ImmunoTools *special* Award 2019

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Human adipose stem cells in peripheral nerve repair: new future perspectives in cell therapy

Background

Peripheral nerve injuries affect several hundred thousand people in Europe every year (1:1000 of population), most often arises secondary to trauma or oncological resection and can be functionally devastating. Nerve injury represents a significant personal and socioeconomic cost often involving young, working age adults. Currently the best practice for surgical repair consists of a tension free direct anastomosis or, in case of a gap, to avoid tension between the stumps, an autologous nerve graft is usually harvested. In these cases, the sural nerve is commonly used as a donor nerve. Unfortunately, a full recovery may never be achieved, particularly with extended lesion and donor site morbidity, such as scar, sensory deficit and potential neuroma formation are recognised complications. In order to minimize donor site morbidity, alternatives to autologous nerve grafts have been tested: firstly, the allografts, but they require an undesirable long-term immunosuppressive therapy. Secondly, several natural and synthetic nerve conduits have been developed and are available, but the outcomes following their use fail to match those of autologous nerve graft.

Cell therapy for peripheral nerve injury

Previous experimental studies in rats have shown that cell transplantation with in a bioartificial conduit is an alternative strategy to create a favourable environment for nerve regeneration.

Schwann cells (SC), providing myelinated nerve fibres and releasing growth factors (GF) in the peripheral nervous system, offer an attractive substrate for axonal migration and further promotion of nerve regeneration.

Despite showing regeneration enhancement, SC have limited clinical applications due to culture times and costs to achieve optimal conditions for transplantation in nerve conduits. Moreover, SC are not easily accessible without nerve biopsy, thus sacrifice of an autologous nerve.

Instead, the adipose tissue has been proposed as an ideal transplantable source of stem cells regarding to its easily accessible (conventional liposuction procedure under local anaesthesia), rapid proliferation in culture and successful integration into host tissue with immunological tolerance.

Regarding neural differentiation, several studies proved trans-differentiation of bone, cartilage and fat into a SC-like cell. A SC-like differentiation of human adipose derived stem cells (hADSC) may have important clinical implications in the field of peripheral nerve regeneration and tissue engineering.

The development of an innovative scaffold in nerve repair

Based on our previous promising results, we are working on combining hADSC cell therapy with topographical cues incorporated into a cutting edge, biocompatible and biodegradable designed nerve conduit.

According to success applications of our partner group in terms of bone defects restoration, we are introducing for the first time in nerve repair the plasma-based chemical treatment with poly-ethyl-acrylate (pPEA) coated with fibronectin to manipulate a grooved poly-caprolactone (PCL) membrane. This approach supports to maintained exposed the growth factors on the interior surface of the scaffold, minimizing the growth factors dosage (and related side effects) and promote regeneration.

Aim

We strongly believe that the combination of these elements could enhance the hADSC neurotrophic effect, their life-long and thus the potential nerve regeneration.

Our next step will be try differentiating the hADSC seeded on the scaffold described above, combining a specific cocktail of specific GFs.

Methods

i) Evaluating stem cells immunophenotype hADSC by flow cytometry (CD45, CD73, CD90, CD105)

ii) Verify the SC-like differentiation protocol to the hADSC on the new scaffold after 14 days:

- Applying the following GFs: BDNF, NGF, GDNF, PDGF-BB, Neuregulin-1b, bFGF

iii) Evaluating Schwann cell specific marker after 14 days:

- protein expression (S100, GFAP and p75),
- protein secretion (Elisa kits for BDNF, NGF and VEGF-A)
- gene expression (p75, GFAP, S100, BDNF, NGF and GDNF) of

Control the inflammation response to promote nerve regeneration

In the context of PNS only a few studies focus on the different roles of the macrophage subtypes at the injury site. We believe that understanding their role and contribution to PNS repair, it will be an interesting starting point.

Aim

We will investigate, in a parallel branch of our research, how modulating the M1/M2 ratio in the nerve injury site, with a drug delivery method, we would affect SC recruitment, axonal growth and scar/fibrosis formation.

Methods

i) Evaluating specific markers of M1 and M2 by:

- flow cytometry (CD86)
- protein secretion (ELISA Kit for IL-10 and TNF-alpha)

ii) Stimulating the differentiation towards M1 or M2 by specific cytokines:

- (INF gamma, IL-1, IL-4, IL-10, IL-13)

ImmunoTools *special* AWARD for **Dr. Martino Guiotto MD** includes 25 reagents

Anti-human antibodies for flow cytometry (FITC or PE):
CD45
CD73
CD86 (anti rat if it is possible)
CD90
CD105

Recombinant human cytokines:
BDNF
NGF beta
FGF-b
FGF-2
Neuregulin-1-b
PDGF-BB

Recombinant rat cytokines (or eventually human if not available):
INF gamma
IL-1
IL-4
IL-10
IL-13

Rat Elisa set (or or eventually human if not available):
IL-10
TNF alpha

DETAILS more AWARDS