support or substrate for stem cell delivery or retention has a number of likely beneficial consequences. Combining cells with scaffolds,

biogels, encapsulation materials or other devices can alter the survival, differentiation, functionality, disposition and the immune interactions of the implanted cells. Any or all of these can be beneficial to repair. For example, the use of hydrogels as a scaffold for stem cells in spinal cord repair has been proposed (Oliveira et al., 2018). The cells can be maintained within the hydrogel, and extracellular matrix components in the gel can retain a niche-like environment for the cells to survive and deliver natural therapeutic molecules. Hydrogel encapsulation can have positive safety features in reducing teratoma formation in pluripotent cell–derived oligodendrocyte progenitor cells (Führmann et al., 2016). Alternatively, when the need is to develop an artificial layer of cells, da Cruz et al. (2018) have engineered an RPE patch comprising a fully differentiated, human embryonic stem cell (hESC)–derived RPE monolayer on a coated, synthetic basement membrane. Two patients have been treated with this cell–material combination with positive effects on the patients' vision.

Notwithstanding the proposed benefits, the combination of cells and materials poses a number of challenges in terms of manufacturing, delivery and the regulatory pathway through preclinical and clinical trials to licencing. Manufacturing on any commercial scale requires considerable new technology enhancements and delivery poses challenges in both devices and real-time imaging. Furthermore, the above advantages, especially long-term survival and immune privilege afforded by the material, require extensive testing in appropriate animal models prior to the initiation of the first clinical trials.

CELLULAR BIOMATERIALS

The field of tissue engineering has yielded a range of biomaterials and manufacturing techniques that can be used to generate cellular biomaterials for use in regenerative medicine. Advances in 3D printing, electrospinning and decellularisation technology in particular have provided biomaterial scaffolds into which therapeutic cells can be seeded (Steffens et al., 2018). This approach to tissue engineering, where the properties of the cellular biomaterial are determined through organising the extracellular material prior to adding cells, is particularly suited to construct tissues that are matrix-rich and need to be strong, stiff or have specific geometry to provide a key function (Brown and Phillips, 2007). For building cell-rich tissues such as those of the nervous system, however, an alternative tissue engineering approach is often more appropriate. The priority in such cases is to create an environment in which cells can interact with each other and adopt an appropriate configuration for restoring function. Due to their similar physical properties to natural tissues, hydrogels are often the substrate of choice for engineering cell-rich soft tissues (Baldwin et al., 2018; Foyt et al., 2018). Cells can be incorporated into polymer material solutions prior to gelation, ensuring that they are distributed throughout the resulting hydrogel and thus overcoming problems associated with trying to seed cells into preformed scaffolds. Furthermore, hydrogel formulation can be adapted to

achieve a wide range of biochemical and physical environments to control the behaviour of the incorporated donor cells and the response of the recipient tissue at a site of implantation (Foyt et al., 2018; Smith Callahan, 2018; Cooke et al., 2018).

CREATING ANISOTROPY IN CELLULAR HYDROGELS

A key challenge in using hydrogels for tissue engineering is to create the cell and tissue-level architecture that is often a hallmark of native tissue and can be crucial to function. It is relatively straightforward to provide an overall shape for a cellular hydrogel through casting it within a mould of the appropriate geometry or through the use of microfluidics and bioprinting techniques (Foyt et al., 2018). Anisotropic structures can be formed at the mesoscale through the parallel positioning of channels or fibres; however, it can be more challenging to achieve organised micro- or nanoscale architectures at the level of cells and matrix molecules. Approaches for structuring cellular hydrogels that have been developed include the use of fluid flow and shear (Cooke et al., 2018; Jang et al., 2015), magnetic and electric fields (Omidinia-Anarkoli et al., 2017) and other micro- and nanofabrication approaches (Kim et al., 2014).

A biomimetic approach to generating aligned cellular hydrogels involves harnessing the natural ability of cells to apply tension to their extracellular matrix. Contraction of hydrogels as a result of cell-generated tension is a well-established phenomenon (Elsdale and Bard, 1972; Grinnell and Petroll, 2010). It causes many researchers problems when trying to construct cell-rich hydrogels because the distortion of the bulk gel size and shape and accompanying increase in cell density can disrupt the intended function of the cellular material. However, this effect can be exploited in the generation of self-aligning cellular hydrogels, where restricting the directions in which hydrogels can contract provides a means to harness and control the direction of cell orientation.

By tethering rectangular cellular collagen gels at opposite ends, tension generated by the cells is aligned parallel to the long axis of the gel. Consequently, the cells and collagen fibrils interact with each other to form an anisotropic structure (Georgiou et al., 2013; Eastwood et al., 1998). The mechanism underpinning this cellular self-alignment process involves the natural binding of cells to extracellular matrix molecules, and a study using antibodies to block specific cell adhesion receptor integrins demonstrated that self-alignment of C6 glioma cells in tethered collagen gels was mediated through α_1 integrins (O'Rourke et al., 2015). Therefore, the sequence of events can be considered to be that (1) cells trapped within a collagen gel form integrin-mediated attachments with the surrounding matrix; (2) cells apply tension to that matrix, with no net initial organisation of forces; (3) the gel deforms in response to the cell-generated contractile forces but is restricted in doing so by the tethering, limiting the deformation to a transverse narrowing, whereas (4) tension builds up parallel to the long axis of the gel. Cells and matrix will both be pulled into alignment as a result of this tension. This sequence of events has

been captured with label-free imaging, using two-photon excitation autofluorescence of the embedded cells coupled to second harmonic generation signals from the collagen fibrils to monitor human dental pulp stem cell-derived cells and collagen matrix reorganisation simultaneously (Sanen et al., 2016).

It is interesting to note that while the cellular self-alignment process in collagen gels relies on natural cell–matrix interactions, the extent to which it is effective can depend on both the cell type and the specific phenotype of the cells. For example, primary rat astrocytes were only able to align within tethered collagen gels after stimulation with TGFβ1 pushed them towards a more reactive phenotype (East et al., 2010), and early attempts to align rat Schwann cells required the addition of a proportion of fibroblasts to produce reliable alignment (Phillips et al., 2005). To understand and accommodate the variabilities in the extent of cellular self-alignment that occur when cell and material parameters are selected, a simple multiwell plate assay was developed in which cells can be screened in terms of their ability to contract small free-floating gels. The extent of contraction is then used to predict the extent of alignment in subsequent tethered gels, facilitating the adaptation of parameters such as cell density and timing to achieve reliable alignment (O'Rourke et al., 2015).

Having initially been developed as a model system for measuring cellular forces and responses in vitro (Eastwood et al., 1998; Mudera et al., 2000), the idea of using tethered gels to construct artificial tissues was developed and patented in 2004 as a 'Self-aligning tissue growth guide' by Phillips and Brown (Phillips and Brown, 2004), with a focus on using the technology to generate a substrate for nerve regeneration (Phillips et al., 2005). Since then, the technology has been refined and combined with a range of potential therapeutic cell types for nerve tissue engineering as described in the next section. In addition to neural tissue engineering, cellular self-alignment has also been applied in the construction of other artificial anisotropic tissues including cornea (Mukhey et al., 2018), central nervous system (East et al., 2010; O'Rourke et al., 2017) and muscle (Jones et al., 2018).

STABILISATION OF ALIGNED CELLULAR HYDROGELS

Maintaining the anisotropic arrangement of cells and matrix in tethered collagen gels requires continuous tension, which can be problematic when the aim is to generate tissue for regenerative medicine. If cells in a tethered gel proliferate and continue to generate more tension, then, at some point, the forces will be sufficient to detach the gel from the tethering points, leading to a loss of tension and collapse of the structure. Furthermore, while implantation of a tethered gel has been achieved in an animal model by anchoring the gel within a stiff tube (Phillips et al., 2005), the practicalities of maintaining physical tethering during and after implantation are considerable. The most promising approach used to date for turning anisotropic tethered cellular collagen gels into viable implantable

constructs is through plastic compression, in which the majority of interstitial fluid is removed from a hydrogel, leaving a thin sheet of dense cellular material (Brown et al., 2005). Performing plastic compression on an aligned tethered gel effectively stabilises the alignment, by increasing the density of cells and matrix to a point whereby the tethering can be released without a loss of organisation. Plastic compression can now be achieved using commercially available RAFT absorbers (Lonza), and the novel combination of plastic compression with cellular self-alignment using stem cells for neural tissue engineering was patented in 2013 as 'Engineered neural tissue' (EngNT) by Phillips and Georgiou (2013).

PERIPHERAL NERVE TISSUE ENGINEERING: REPLACING THE AUTOGRAFT

Peripheral nerve injury (PNI) has diverse causes; trauma, surgery (tumour removal) and compression syndrome can all lead to loss of sensation and muscle control and cause chronic pain. A peripheral nerve, if cut, can regenerate, and a direct suture repair is optimal for minimal gaps. However, any significant gap must be bridged, otherwise poor functional recovery results in permanent loss of muscle control and feeling, the disability can be severe with lifelong pain (Safa and Buncke, 2016). PNI incidence is 2%–5% of trauma cases, affecting ~1 million people in Europe and United States p.a. of whom 600,000 have surgery, but only 50% regain function. PNI has high healthcare, unemployment, rehabilitation and social costs, e.g., forearm PNI patients average 273 sick days (Noble et al., 1998; Belkas et al., 2004). PNI more often affects young people; their disability greatly impacts lifetime productivity. A wrap or empty tube to bridge cut nerve ends helps guide and support nerve growth but is only effective for short gap <30 mm repair. An autograft harvested from healthy nerve is the 'gold standard' for long gaps (>30–50 mm) (Battiston et al., 2017). A specialist plastic surgeon must harvest the nerve; it takes 30–60 min and costs c.$7K (Brattain, 2013). Limited donor nerve amount or size reduces repair efficacy, especially for larger nerves. Donor site damage, loss of sensation, the risk of chronic pain, plus the poor or variable outcomes pose a significant clinical challenge, so more effective long-gap repair options are needed. Columns of Schwann-like cells (to support axonal growth) and matrix architecture are vital to guide nerve regeneration across long gaps. The living nerve growth guide, EngNT, mimics nerve structure; it has a core of aligned cells/collagen matrix in an outer collagen sheath and can be tailored to size. Early animal data show EngNT supports and guides neural outgrowth and nerve regeneration (Georgiou et al., 2013, 2015; Gonzalez-Perez et al., 2018; O'Rourke et al., 2018; Sanen et al., 2017; Schuh et al., 2018). We propose EngNT as an off-the-shelf autograft substitute. It should save the time, cost and risk of autograft surgery and improve clinical outcomes compared to empty tubes or acellular growth guides, e.g., acellular cadaveric nerve (Avance, Axogen Inc., Alachua FL), which lacks guide cells.

Products for short-gap nerve repair are collagen-based wraps or empty tubes, e.g., Stryker (NeuroMatrix, Neuroflex), Integra Life Sciences (NeuraGen, NeuraWrap) and Cook (AxoGuard). The worldwide market for short-gap repair is estimated at £32m (Sanen et al., 2017). But patients with PNI >30 mm are poorly served, apart from autografts only AxoGen's Avance is available. None of the scaffold or tubular materials (plus cells/growth factors) in development is as effective as an autograft. The potential market for EngNT is all patients with long-gap PNI, not just trauma. In the United States, trauma PNI leads to 8.5m restricted activity days and 5m bed/disability days, and upper extremity paralysis affects 360K in US and 300K in Europe (Belkas et al., 2004). Up to ~50% have autografts (140–180K US procedures), but lack of suitable donor tissue or surgeon availability will limit autograft numbers. PNI from cancer surgery can have serious side effects, e.g., impotence in prostate surgery. So an effective off-the-shelf EngNT nerve repair device could benefit several large patient groups who are not currently treated, including diabetic and older patients. We believe EngNT could significantly increase nerve repair procedures; benefits are (1) A repair, made at the same time as trauma or tumour surgery, saves time and cost of a second surgery. (2) A short injury-to-repair time improves outcomes. (3) A nerve can be repaired without referral to a specialist. AxoGen's estimated market potential for Avance is £325m–£650m, but little return on investment data exist for PNI products. The majority of patients who may benefit from PNI repair products are young: drivers, cyclists, the military and many younger cancer patients undergoing major resection. A strong health economic case can be made for restoring employment capacity in such patients, based on cost per quality adjusted life year. Furthermore, the EngNT is likely to be more suitable than autografting for treatment of long-gap PNI in patients limited in their access to well-equipped medical/surgical facilities and staff as specialist surgical input is not as necessary in comparison to that required for autografting.

In Fig. 10.1, a comparison of devices and procedures used in the treatment of PNIs shows that short gaps, <10 mm, are most commonly repaired by direct repair or by

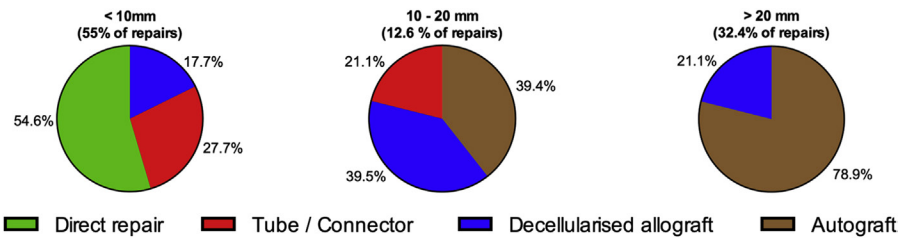

Figure 10.1 ***Breakdown of nerve repair interventions by gap length levels.*** It shows the proportion of gap lengths and the distribution of surgical procedures according to gap size, i.e., short, <10 mm, medium, 10–20 mm, and long >20 mm. *(Data based on Brattain K. Analysis of the peripheral nerve repair market in the United States. Magellan Medical Technology Consultants Inc.; 2013.)*

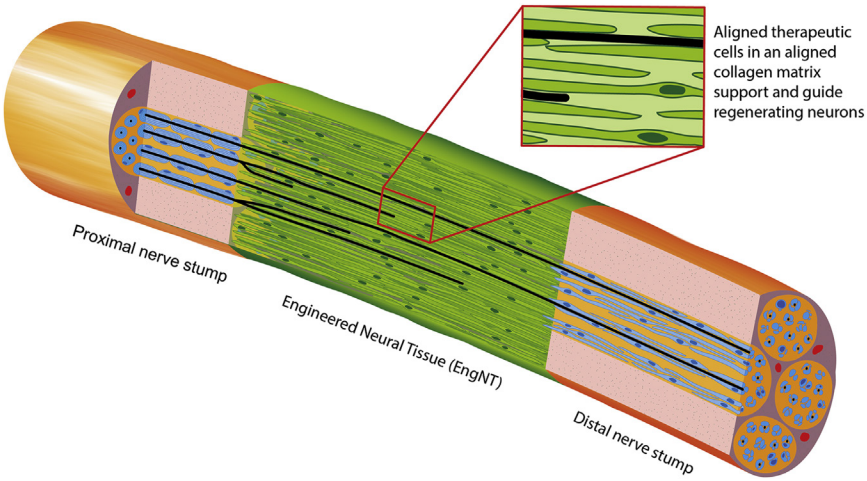

Figure 10.2 ***Engineered neural tissue can be implanted to support regeneration of neurons across a gap in a damaged peripheral nerve.*** The living EngNT material contains highly aligned cells embedded within an aligned hydrogel matrix. Once implanted, it forms a bridge between the proximal and distal nerve stumps, supporting and guiding neuronal regeneration across the gap. *(Figure supplied by Sarah Hannis.)*

simple devices, either tubes or decellularised allograft cadaveric nerve. For the larger gaps, the most common procedure is to remove a nerve from the patient, usually the sural nerve, and graft the dissected nerve as the replacement. An autograft is used because it has all the characteristics that will promote axon growth and will not be subject to immune rejection. The advantage of the engineered neural tissue construct containing aligned nerve cells in a stabilised collagen biogel (Georgiou et al., 2013) is to offer a substitute for long-gap repair that eliminates autograft surgery morbidity (Fig. 10.2).

ENGINEERED NEURAL TISSUE TO REPLACE THE AUTOGRAFT: THERAPEUTIC CELLS?

The nerve autograft provides a suitable environment to support and guide neuronal regeneration (Safa and Buncke, 2016; Deumens et al., 2010). Effectively it is a section of denervated peripheral nerve tissue, with predominantly the same structural and cellular composition as an acutely denervated distal nerve stump. The key supportive feature is the columns of Schwann cells that persist after a nerve has been cut, forming the Bands of Büngner that provide pathways for axonal growth (Ribeiro-Resende et al., 2009). The Schwann cells in a nerve graft or distal nerve stump respond to denervation by undergoing adaptive cellular reprogramming to adopt a specialised repair phenotype (Jessen and Mirsky, 2016). This has been studied extensively in rodents, but less is known

about the equivalent cellular changes that take place in human nerves. Nevertheless, it is reasonable to assume that the effectiveness of the human nerve autograft is underpinned by the presence of aligned columns of Schwann cells that adopt a repair-supportive phenotype, so recreating this is a key aim for nerve tissue engineering. Furthermore, while decellularised nerve grafts can support successful regeneration and are available for clinical use in certain situations, an autograft containing living Schwann cells remains the superior option for repairing nerves with longer gaps and larger diameters (Rbia and Shin, 2017). Building living constructs with features that mimic the Bands of Büngner in the nerve graft is a tempting challenge for the tissue engineer with numerous examples of Schwann cell-seeded grafts being tested in animal models over many years (Daly et al., 2012). However, moving this technology into clinical application is severely limited by the need for a suitable source of therapeutic cells. Deriving autologous Schwann cells from patients is problematic because it requires the destruction of additional nerve tissue and expansion capacity is limited, so efforts have focused on exploring whether stem cells can be used instead, in many cases through differentiating them into Schwann cells. Potential sources of stem cells that have been considered include adipose, bone marrow, embryonic, umbilical cord, dental pulp, neural and skin tissue and iPSCs (Bhangra et al., 2016; Armaiz Flores and Wang, 2018). Interestingly, while the logical approach to developing therapeutic cells for nerve repair might be to generate cells that resemble the repair phenotype Schwann cell (Jessen and Mirsky, 2016), there is some evidence that cells at an earlier stage of differentiation may be more effective. This was demonstrated in a recent study where iPSCs were differentiated to form neural crest stem cells (NCSC) or Schwann cells, and the NCSCs showed better engraftment and improved functional recovery in a rat sciatic nerve model (Huang et al., 2017). It is worth speculating what properties the 'ideal' cell for use in nerve tissue engineering would need to exhibit. For regeneration support then the specialised repair phenotype Schwann cell that releases neurotrophic factors and forms a physical guidance substrate would be useful during the initial phase, but in the longer term these features would be less desirable and cells able to myelinate axons and support efficient conduction may be more appropriate. Long-term stable engraftment of cells may be beneficial for long gaps where regeneration support might be required for a long duration, whereas in a construct designed to stimulate host cell infiltration perhaps a short-lived therapeutic cell would be more suitable. Release of neurotrophic factors to promote regeneration is of benefit, but at the distal stump this might be detrimental if regenerating neurites are discouraged from leaving the graft environment as a consequence. These are just a few considerations and many more such as the ability of implanted cells to modulate immune and inflammatory responses, support appropriate levels of graft vascularisation and deposit appropriate extracellular matrix components are also important. Given these complex requirements, it is unsurprising that no single ideal cell type has yet been adopted for nerve tissue engineering, but with advances in cell and tissue engineering

there are powerful new tools available to adjust the phenotype and behaviour of cells, such as ex vivo gene therapy and the use of biomaterials to control cell behaviour, opening up new possibilities for improved future options (Busuttil et al., 2016).

For now, researchers tend to take a pragmatic approach to therapeutic cell choice, balancing repair efficacy with clinical suitability and availability. The development of EngNT as an aligned cellular nerve repair biomaterial started in the same way as many tissue-engineered approaches, using rat Schwann cells (specifically in this case an immortalised Schwann cell line SCL 4.1/F7) as the cellular component (Georgiou et al., 2013). The concept was then tested using potential autologous stem cell options as a source of Schwann cells through differentiation of rat adipose–derived stem cells (Georgiou et al., 2015), human dental pulp stem cells (Sanen et al., 2017; Martens et al., 2014) and rat bone marrow–derived stem cells (Gonzalez-Perez et al., 2018). The use of autologous stem cells in nerve tissue engineering is hampered by limitations such as variability between patients, time required for preparation and lack of commercial attractiveness. There is substantial interest, therefore, in the use of allogeneic cells to construct engineered tissue that can be available immediately as an 'off-the-shelf' therapeutic product.

ALLOGENEIC OFF-THE-SHELF REPLACEMENT NERVE TISSUE

The use of an allogeneic cell source provides some appealing benefits for the commercial and clinical translation of the EngNT technology because it offers the possibility of being immediately available as an 'off-the-shelf' therapeutic product. This not only overcomes the delay associated with harvest and expansion of autologous cells, which is undesirable in situations where rapid nerve repair is required, but also it opens up the possibility for the living artificial tissue to be manufactured at scale as a robust and consistent advanced therapy medicinal product. Important progress to test this concept was made by constructing EngNT using ReNeuron's clinical-grade CTX0E03 conditionally immortalised clonal human NSC line. The conditional immortalisation uses the c-mycER^{TAM} transgene, generating a fusion protein that drives cell proliferation only in the presence of 4-hydroxytamoxifen (4-OHT). This allows stable and continuous cell expansion and, as the fusion protein cannot function in the absence of 4-OHT, it improves safety after implantation (Pollock et al., 2006). CTX0E03 cells have been differentiated and subsequently used to construct EngNT-CTX for implantation to repair the sciatic nerves of athymic nude rats (O'Rourke et al., 2018). In addition to establishing the feasibility of using CTX0E03 cells for nerve repair, refinement of the EngNT manufacturing process was conducted with a view to comply with Good Manufacturing Practices. This includes adopting bovine sources of collagen for hydrogel formulation, using media free from components such as antibiotics and serum and streamlining the two-step processes of cellular self-alignment and RAFT stabilisation using purpose-built moulds.

Recogniing the likely cost of bringing EngNT-CTX to a clinical trial and following support from the UCL Technology Fund, the UK government through its Innovate UK

grant scheme and the UKI2S Seed Fund, the start-up company Glialign Limited (London, UK) was established by the authors to demonstrate the feasibility of the EngNT product in long-gap repair from a preclinical safety and efficacy, as well as cost of goods and manufacturing/regulatory perspective.

PERSPECTIVES

The Glialign project is young and still at the proof of concept stage. While this is a stem cell project, we think the method of presentation of the cells is unique and significant from a therapy perspective. Instead of installing stem cells into heterogenous host environments that characterise most diseased or damaged tissues for which stem cells are normally intended, we present the stem cells in a tissue format for which the cells have already formed their structure/function which is naturally regenerative to nerves. If the initial positive in vitro and long-gap repair animal data play out well, we anticipate moving to a first open label safety trial in PNI patients where a signal of efficacy will be easy to identify. We see this application of stem cells and their formation of engineered nerve tissue as of potential benefit in many other unmet medical needs where the requirements for neural tissue to repair defects after disease or trauma are necessary.

REFERENCES

Armaiz Flores A, Wang H. The use and delivery of stem cells in nerve regeneration: preclinical evidence and regulatory considerations. Ann Plast Surg 2018;80(4):448–56.

Baldwin M, et al. Augmenting endogenous repair of soft tissues with nanofibre scaffolds. J R Soc Interface 2018;15(141).

Barker RA, Drouin-Ouellet J, Parmar M. Cell-based therapies for Parkinson disease—past insights and future potential. Nat Rev Neurol 2015;11:492.

Battiston B, et al. Peripheral nerve defects: overviews of practice in Europe. Hand Clin 2017;33(3):545–50.

Belkas JS, Shoichet MS, Midha R. Peripheral nerve regeneration through guidance tubes. Neurol Res 2004;26(2):151–60.

Bhangra KS, et al. Using stem cells to grow artificial tissue for peripheral nerve repair. Stem Cell Int 2016;2016:7502178.

Bing Y, et al. Multipotent adult progenitor cells enhance recovery after stroke by modulating the immune response from the spleen. Stem Cell 2017;35(5):1290–302.

Brattain K. Analysis of the peripheral nerve repair market in the United States. Magellan Medical Technology Consultants Inc.; 2013.

Brown RA, et al. Ultrarapid engineering of biomimetic materials and tissues: fabrication of nano- and microstructures by plastic compression. Adv Funct Mater 2005;15(11):1762–70.

Brown RA, Phillips JB. Cell responses to biomimetic protein scaffolds used in tissue repair and engineering. Int Rev Cytol 2007;262:75–150.

Busuttil F, Rahim AA, Phillips JB. Combining gene and stem cell therapy for peripheral nerve tissue engineering. Stem Cell Dev 2016;26(4):231–8.

Cooke ME, et al. Structuring of hydrogels across multiple length scales for biomedical applications. Adv Mater 2018;30(14):e1705013.

da Cruz L, et al. Phase 1 clinical study of an embryonic stem cell–derived retinal pigment epithelium patch in age-related macular degeneration. Nat Biotechnol 2018;36:328.

Daly W, et al. A biomaterials approach to peripheral nerve regeneration: bridging the peripheral nerve gap and enhancing functional recovery. J R Soc Interface 2012;9(67):202–21.
Deumens R, et al. Repairing injured peripheral nerves: bridging the gap. Prog Neurobiol 2010;92(3):245–76.
East E, et al. Alignment of astrocytes increases neuronal growth in three-dimensional collagen gels and is maintained following plastic compression to form a spinal cord repair conduit. Tissue Eng Part A 2010;16(10):3173–84.
Eastwood M, et al. Effect of precise mechanical loading on fibroblast populated collagen lattices: morphological changes. Cell Motil Cytoskelet 1998;40(1):13–21.
Elsdale T, Bard J. Collagen substrata for studies on cell behavior. J Cell Biol 1972;54(3):626–37.
Foyt DA, et al. Exploiting advanced hydrogel technologies to address key challenges in regenerative medicine. Adv Healthc Mater 2018;7(8):e1700939.
Führmann T, et al. Injectable hydrogel promotes early survival of induced pluripotent stem cell-derived oligodendrocytes and attenuates longterm teratoma formation in a spinal cord injury model. Biomaterials 2016;83:23–36.
Gage FH. Mammalian neural stem cells. Science 2000;287(5457):1433–8.
Gao F, et al. Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis 2016;7:e2062.
Georgiou M, et al. Engineered neural tissue for peripheral nerve repair. Biomaterials 2013;34(30):7335–43.
Georgiou M, et al. Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve. Biomaterials 2015;37:242–51.
Gonzalez-Perez F, et al. Schwann cells and mesenchymal stem cells in laminin- or fibronectin-aligned matrices and regeneration across a critical size defect of 15 mm in the rat sciatic nerve. J Neurosurg Spine 2018;28(1):109–18.
Grinnell F, Petroll WM. Cell motility and mechanics in three-dimensional collagen matrices. Annu Rev Cell Dev Biol 2010;26:335–61.
Holm MM, Kaiser J, Schwab ME. Extracellular vesicles: multimodal envoys in neural maintenance and repair. Trends Neurosci 2018;41(6):360–72.
Huang CW, et al. The differentiation stage of transplanted stem cells modulates nerve regeneration. Sci Rep 2017;7(1):17401.
Jang JM, et al. Engineering controllable architecture in matrigel for 3D cell alignment. ACS Appl Mater Interfaces 2015;7(4):2183–8.
Jessen KR, Mirsky R. The repair Schwann cell and its function in regenerating nerves. J Physiol 2016;594(13):3521–31.
Jing H, He X, Zheng J. Exosomes and regenerative medicine: state of the art and perspectives. Transl Res 2018;196:1–16.
Jones JM, et al. An assessment of myotube morphology, matrix deformation, and myogenic mRNA expression in custom-built and commercially available engineered muscle chamber configurations. Front Physiol 2018;9(483).
Kalladka D, et al. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. Lancet 2016;388(10046):787–96.
Kim J, et al. Biologically inspired micro- and nanoengineering systems for functional and complex tissues. Tissue Eng Part A 2014;20(15–16):2127–30.
Maherali N, Hochedlinger K. Guidelines and techniques for the generation of induced pluripotent stem cells. Cell Stem Cell 2008;3(6):595–605.
Martens W, et al. Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. FASEB J 2014;28(4):1634–43.
Mudera VC, et al. Molecular responses of human dermal fibroblasts to dual cues: contact guidance and mechanical load. Cell Motil Cytoskelet 2000;45(1):1–9.

Mukhey D, et al. Controlling human corneal stromal stem cell contraction to mediate rapid cell and matrix organization of real architecture for 3-dimensional tissue equivalents. Acta Biomater 2018;67:229–37.
Noble J, et al. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. J Trauma 1998;45(1):116–22.
O'Rourke C, et al. Optimising contraction and alignment of cellular collagen hydrogels to achieve reliable and consistent engineered anisotropic tissue. J Biomater Appl 2015;30(5):599–607.
O'Rourke C, et al. Adapting tissue-engineered in vitro CNS models for high-throughput study of neurodegeneration. J Tissue Eng 2017;8. https://doi.org/10.1177/2041731417697920.
O'Rourke C, et al. An allogeneic 'off the shelf' therapeutic strategy for peripheral nerve tissue engineering using clinical grade human neural stem cells. Sci Rep 2018;8(1):2951.
Oliveira JM, et al. Hydrogel-based scaffolds to support intrathecal stem cell transplantation as a gateway to the spinal cord: clinical needs, biomaterials, and imaging technologies. npj Regen Med 2018;3(1):8.
Omidinia-Anarkoli A, et al. An injectable hybrid hydrogel with oriented short fibers induces unidirectional growth of functional nerve cells. Small 2017;13(36).
Ottoboni L, Merlini A, Martino G. Neural stem cell plasticity: advantages in therapy for the injured central nervous system. Front Cell Dev Biol 2017;5(52).
Phillips JB, Brown RA. In: London UC, editor. Self-aligning tissue growth guide. 2004.
Phillips JB, et al. Neural tissue engineering: a self-organizing collagen guidance conduit. Tissue Eng 2005;11(9–10):1611–7.
Phillips JB, Georgiou M. Engineered neural tissue. 2013.
Pollock K, et al. A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke. Exp Neurol 2006;199(1):143–55.
Rbia N, Shin AY. The role of nerve graft substitutes in motor and mixed motor/sensory peripheral nerve injuries. J Hand Surg Am 2017;42(5):367–77.
Ribeiro-Resende VT, et al. Strategies for inducing the formation of bands of Bungner in peripheral nerve regeneration. Biomaterials 2009;30(29):5251–9.
Safa B, Buncke G. Autograft substitutes: conduits and processed nerve allografts. Hand Clin 2016;32(2):127–40.
Sanen K, et al. Label-free mapping of microstructural organisation in self-aligning cellular collagen hydrogels using image correlation spectroscopy. Acta Biomater 2016;30:258–64.
Sanen K, et al. Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: potential for peripheral nerve repair? J Tissue Eng Regen Med 2017;11(12):3362–72.
Schuh C, et al. An optimized collagen-fibrin blend engineered neural tissue promotes peripheral nerve repair. Tissue Eng Part A 2018. https://doi.org/10.1089/ten.tea.2017.0457.
Sinden JD, et al. Human neural stem cell therapy for chronic ischemic stroke: charting progress from laboratory to patients. Stem Cell Dev 2017;26(13):933–47.
Smith EJ, et al. Implantation site and lesion topology determine efficacy of a human neural stem cell line in a rat model of chronic stroke. Stem Cell 2012;30(4):785–96.
Smith Callahan LA. Gradient material strategies for hydrogel optimization in tissue engineering applications. High Throughput 2018;7(1).
Steffens D, et al. Update on the main use of biomaterials and techniques associated with tissue engineering. Drug Discov Today 2018;23(8):1474–88.
Yamanaka S. Induced pluripotent stem cells: past, present, and future. Cell Stem Cell 2012;10(6):678–84.