BIOENGINEERING THE ARTIFICIAL NERVE

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SUNTO. – Attualmente, la riparazione di un nervo periferico a seguito di un danno traumatico, richiede una operazione chirurgicamente complessa e spesso risulta in un recupero non ottimale con conseguente deficit funzionale. La neurobiologia della rigenerazione del nervo non può essere risolta unicamente con le tecniche chirurgiche esistenti. La bioingegneria rappresenta una possibile soluzione a questo problema, con l'applicazione di condotti neuronali che potenzialmente possono migliorare la rigenerazione dei nervi. L'utilizzo di nuovi bio-materiali e di metodi di fabbricazione sempre più raffinati, usando micro- e nano-tecnologie, hanno portato ad un ulteriore perfezionamento dei condotti. Un successivo progresso nella rigenerazione del nervo è l'utilizzo di cellule trapiantate all'interno dei condotti, creando così un nervo bio-artificiale da innestare nel nervo danneggiato. Le cellule staminali adulte sono l'alternativa ideale alle cellule di Schwann, ed in particolare il tessuto adiposo è la fonte più promettente di cellule staminali per la medicina rigenerativa. Le cellule staminali, isolate facilmente da questo tessuto in donatori adulti, si moltiplicano rapidamente e possono essere pertanto differenziate in cellule di Schwann. Per questa ragione, queste cellule rappresentano una nuova strategia nell'applicazione clinica e per il miglioramento nella rigenerazione dei nervi periferici.

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ABSTRACT. – At present, peripheral nerve injury represents a complex challenge for the surgeons, and the repair of these injuries often results in sub-optimal recovery and functional deficit. The neurobiology of nerve regeneration cannot be adequately addressed by the existing surgical techniques, and it has become apparent that tissue engineering and the creation of nerve conduits have significant potential to improve the results of nerve reconstruction. The use of novel biomaterials and more refined fabrication methods, by using micro- and nano-technology, are a promising development for

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these experimental constructs. Also, the use of cells transplanted into the conduit to create a bioartificial nerve graft represents a new development. Adult stem cells constitute the ideal alternative to Schwann cells, and among the various sources of stem cells with potential application for regenerative medicine the adipose tissue has been proven to be the most promising. Adult adipose-derived stem cells are easily obtained, they expand rapidly and can be differentiated to Schwann cells, hence they represent a new strategy for clinical application in order to improve the results of nerve re generation.

1. Introduction

Injuries to peripheral nerves constitute a substantial clinical problem, although the peripheral nervous system possesses an intrinsic capacity to regenerate, which allows the nerve to regenerate spontaneously when there is nerve continuity after damage.[1] However, the functional recovery is often not satisfactory, leaving patients with diability and poor quality of life. This is exacerbated by the delay that may occur between injury and repair, which causes peripheral atrophy in the muscles lacking innervation, and neuronal cells death of those neurons that have lost contact with the periphery.[2]

Current surgical techniques for nerve repair consist in joining the proximal and distal stumps of the damaged nerve. When the nerve gap is nor repairable without creating tension, a nerve autograft is carried out, using a nerve from another area of the body, typically the sural nerve in the leg. The nerve autograft contains all the elements that are required to promote nerve regeneration, but often this approach is not ideal in term of size match for the recipient nerve. Also, autografting involves donor site morbidity such as scarring, sensory loss and formation of neuroma. An alternative solution would be the use of a bioenegineered nerve graft. This approach consists in the creation of a construct composed of a nerve guide, or conduit,[3] enriched with the elements normally presents in a nerve autografts which can improve the outcome of regeneration, such as transplanted cells, extracellular matrix proteins and growth factors.

2. Nerve conduits

A simple biologically inert tube in which the nerve stumps are inserted to bridge the gap would help in directing the regenerating

axons, concentrate the regenerative factors and block scar formation to the nerve. A range of bio-compatible materials have been tested experimentally as empty tubes with various degrees of success.[4,5,6] A direct comparison between different studies is difficult due to the variability in animal models, nerve gap size, surgical technique and outcome measures. However, all have indicated that the entubulation of the nerve is a feasable approach for the repair of a gap injury. Some materials have also been used clinically, although their use is limited to specific applications and not always they produce good results.[7]

More recent studies have developed modification of the internal structure of the conduit, for example adding internal fibres or porous structures to enhance directional axonal growth,[8,9,10,11] but further work will be needed before full clinical application will be possible.

An alternative way to making the conduits bioactive is the incroporation of elements that partecipate actively to the regeneration process, such as the targeted delivery of exogenous neurotrophic factors,[12] as they have a great impact upon the initiation of the regeneration process. Despite experimental evidence that isolated factors improve regeneration following their incorporation into nerve constructs, they are not effective for a clinical application since each neuronal sub-population exhibits specificity for individual neurotrophic factor.[13] Hence a cocktail of growth factors would be necessary for optimal effect, but this mix would need to be changed for each type of injured nerve.

Cell transplanted within a conduit rapresent a further alternative. Schwann cells are the obvious choice given their active role in peripheral nerve injury and regeneration,[14] and several experiments have demonostrated the improvement obtained by adding these cells to a conduit.

3. Transplanted cells

Autologous Schwann cells are the favourable choice for seeding in a nerve conduit, as they are normally present in the autologous nerve graft, and they drive the regeneration process by secreting growth factors and producing extracellular matrix proteins. They also form structures called bands of Büngner, which are essential to guide the regenerating axons towards the target organs.[15] Autologous Schwann cells,

both human and animal [16,17] have been successfully cultured and have been shown experimentally to enhance regeneration across nerve gaps.[18] However, the practicalities for their use are not ideal, as it takes several weeks to culture sufficient autologous cells to fill even a short conduits bridging a nerve gap, and this delay would lead to increase neuronal death and nerve atrophy.[2]

Allogeneic Schwann cells could be cultured and stored for rapid use of nerve injury repair, but they are rejected too quickly to give a significant improvement to the regeneration process,[19] unless immunosuppression is carried out. Immunosuppression is not detrimental to nerve regeneration [20] and it has been tested clinically.[21] However, ethical issue relating to transplantation and, more importantly, the potential of infection to the patient have precluded a widespread use of this approach.

For all these reasons, the research has moved towards the use of alternative cells, in particular stem cells, which presents all the advantages of an ideal candidate cell for regenerative medicine.

4. Stem cells

Stem cells are undifferentiated precursors that can divide into cells with identical potential, in a process called self-renewal. Also they can differentiate to a variety of cell lineages.[22] Embryonic stem cells are defined as pluripotent, as they can generate all the three germ layers of the embryo, *i.e.* ectoderm, mesoderm and endoderm.[22] Although they have shown great potential for regenerative medicine, embryonic stem cells present moral and ethical issues, the risk of solid tumour formation and possible differentiation into undesirable cells.[22,23] Hence their clinical use has been somehow limited and controversial.

Most adult organs contain small populations of adult stem cells, originally considered unipotent as generating only one type of differentiated cells. Their role is primarily to repair and replace the functional cells of the tissue to which they belong in case of damage or injury.[24] New findings on cell plasticity have shown that adult stem cells can trans-differentiate into cells of a different lineage under specific stimulation.[24,25] Adult stem cells are distributed throughout the body, including bone marrow, fat, skeletal muscle, liver and skin.[25] Bone marrow derived stem cells (MSC) were the first cell population to be

characterised and used experimentally and clinically, as they can differentiate *in vitro* into mesenchymal cells leading to the formation of bone, cartilage and fat.[25,26] Under specific stimulation, MSC can also differentiate into Schwann cell-like cells, with similar molecular markers, morphology and secretion of growth factors.[27,28] Experimentally, MSC have also been shown to promote nerve regeneration after injury.[24,29]

The harvest of MSC from bone marrow is a highly invasive procedure, with pain at the harvest site and with limited volume of harveste fluid and cells.[30] However, humans have abundant subcutaneous fat deposits containing stem cells, defined as adipose-derive stem cells, which can be easily isolated by conventional liposuction procedures avoiding the problems related to the harvest of MSC.[31]

5. Adipose-derived stem cells

Adipose-derived stem cells (ASC) present similar characteristic to MSC, such as self-renewal capacity and ability to differentiate into multiple lineage.[32] ASC have the advantage to be more numerous in the processed lipoaspirate compared to the MSC in the bone marrow,[33] and given the amount of fat that can be harvested subcutaneously ASC represent a more attractive source of stem cell for regenerative medicine.

Different groups have also demonostrated that ASC can be differentiated *in vitro* into Schwann cell-like cells, similarly to MSC.[31,34] Interestingly, depending on the anatomical site of harvest, ASC have shown different properties in culture models. Human ASC obtained from superficial fat deposits show higher yields, faster proliferation and increased promotion of neurite outgrowth when compared to ASC obtained from deeper layer.[35] Also, the harvest site and donor age influence the growth rate, differentiation and neurotrophic factor secretion of mouse and rat ASC.[36,37,38] These studies indicate that more has to be done in order to characterise and standardise ASC for a possible clinical use.

Experimental studies have also confirmed the ability of differentiated ASC to promote nerve regeneration, when the cell are seeded into fibrin conduit bridging a short gap in the rat sciatic nerve.[39, 40] Good regeneration results were also obtained in experiment using poly-

mer conduits [41] seeded with differentiated ASC, showing increased function index and prevention of neuronal cell death respectively.

6. Conclusions

There are increase evidence that ASC may represent a potential tool for the treatment of nerve injury. In a clinical scenario, fat could be harvested from the patients suffering from nerve injury to extract and culture ASC. These can then be differentiated to Schwann cell-like before seeding them in a nerve conduit, to be grafted at the site of injury creating an "artificial nerve" substituting the presently used nerve autograft.

Considerable technical progress has been made and good experimental results have defined the elements required for nerve repair and regeneration. However, many factors are still to be clarified before this can be achieved, such as shortening the presently time of culture and differentiation of the ASC, or alternatively finding a way to differentiate these cells within the conduit after being seeded as undifferentiated stem cells. Emerging technologies may also have a further impact on the design of the nerve conduit, including internal nanatotechnology design to modulate cell behaviour or even differentiation. Although clinical translation of nerve conduit has already began using inert constructs, the results are still below optimal and it will be only when a bioactive conduit will be available to obtain the best nerve regeneration results.

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