Stem Cells: A New Hope for Neural Regeneration?

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"Regeneration is a basic phenomenon in life and nervous tissue is not an exception[1]"

Over the centuries, man has tried to achieve satisfactory nervous system regeneration. In 200BC, Galeno portrayed the first known attempt at regeneration on an injured nerve. In 600 BC, Argino described the first neurorraphy but only in 1873, more than one millennium later, Hueter reported what we know as "some success" in the surgical repair of an injured nerve.[2]

Much is speculated about "technological leaps" which occur during wars and in medicine. In the case of neural regeneration, this forward progress did really exist. During the Second World War in the twentieth century, because of the multitude of patients generated from battlefields, neural regeneration reached its highest summit, moreover with the disclosure and release of the "Senior Consultant in Neurologic Surgery to the European Theater of Operations" in 1942[3], in which it was established that "a premature suture without strain" is the best functional injured nerve recuperation method. However, even after approximately 80 years and having spent millions of dollars on research, the functional recovery results of a nerve continue to be quite unsatisfactory[4,5,6] as well as being very similar to the ones described in the last century.[3]

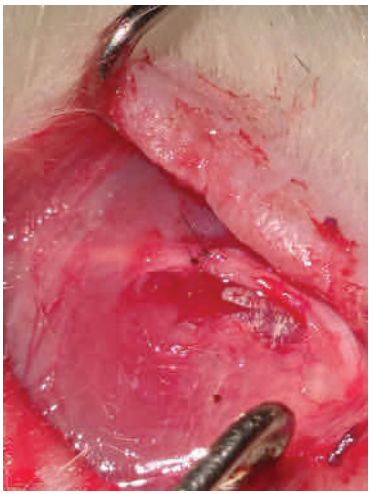

Figure 1. Example of suture end-to-end in the mandibular branch of the facial nerve in rats – "gold standard" technique for neural reparation.

When a nerve suffers a neurotmesis, meaning a total rupture, spontaneous recovery is not expected and the best functional results are obtained by using the end-to-end suture of the stumps (**Figure 1**), the modern "gold standard" current method for neural reparation.[7,8,9,10] However, when there is a great loss of a natural neural segment, and the end-to-end technique cannot be applied, one may use either homologous or heterologous grafting for the correcting of gaps formed between the stumps. These insertions serve both as a causeway for neural growth as well as biological sustenance of axonal stumps, granting them chemical substratum which promotes growth while preventing their degeneration.[9,10,11] However, using these grafts implies the need for immunosuppression (heterologous grafts)[12] and natural

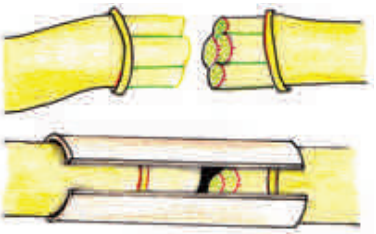

Figure 2. Example of tubing technique.

sequelae from the donor area[13] (homologous grafts). Acknowledging these facts, new neural reparation techniques emerged with the use of conduits such as the tubing technique (**Figure 2**).[14,15] At first, these conduits were made of homologous materials like fascia muscle,[16] however, with research advances, today it is possible to use synthetic conduits filled with specific neural growth factors,[17] which prolong neural nutrition, therefore, becoming true "supporting cells" and looking very much like Schwann cells (SC), which are responsible for success in peripheral nerve regeneration.[2,10,18,19,20]

As described before, the importance that Schwann cells have on peripheral nerve regeneration causes us to come across a great problem: the proliferation of endogenous SC, derived from the axonal segments, is insufficient to meet the necessary demand in the neural regeneration process, making it important to have an exogenous supplementation.[19,20,21] With the purpose of supplying this demand, several alternative SC sources have been studied, but stem cells are the most promising within all other promises.[22]

Stem Cells. Stem cells are cells that bear the capability of self-replication and differentiation. In other words, not only can they create other stem cells, but they can also differentiate themselves in any other kind of cell[23,24] (**Figure 3**). Unlike what most people may think, stem cells may be found in any human body tissue, however, some tissues such as hematopoietic and connective adipose possess a greater number of stem cells that are more specialized tissues such as nervous tissue.[2,25] Nowadays, three types of stem cells are described: a) **The Embryonic Stem Cells**, which are also called pluripotent cells, for they bear the capabil-

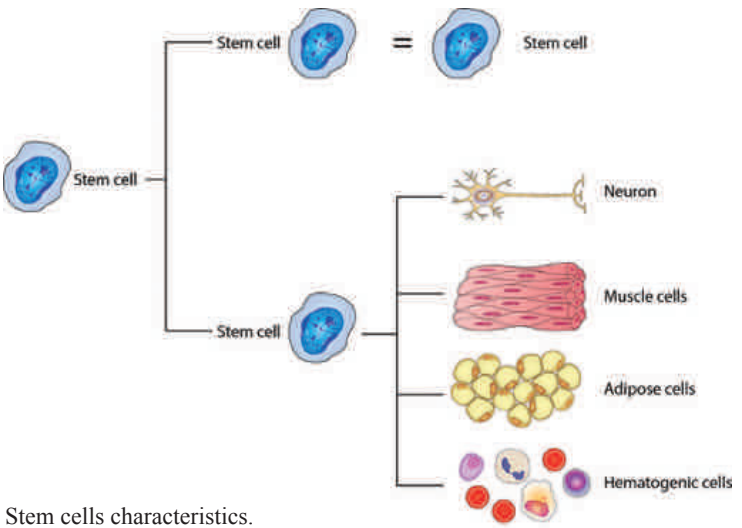

Figure 3. Stem cells characteristics.

ity of differentiation within any kind of adult cells; b) **The Adult Stem Cells**, also known as multipotent cells, for they possess a smaller capacity for differentiation ability than that of the Embryonic cell; lastly, c) **The Induced Pluripotent Stem Cells** (**Figure 4**), which are adult cells being reprogrammed by viral vector through their DNA modification.[25]

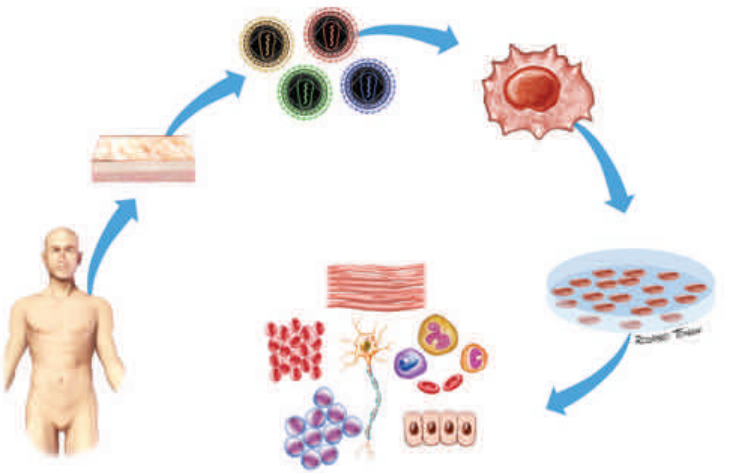

Figure 4. The figure shows how the reprogramming of skin cells is made through the insertion of viral vectors. A normal adult cell is infected with vírus carrying 4gens; oct-4, sox-2, klf-4 and c-Myc. These cells "return" to the stem cell condition, and then we are able to duplicate and differentiate them in other tissues.

Different sources of stem cells such as: capillary folic stem cells; stem cells derived from adipose tissue;[27] neural crest stem cells;[28] embryonic stem cells;[29] dental pulp stem cells[30] and many others have been widely used in neural regeneration research. Each and every one of these sources representing their own peculiar advantages and disadvantages. However, one of the most studied and researched sources of stem cells, are the stromal cells from bone marrow. These cells are known to be multipotent stem cells[31,32,33] and satisfy the requirements of an ideal cell transplantation such as: easy access, rapid in vitro expansion and low immunogenicity.[33,34] Furthermore, when well cultivated, the mesenchymal stem cells, better known as multipotent mesenchymal stromal cells[2] have the capability to differentiate themselves in several lineages, including ectodermal, and to express phenotypes of SC and glial cells, making them one of the most promising alternatives in the treatment of nerve system injuries.[32,35,36,37]

Stem Cells in neural regeneration. Ever since its first formal presentation in 1978,[38] the use of stem cells in the treatment of diseases has grown exponentially. It is important to note that with the emergence of imposing legislation on the use of stem cells; very few studies on human beings have been published in the last decade. However, over 4000 studies involving patients and the utilization of stem cells have been registered as clinical trials.[39]

In this exact moment, very few diseases can really be treated with stem cells. As examples, we can point out some hematopoietic diseases such as some types of leukemia and falciform anemia as well as the treatment of severe burns through the grafting of healthy skin extracted from the culture of stem cells derived from small pieces of healthy skin. However, the advance in research leads us to think that very soon, diseases once believed to be incurable such as type 1 diabetes[40], Parkinsons[41] and some types of blindness[42] can, and will, be cured.

In 2004, Mazzini *et al*.[43] implanted autologous mesenchymal stem cells in the spinal cords of seven patients diagnosed with lateral amyotrophic sclerosis. The results were positive: four patients presented intense improvement and two patients showed moderate improvement in muscular strength near the lower limbs.

Caylan *et al*. (2006)[44] published a case in which a 20-year-old woman presented a traumatic iatrogenic facial paralysis after a mastoidectomy for chronic medium otitis. First, the authors repaired the 8-10mm gap in the nerve (mastoid segment) with an autologous graft (greater auricular nerve) immediately after the lesion. After 42 days and no clinical and/or electroneurophysiological improvement (House-Brackmann [HB] VI), the patient was submitted to a new surgical procedure for the implantation of undifferentiated autologous mesenchymal stem cells, thus obtaining important results. After seven days, there was an improvement of two degrees in the HB scale (HB VI for HB IV) in addition to an electroneurophysiological improvement with the appearance of polyphasic potentials. After five months, the authors described the patient as having evolved to HB III.

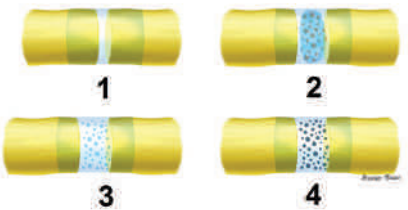

Figure 5. Groups studied by Salomone et al (2012)[2,31]. [1]Both parts of the nerve where connected by using an empty silicone tube. [2] The tube was filled with an acellular gel. [3] The space was filled with non-differentiated stem cells. [4] The tube was filled with already differentiated stem cells in Schwann cells.

Salomone *et al*. (2012)[2,31] evaluated the facial nerve regeneration on rats after neurotmesis and the implantation of undifferentiated stem cells or differentiated stem cells in SC (**Figure 5**). It was concluded that both types of stem cells in the research benefitted neural regeneration; however, the undifferentiated cells were more effective in neural regeneration than those differentiated in SC. These results diverged from most of the other published researches because of two reasons: first, because of the objective method used in functional evaluation[45] (electromyography). Second, because of the greater need that SC differentiated stem cells have for a biological substratum in order to survive.

Yan *et al.* (2004)[46] used neural stem cells in conduits composed by autologous fascia in order to repair a 6mm gap on rabbits facial nerves. Six weeks after the surgical procedure, they compared this group of rabbits to a group in which only the fascia was applied. The authors reported an improvement in the functional response, with significant difference in the latencies and in the axonal growth within the group which utilized stem cells.

Guo *et al.* (2006)[47] surveyed the regeneration of rabbits' facial nerves by using immunohistochemistry, electromyography and (quantitative) histology 12 weeks after the neural stem cells extracted from guinea pigs were implanted. They describe a decrease in latency, an increase in amplitude, an increase in quantity and in the diameter of axonal fibers significantly larger in the group that was treated with stem cells, when compared to the group treated only with physiological saline. They concluded that guinea pigs' neural stem cells promoted the regeneration of facial nerves in rabbits.

Zhang *et al*.(2008)[48] implanted stem cells previously extracted from the cerebral cortex of rats, and soaked in a hyaluronic solution and/or collagen inside collagen conduits for evaluating the regeneration of sectioned facial nerves in rabbits. The researchers examined the electrophysiological and histological results after 12 weeks, concluding that these stem cells facilitated the reinnervation of damaged axons. Furthermore, the researchers mention that there was no rejection, proving the low immunogenicity in this type of stem cell.

Satar *et al.* (2009)[49] studied the buccal branch regeneration on rats when in contact with gel foam soaked mesenchymal undifferentiated stem cells, after an immediate epineural end-to-end transection and suture. The authors evaluated the neural regeneration through qualitative histological study and concluded that the group treated with stem cells obtained better results, including an improvement in the axonal organization and in myelin thickness.

A number of other studies have demonstrated the potential benefits from the use of stem cells in neural regeneration, proving that, **YES**, stem cells are a real alternative in the treatment of neural lesions.[22]

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