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Mesenchymal stem cells from the Wharton's jelly of the umbilical cord (UC-MSCs) and from dental pulp (DPSCs) used for neuro-muscular regeneration – A longitudinal study from in vitro characterization to animal experimentation

Recent advances in Tissue Engineering considering the peripheral nerve system have greatly promoted the generation of nerve conduits, which may be implanted filled with growth factors and/or cellular systems. Mesenchymal stem cells (MSCs) comprise a rare population of multipotent progenitors with a great therapeutic potential, support hematopoiesis and enhance the engraftment after co-transplantation. Currently, bone marrow (BM) represents the main source of MSCs. However the number of BM-MSCs significantly decreases with age and the HLA compatible donors are very difficult to find. The MSCs obtained from the umbilical cord tissue (UCT) are a promising alternative: i) the number of cells per volume is higher, ii) because of the low expression of HLA-ABC antigens and the absence of HLA-DR expression, a complete or high HLA profile match for allogenic use is not necessary, which permits to greatly enlarge the number of available donors, and the use in xenografts iii) are easier to obtain, cryopreserve, and the collection is ethically approved by national and international laws, iv) the number of high quality samples cryopreserved is increasing in public and private cord blood banks in Portugal and Europe. More recently, also the MSCs isolated from the dental pulp have been intensively studied. Our multidisciplinary team has a crucial role in the development of new biomaterials, cells therapies, and in pre-clinical trials considering appropriate animal models and welfare where the main objective of the Regenerative Medicine research sub-unit from CECA-ICETA is to evaluate the therapeutic effect (by morphological and functional analysis) of biomaterial nerve conduits used as a scaffolds for UC MSCs and DPSCs, on neuro-muscular regeneration after axonotmesis and neurotmesis injuries. It is a transversal and integrated study considering the development and characterization of new biomaterials, the isolation and expansion of MSCs under GMP conditions and pre-clinical studies [1-5]. The in vivo trials include 2 animal models, the rat for initial validation of the scaffold and the ovine which allows studying critical defects. The therapeutic effect of UC-MSCs and DPSCs does not simply reside on their capacity to replace the original cells of damaged tissues, but also by secreting growth factors and cytokines that modify the microenvironment and induce activity of endogenous progenitor cells and by modulation of the inflammatory process. Due to this fact, the UC-MSCs and DPSCs secretome and metabolic profile and the therapeutic effect of conditioned medium (CM) has been studied in detail [1-5]. The team has a very strong experience working with extra-fetal stem cells and 2 elements (ACM, JDS) are founders shareholders of a private cord blood bank (www.criovida.pt; www.biosckin.com) approved by Direcção Geral de Saúde (DGS). Also several international publications in the field and close collaborations with some of the strongest groups working in Regenerative Medicine are the outputs for the past 10 years [1-5]. The biodegradable biomaterials filled with both MSCs or CM and different vehicles are being nowadays tested in the rat sciatic nerve across 10 mm-gap (neurotmesis) or in 3 mm axonotmesis lesion for an initial scaffold in vivo validation. Afterwards, the same scaffold will be tested in critical nerve injuries, using the peroneal nerve in sheep, which reproduces more closely, the neurosurgery clinical cases [1-5]. The regenerated nerves are usually processed for light, confocal and electronic microscope analysis, imunohistochemistry, and stereological studies after 20 and 12 weeks, for neurotmesis and axonotmesis injuries, respectively. The functional recovery is always assessed serially using video recording of the gait for biomechanical analysis, by measuring extensor postural thrust (EPT), sciatic functional index (SFI) and static sciatic functional index (SSI), and the withdrawal reflex latency (WRL) [1-5]. Also, the muscular regeneration after neurogenic atrophy has been evaluated by morphometric analysis and functional assessment. The axonotmesis and neurotmesis injury models are also widely used in the evaluation of muscle regeneration via the denervation/reinnervation process, since this phenomena is caused by blockage of the nerve impulse, with consequent loss of stimulation at the level of the neuromuscular junction. The rapid restoration of the motor function unit is crucial for successful reversal of muscular atrophy and this factor has been intensively studied by our research group [1-5]. Our research projects allow to adequate GMP isolation, and expansion protocols of UC MSCs and DPSCs to clinical use, through in vitro studies including cytocompatibility, multi-lineage differentiation capacity, imunocytochemistry, flow cytometry, secretome, metabolic profile, and RT-PCR. The in vivo trials allow evaluation of neuro-muscular functional and morphologic recovery, including the ovine model to study critical defects. Also, the importance of a longitudinal and complete study concerning tissue engineering of the peripheral nerve, which includes a multidisciplinary team able to develop biomaterials, to prepare cellular cultures for cell therapies, and to elaborate in vitro analysis and pre-clinical trials concerning animal welfare and the most appropriate animal model is enhanced by the Regenerative Medicine sub-unit from CECA.

Biography

Ana Colette Mauricio has a degree on Veterinary Medicine since 1995, a PhD on Veterinary Sciences since 1999 from Faculdade de Medicina Veterinária (FMV) - Universidade Técnica de Lisboa (UTL) and Habilitation in Veterinary Sciences (ICBAS-UP) since 2011. At the present, she is an Associated Professor with Habilitation, from the Veterinary Clinics of ICBAS – UP. She also belongs to the Scientific Council of ICBAS-UP and to the Ethic Comission for Health Sciences from University of Porto (UP). She belongs to the Scientific Committee of the Veterinary Sciences Doctoral Program at ICBAS–UP. She is the Scientific Coordinator of Regenerative Medicine and Experimental Surgery sub-unit from CECA-ICETA fro UP. She is one of the founding shareholders of Biosckin, Molecular and Cell Therapies, S.A since 2007 for development of new cell therapies and medical devices. For the past 12 years she coordinates a multidisciplinary research group of Experimental Surgery and Regenerative Medicine, working with several biomaterials and cellular therapies. She coordinates a multidisciplinary team, including Veterinaries, Engineers, Medical Doctors that through Experimental Surgery have a crucial role in the development of biomaterials and cellular therapies, allowing a close share of knowledge between biomaterials design, development of cellular systems, and surgeons needs when related to specific clinical cases. This group has several recent relevant publications in the research areas of nerve, bone, musculoskeletal and vascular tissue regeneration. She is the supervisor of several PhD, Post-Doctoral and Master students, she is the co-author of a large number of scientific articles (Maurício AC) and scientific book chapters. She was the principal researcher of several national and international scientific projects.

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