IDEAPUZZLE
DESIGN AND DEFEND YOUR PHD

Combined use of olfactory mucosa mesenchymal stem cells and biomaterials in regenerative therapies after peripheral nerve injury

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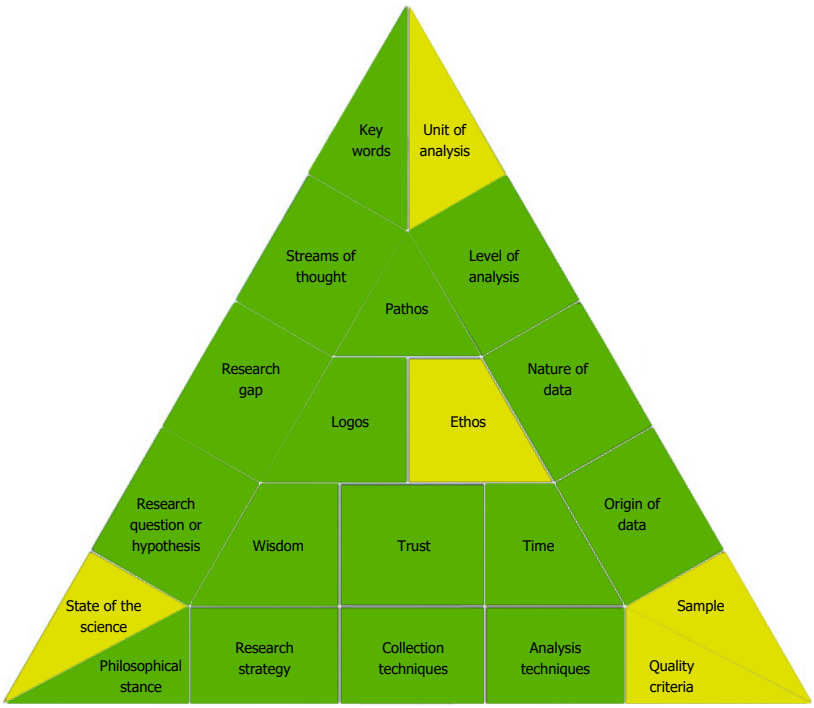
Self-evaluation: 88%



Key words

1/21. Theoretical question: which are the two main key words of your research?

The main objective of my project is to establish the therapeutic effects of the use of Olfactory Mucosa Mesenchymal Stem Cells (OM-MSC´s) in the treatment of peripheral nerves after injury. In this way, the two main keywords of my project are:
1. Olfactory Mucosa Mesenchymal Stem Cells - OM-MSCs are members of the superfamily of the mesenchymal Stem cells (MSCs), cells derived from neural crest that can be found in the olfactory mucosa (Veron et al., 2018). They are multipotent cells, and therefore a potential source of stem cells to treat various types of tissue lesions as they propagate rapidly without risks of tumorigenicity (Delorme et al., 2009).
2. Peripheral Nerve Injury - Peripheral nerve injuries (PNI) are a common occurrences associated with severe physiological and occupational changes in the individual who suffered the lesions (Wojtkiewicz et al., 2015). These injuries can originate in several traumatic or iatrogenic phenomena (Antoniadis et al., 2014). The consequent motor, sensory and autonomic changes lead to substantial functional deficits in the denervated body segments (Navarro et al., 2016).

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Journal of Neuroscience.
2016;43(3):271-286.

Self-evaluation: 100%

Streams of thought

2/21. Theoretical question: which are the two main streams of thought of your literature review?

The two streams of thought of my research project are the promotion of peripheral nerve regeneration after injury and the use of mesenchymal stem cells as potential therapeutic sources for the repair and regeneration of various tissues, namely peripheral nervous tissue.

The peripheral nervous system (PNS) presents a better reparative and regenerative capacity than the central nervous system (CNS), and this difference is based essentially on the characteristics of the functional environments in each one of the systems (Lutz & Barres, 2014), the age of the injured individual, the type of injury observed and the integrity of the neural cell body of the injured nerve (Faroni, Mobasseri, Kingham, & Reid, 2015). Nevertheless, ineffective functional recovery is common in the injured peripheral nerve, particularly due to phenomena of chronic axotomy, chronic Schwann cell denervation (Sulaiman & Gordon, 2013) or severe disruption of endoneurial tubes that prevent normal progression of the regenerative process (Burnett & Zager, 2004). To date, several therapies with different levels of efficacy have been weighed and tested, from conservative and pharmacological methods to the use of cell therapies, growth factors, genetic therapies and surgical interventions. Despite this, the ideal and fully effective method for promoting peripheral nerve regeneration after injury has not yet been established.

Cell-based therapies have been proposed as a promising alternative to treat a variety of neurologic injuries and the use of stem cells that can differentiate into appropriate cell types in the affected area has developed rapidly in the last years (Casañas et al., 2014). Stem cells are undifferentiated cells capable to proliferate and produce both new stem cells and different types of cells and tissues (Kobolak et al., 2016). More specifcally, MSCs are multipotent, heterogenic stromal cells derived from the mesoderm (Caceres et al., 2016) and were initially characterized as presenting adherence to plastic culture dishes, fibroblast-like shape and a unique ability to differentiate into multi-lineage MSCs, phenotypes and specialized tissues (Dominici et al., 2006). MSCs are able to differentiate not only into osteoblasts, adipocytes and chondrocytes but also in other cells and tissues with mesodermal origin (ligaments and tendons, cardiomyocytes, muscle) and also ectodermal and endodermal origins (skin, retinal epithelial pigment, lungs, hepatocytes, renal tubules, pancreatic islets, hair follicle, sebaceous gland ducts and neural cells) (Kobolak et al., 2016). MSCs can be obtained from a vast array of tissues that include adipose tissue, lungs, bone marrow, umbilical cord (Wharton's jelly and umbilical cord blood), synovium, amniotic fuid, fetal blood, dental pulp, skeletal muscle, circulatory system (Caceres et al., 2016) and olfactory mucosa (Ge et al., 2016). Applied to regenerative medicine, MSCs present exceptional features that make them great options, such as easy expansion, differentiation into different cell types, immune-privileges and immune modulation, tropism to injured sites, trophic stimulation and modulation of tissues functions (Scatena et al., 2017). In addition to being able to secrete neurotrophic factors and provide an environment conducive to neurogenesis and proliferation of Schwann cells in nerve injury sites, they can themselves differentiate into cells with Schwann cell phenotype and modulate the local inflammatory process and the Wallerian degeneration (Kingham et al., 2007), being a precious addition to the use of biomaterials and growth factors in therapeutic techniques after PNI.

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neurite outgrowth in vitro. Experimental Neurology. 2007;207(2):267-274.

Self-evaluation: 100%

Research gap

3/21. Theoretical question: which is the main gap that your research addresses?

MSCs have been widely applied in the regeneration of several tissues, with greater or lesser effectiveness (Robey 2017), namely in the regeneration of the peripheral nerve after injury (Gärtner et al., 2012). OM-MSCs, having been identified more recently, are also considered a promise in regenerative medicine due to their characteristics that make them great candidates to be applied in damaged tissues: high versatility, vast distribution, advantageous localization, absence of susceptibility to chromosomal abnormalities or tumorigenicity (Shafee et al., 2011), high mitotic activity, self-renew in long-term cultures and lack of apoptotic activity (Marshall et al., 2005). The olfactory mucosa itself is a great cell source since its renewal continues throughout life and OM-MSC potency is not even affected by the age of the donor (Huang et al., 2015). These cells have already been used, and their therapeutic effect confirmed, in the treatment of neurodegenerative diseases of the central nervous system (Murrell et al., 2008), hippocampal lesions, (Nivet et al., 2011) in the regeneration of cranial nerves (Pandit et al., 2011; Bas et al., 2013) and in cases of spinal cord trauma (Xiao et al., 2006). Its immunosuppressive effect in autoimmune diseases (Rui et al., 2016) and regenerative promotion in myocardial tissue after infarct (McDonald et al., 2010) were also evaluated. So far, the effect of OM-MSCs and peripheral nerve regeneration after injury has only been tested in one study (Roche et al., 2017).

Given the evidence that currently available treatments for peripheral nerve injury are still far from being effective and guarantee good outcomes in treated individuals, it is essential to seek and explore alternatives. OM-MSCs are not yet fully characterized, and new works and clinical trials are needed to determine their efficacy in different types of nerve damage, either alone or in combination with specific biomaterials. This is the main gap that my research project intends to fill.

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Self-evaluation: 100%

Research question or hypothesis

4/21. Theoretical question: which is the main question or hypothesis of your research?

The question that my research project intends to answer is: could the combined use of MSCs from the olfactory mucosa and biomaterials be an effective therapy in the promotion of peripheral nerve regeneration after injury?

Self-evaluation: 100%

State of the science

5/21. Theoretical question: which is the current answer to your research question or hypothesis?

The results obtained in previous studies concerning the treatment of peripheral nerve lesions after injury and the therapeutic use of OM-MSCs are still not completely conclusive. PNI continue to be an important clinical concern and most of the available treatments are associated with chronic disabilities and significant health care expenditures (Kubiak et al., 2018). Despite all the advances made to date, it is still necessary to explore new alternatives and to validate the treatments already available to augment regeneration and improve functional outcomes after injury. OM-MSCs have already been used in some studies where it has been demonstrated its efficacy in the regeneration of nervous tissue (Xiao et al., 2007), its anti-inflammatory effect (Rui et al., 2016) and its therapeutic effect in other tissues (McDonald et al., 2010). In the same way, they have already been used effectively in the regeneration of the peripheral nerve in neurotmesis lesions in combination with a specific biomaterial (Roche et al., 2017). These data, however, are still limiting and do not allow general and unequivocal conclusions.

In summary, the answer to the question raised by my research project is still unclear: it is possible to stimulate the regeneration of the

peripheral nerve with different methods, among them the use of MSCs, and OM-MSCs also have a beneficial effect on their reparation. Nevertheless, it is necessary to explore new combinations of OM-MSCs with different biomaterials and to validate their efficacy and also test their efficiency in other types of lesions than neurotmesis.

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Self-evaluation: 50%

Philosophical stance

6/21. Methodological question: which is the philosophical stance of your research?

The philosophical stance of my research project is quantitative objectivism. In this work we are testing the theory that OM-MSCs are capable of promoting the regeneration of the peripheral nerve in different lesions and in association with different biomaterials, based on the fact that these cells have already demonstrated regenerative efficacy in other tissues and with other biomaterials in previous works. Additionally, the work may help to formulate the theory that MSCs collected from niches with neurological interaction, such as the olfactory mucosa, may have more tendency and efficacy in the regeneration of nervous tissue.

Self-evaluation: 100%

Research strategy

7/21. Methodological question: which is the qualitative, quantitative or mixed method of your research?

The research strategy of my project is based on a quantitative and longitudinal research study (Pope et al., 2017).
The effects of the therapeutic application of OM-MSCs will not only be determined but will also be quantified in the individuals intervened by functional, histomorphometric and histopathological methods, justifying the quantitative nature of the study (Tavakol et al., 2014). On the other hand, and since different individuals will be tested several times throughout the study in order to determine the temporal variations of the effect of the established cellular therapeutics, the study also has a longitudinal character (Caruana et al., 2015).

References:
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Self-evaluation: 100%

Collection techniques

8/21. Methodological question: which are the data collection techniques of your research?

All data are collected through standardized procedures performed by veterinarians and other health professionals, trained in handling of laboratory animals and with proven experience in performing the necessary procedures. The performance of functional tests aims at postural and kinematic assessment as well as the determination of sensory and motor function at the level of the members submitted to PNI (de Medinaceli et al., 1982; Varejão et al., 2004). The histomorphometric evaluation of the peripheral nerve aims to evaluate the cross-sectional area of the nerve, the total fiber number, fiber density, fiber diameter, axon diameter, myelin thickness and axon/fiber ratio (Bozkurt et al., 2012). The histopathological evaluation aims at determining signs of inflammation, infection or adhesions at the site of peripheral nerve intervention and/or biomaterial application (Raimondo et al., 2009).

References:
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Self-evaluation: 100%

Analysis techniques

9/21. Methodological question: which are the data analysis techniques of your research?

The data analysis technique will be based on a multivariate statistical analysis, in which the different variables under study (variables of the functional analysis, histomorphometric and histopathological evaluation) will be analyzed to determine the effects of the use of OM-MSCs with and without biomaterials on peripheral nerve regeneration.
The software used to perform statistical analysis will be SPSS version 25 (IBM® SPSS® Statistics) and GraphPad Prism 6 (GraphPad Software Inc).

Self-evaluation: 100%

Quality criteria

10/21. Methodological question: which are the tactics of your research to ensure scientific quality criteria?

My research project is based on previously identified and characterized facts, so the adopted criteria are objectivist (Solli et al., 2018).
The population used will be a small sample of the population under study, with the theory to be developed using descriptive statistics. In this way, external validation will be performed by convergent validation.
The internal validity is considered through the predicted causal relationship between the therapeutic use of OM-MSCs and the regeneration of the peripheral nerve after injury. For this, it is considered that the three conditions of internal validation by causal relation are present: 1) variations in the cells used or the type of nerve injury will vary the desired therapeutic efficacy; 2) expected regeneration will not be observed without the use of OM-MSCs; 3) No relevant influence of external factors on the therapeutic efficacy of OM-MSCs on the injured nerve is expected.
The research project does not involve, in its current phase, human patients in the therapeutic application of OM-MSCs. As such, construct validity is not considered.
Once again, as my research project is based on previously identified and characterized facts, the convergent validity will be based on the triangulation between the data collection technique, the data source and the stream of thoughs.
Reliability is guaranteed through the nature of measurements and tests performed. Both functional tests and histomorphometric and histopathological evaluations will always be performed by the same operator (or by a restricted group of operators) in order to ensure that there is no interoperator influence on data collection and interpretation of results.

References:
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Self-evaluation: 50%

Unit of analysis

11/21. Empirical question: which is the unit of analysis of your research?

The analysis units under study, in order to infer the effects of therapeutic use of OM-MSCs on peripheral nerve regeneration after PNI, are sciatic nerves of rats (Rattus norvegicus) and sheep (Ovis aries) submitted to surgical injury. In the functional evaluation, each individual is submitted to tests at different times between the induction of the lesion and the end of the study (12 or 20 weeks, depending on the type of lesion induced). The histomorphometric and histopathological evaluation are performed only after the end of the functional evaluation phase. Thus, not only the global regenerative effect at the end of the study period but also the trajectory over the time for functional evaluation are evaluated.

Self-evaluation: 50%

Level of analysis

12/21. Empirical question: which is the level of analysis of your research?

Since each animal under study will be evaluated for its functional capacity as well as for the level of histomorphometric organization of the nerves and the organic histopathological changes, the level of analysis of the units of analysis is individual.

Self-evaluation: 100%

Nature of data

13/21. Empirical question: which is the nature of the data of your research?

Quantitative data collected during the functional assessment include animal weight, limb retraction velocity during sensory evaluation, and disposition of the digits on the support surface during gait. During the histomorphometric evaluation, the following parameters are determined: the cross-sectional area of the nerve, the total fiber number, fiber density, fiber diameter, axon diameter, myelin thickness and axon/fiber ratio. During the histopathological evaluation, the degree of inflammatory reaction and fibrous adherences at the level of the intervened nerve and the inflammatory reaction at the systemic level will be evaluated parameters.

Self-evaluation: 100%

Origin of data

14/21. Empirical question: which is the origin of the data of your research?

All data used in my study will be collected by the researchers and operators involved in the same, and are not recycled from previous studies. As such, as to their origin, the data are primary (Velentgas et al., 2013).

References:
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Self-evaluation: 100%

Sample

15/21. Empirical question: which is the sample of your research?

The samples under study are the peripheral nerves of Rats and Sheeps submitted to PNI. Since several samples are used in the study (several animals) but few variables (use or not to use OM-MSCs, different types of PNI, use or not to use associated biomaterial), the sample is considered descriptive, thus allowing a descriptive generalization and a logic of replication and extension. The number of samples used was established according to the principle of theoretical saturation, that is, the maximum number of animals and samples above which the study does not benefit in terms of the information obtained was determined.

Self-evaluation: 50%

Pathos

16/21. Rhetorical question: which are the positive and negative emotions of your research?

PNI is a common phenomenon and transversal to human and veterinary medicine. The causes of these occurrences are multiple, and may be traumatic or result from human interventions. Being able to manifest themselves with different degrees of severity, these nervous lesions are, in the majority, incapacitating from the physiological and labor point of view, and the treatments applied today are still far from being totally successful in their resolution. Finding new and more effective therapies to promote peripheral nerve regeneration after injury is essential. Of all the new and potential therapies to be explored, the use of MSCs in all their strands are those that have suffered the most advances in recent years. Similarly, OM-MSCs, due to their location, biological characteristics and evidence of effective potentiation for peripheral nerve regeneration, are also a essential line of research that will allow advances in regenerative medicine of the injured nerve. The results and evidences observed at the end of the study may allow great advances in understanding the therapeutic effects of these cells in the resolution of peripheral nerve lesions and contribute to the establishment of regenerative medicine as an important branch of modern medicine.

My project may raise some questions from the ethical point of view, due to the use of animal models in the induction of nerve lesions. Nevertheless, all the project and the procedures are approved by the ethics committees of all the institutions involved and by the General Direction of Veterinary in Portugal. Likewise, all operators involved in animal manipulation are Veterinarians and health professionals with a thorough knowledge of the principles of the procedures to be carried out, specific training in the use of laboratory animals for scientific studies and all guide-lines have been stablished from to avoid any unnecessary manipulation, intervention or suffering.

Self-evaluation: 100%

Logos

17/21. Rhetorical question: which is the scientific logic of your research?

The logic adopted in this project is hypothetic-deductive, since it will test the therapeutic efficacy of the use of OM-MSCs, a group of cells belonging to the great type of cells called MSCs that have been previously studied and have shown a pro-regenerative capacity, in order to attest its efficacy in the regeneration of the peripheral nerve after induced injury. The predisposition of the peripheral nerve to improve its levels of regeneration and repair when associated with cell-based therapies is also known.

Self-evaluation: 100%

Ethos

18/21. Rhetorical question: which are the limitations of your research?

Theoretical limitations - Although there are several strong evidence of the effectiveness of cell therapies and MSCs in regeneration and tissue repair after injury, a fully effective regeneration of the peripheral nerve has not yet been achieved through this therapeutic pathway. Likewise, even though OM-MSCs have already been extensively studied, there are still several components of their molecular biology that are not yet fully understood. Thus, the present project may or may not fill some of these lack of information.

Methodological Limitations - Lesions induced in the peripheral nerve at laboratory level are controlled, clean and standardized. Although these characteristics facilitate the monitoring of the regenerative phenomenon and the characterization of the therapeutic effect of the cells used, it does not completely mimic a real lesion that usually arises associated with other components such as wounds involving soft tissues,

haemorrhages or concomitant fractures. Likewise, when using animal models such as the rat and the sheep, neither the conditions nor the physiology of man are completely mimicked, and it is necessary at a later stage to carry out clinical trials in human patients to infer the therapeutic capacity of these cells in the human species.

Empirical limitations - The main empirical limitations are related to monetary restraints, short periods of time, structural restrictions and ethical issues that prevent the use of a superior number of animals in the therapeutic trials.

Self-evaluation: 50%

Wisdom

19/21. Authorial question: which is your education and experience related with your research?

I am a Master in Veterinary Medicine graduated from the University of Trás-os-Montes and Alto Douro, Vila Real Portugal, in 2015. Between 2015 and 2017 I worked in a Veterinary Hospital where I practiced clinical and surgical procedures in small animals, particularly in emergency periods and critical care régimen. This clinical experience allowed me to acquire many knowledge of medicine and veterinary surgery, giving me the tools to participate in all the medical and surgical procedures necessary for the progression of the project. The experience in an emergency environment also allowed me to perceive the alarming frequency of PNI and their harmful consequences in small animals, which mimic the occurrence and consequences in other species and in man.

In 2017 I became a fellow of the Foundation for Science and Technology in Portugal, winning a PhD scholarship. Simultaneously I started the PhD in Veterinary Sciences at the Abel Salazar Institute of Biomedical Sciences, University of Porto, Portugal. I was integrated in a multi-thematic team, consisting of health professionals of various origins (namely Veterinary Doctors, Human Medicine Physicians, Pathologists and Pharmacists) with extensive experience in Regenerative Medicine, cell therapies and biomaterials. In this way I am accompanied directly by experienced people who can guide, teach and advise me on the different methods of approach to each phase of my project. To improve my knowledge in the species to be used as animal models, I attended a laboratory animal science course in order to be credited with the category Felasa C. In this way, I not only acquired extensive knowledge about legislation, ethics, animal welfare, general and reproductive biology and physiology, health and its maintenance, recognition of signs of pain, suffering and acute and chronic stress, methods of euthanasia and human endpoits and minimally invasive procedures as well as obtained authorization from the General Direction of Agriculture and Veterinary of Portugal to carry out procedures on laboratory animals.

The daily laboratory work and participation in national and international congresses have allowed me to gain the experience and the willingness to progress with my project, both in the practical and laboratory components as well as in the writing of scientific literature and interpretation of the obtained data and observed results.

Self-evaluation: 100%

Trust

20/21. Authorial question: who are the partners of your research?

At the moment I am integrated in the Laboratory of Veterinary Cell Therapies of the Department of Veterinary Clinics of the Institute of Biomedical Sciences Abel Salazar of the University of Porto. At the same time I am affiliated to the Center for Animal Science Studies (CECA / ICETA-Porto / ICETA) of the University of Porto.

My Supervisor has a PhD in Veterinary Medicine and is the director of the PhD program in Veterinary Sciences of my Institution, professor of several curricular units of the Integrated Master in Veterinary Medicine and has extensive pedagogical and research experience in clinical and veterinary surgery, in Regenerative Medicine and Biomaterials. Both my co-supervisors are PhDs and have long experience in regenerative medicine, with special emphasis on peripheral nerve regeneration.

The whole research group where I am integrated consists of multi-thematic health professionals with different backgrounds, including Veterinarians, Pharmacists, Pathologists, Biologists, Biochemists and Material and Biomedical Engineers. The internal and external to the institution cooperations allows me to maximize the resources available for the progression of my project and to receive a guided follow-up by experienced professionals in their respective areas of knowledge.

With all the people who accompany me, I can enjoy all the supervision, accompaniment and knowledge that will allow me to finish my project according to the guidelines established or with small changes resulting from the advice and suggestions from these experienced professionals.

Self-evaluation: 100%

Time

21/21. Authorial question: which is your availability of time and resources for your research?

I am a fellow of the Foundation for Science and Technology of Portugal. My PhD scholarship encompasses an exclusivity clause, so I have at present 100% dedication to the realization of my research project. The facilities and resources provided by the host institutions where I am integrated are sufficient to ensure the overwhelming majority of the procedures and stages of my project, and those which are not covered can be completed elsewhere through internal and external cooperation with other institutions or research groups and whose cooperation with our

group has already happened in the past.

Self-evaluation: 100%