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Asma Alanazi et al., J Biomedical Sci 2019, Volume 08

Comparative adhesive and migratory properties of mesenchymal stem cells from different tissues

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Mesenchymal stem cells (MSC) are used in therapy, often by injection into the blood. We aimed to compare the adhesive and migratory properties of MSC from umbilical cords (UCMSC), bone marrow (BMMSC) or trabecular bone (TBMSC), which might influence delivery to injured tissue.MSC were perfused through glass capillaries coated with matrix proteins, collagen or fibronectin, or albumin. Adherent cells were counted microscopically and their spreading analysed over time. MSC migration through 8µm pore filters coated with the same proteins was analysed. The number of MSC adhering to collagen was greater than fibronectin, decreased as wall shear rate increased from 17 to 70s-1, and was in the order UCMSC>BMMSC>TBMSC. Conversely, spreading was more effective on fibronectin and was in the order BMMSC>TBMSC UCMSC. Migration was promoted by coating the lower surface of filters with either matrix protein, with UCMSC migrating more efficiently than BMMSC. MSC show origin-dependent variations in their efficiency of capture from flow and subsequent spreading or ability to migrate on matrix proteins. UCMSC showed most efficient capture from flow, which was followed by less spreading, but more rapid migration. These responses might be associated with more effective delivery from the circulation into damaged tissue.

Biography

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M. Basile et al., J Biomedical Sci 2019, Volume 08

Chemotactic properties of human amniotic fluid-derived stem cells (hAFSCs) in bone healing

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Current treatment of large bone defects is based on autologous or allogenic bone grafts that still have several limitations. In the past few years, human amniotic fluid stem cells (hAFSCs) were evaluated for their osteogenic potential in the repair of bone defects due to the absence of ethic controversy and risk of teratocarcinoma formation. Thus, the aim of this study was to investigate the role of hAFSCs in the regeneration of critical-size bone defects in calvaria mouse model. For this purpose, we transduced hAFSCs with cherry red fluorescent protein and used a recipient transgenic mouse model carrying GFP fluorescent reporter to follow the fate of hAFSCs transplanted in vivo into Healos® construct and distinguish donor and host cells at the implant site. Our results showed that transduced hAFSCs can be tracked in vivo directly at the site of transplantation. Cherry red fluorescent hAFSCs were not present in the implant site after 3 and 6 weeks. Instead, the presence of a greater number of GFP-positive cells in the scaffold at the same time-intervals indicates that hAFSCs can recruit host cells during the repair process. Moreover, we observed that hAFSCs are able to attract mouse bone marrow stromal cells (mBMSCs) in vitro, suggesting a possible chemotactic property of their releasing soluble factors. These observations help clarify the role of hAFSCs in bone tissue repair.

Biography

Mariangela Basile graduated in 2015 in Pharmaceutical Chemistry and Technology, UdA Chieti, Italy. In 2013 active collaboration in conducting scientific research into Department of Pharmacy, General Pathology Unit, UdA Chieti, Italy. Since 2016 PhD student in Translational Medicine, Cell Biology Lab, Dept. of Medicine and Ageing Sciences, Inst. of Normal Human Morphology, UdA Chieti, Italy. In 2017/2018, research assistant at Center for Regenerative Medicine and Skeletal Development, Reconstructive Science Department, UConn Health Center, Farmington, Connecticut, USA.

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Radha Santonocito et al., J Biomedical Sci 2019, Volume 08

Tissue bioadhesives: A study on recombinant mussel protein Pvfp-5ß

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Many marine organisms rely on natural adhesives to attach to various surfaces under wet conditions for their life-cycle, movement and self-defense in aqueous tidal environments. Mussel adhesive proteins have received increased attention in recent years for their potential applications in several fields, such as medicine, biomaterials and biotechnology being biocompatible and able to elicit minimal immune response. The Asian green mussel Perna viridis secretes several byssal plaque proteins. The Perna viridis foot protein-5β (Pvfp-5β) is the first protein to initiate interaction with the substrate, displacing interfacial water molecules before binding to the surface. Here, we present a study of recombinant Pvfp-5β, in which we established the first recombinant expression in E. coli of the protein. We characterized recombinant Pvfp-5β and showed that, despite the circular dichroism spectrum with features of a random coil, the protein is correctly folded as demonstrated by mass spectrometry and nuclear magnetic resonance. We evaluated the cell viability and cell adhesion capacity of Pvfp-5β using NIH-3T3 and HeLa cell lines. Our results revealed that the protein has no cytotoxic effect at the investigated protein concentrations and a good cell adhesion strength on both glass and plastic plates. Overall, we show that the adhesive properties of recombinant Pvfp-5β make it an efficient surface coating material, suitable for biomedical applications including regeneration of damaged tissues.

Biography

Dr. Radha Santonocito has completed her master's degree in Biodiversity and Evolution at the age of 26 years from University of Palermo. She is a research fellow at the Institute of Biophysics (IBF) of the National Research Council of Italy (CNR).

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Y. Khalifa et al., J Biomedical Sci 2019, Volume 08

The effect of nanoparticle labeled bone marrow-derived mesenchymal stem cells as a therapeutic strategy for experimentally induced liver fibrosis in albino rats

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Objectives: This study aims at exploring the therapeutic efficacy of superparamagnetic iron oxide nanoparticles (SPIO) labeled bone marrow derived mesenchymal stem cells (BM-MSCs) on carbon tetrachloride (CCl4) induced liver fibrosis in adult female albino rats.

Material and methods: MSCs were obtained from 10 male Sprague Dawley rats and 50 female rats were assigned into 2 groups; control group (CG) and experimental group (EG). EG was subdivided into three subgroups. Induction group by intraperitoneal injection of CCl4 for 8 weeks, MSCs treated + CCl4 group (MSCs+CCl4G) received SPIO- BM-MSCs simultaneously with CCl4 administration to assess the effect of SPIO-BM-MSCs on the prevention of progression of liver fibrosis with the persistence of the cause. MSCs treated group (MSCsG), received SPIO-BM-MSCs after withdrawal of CCl4. The rats were sacrificed after 8 weeks and assessed by histological examination, liver function tests, transforming growth factor-beta (TGF-ß1) immunofluorescence staining, PCR for quantification of the gene expression levels of matrix metalloproteinase-1(MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1).

Results: SPIO labeled MSCs were engrafted in the fibrotic liver and MSCs improved liver functions, enhanced the gene expression of MMP-1, whereas TIMP-1 gene expression was depressed. Histological and morphometric studies confirmed these results.

Conclusion: BM-MSCs prove to be a promising therapy for liver fibrosis.

Biography

Khalifa is an assistant lecturer of Histology and Cell Biology (23/9/2014- now), Department of Histology and Cell Biology, Faculty of Medicine, Alexandria University, Egypt. She is a member of the stem cell research group, Faculty of Medicine, Alexandria University, Egypt. (Oct 2015- present). In September 2014, she was registered for Doctorate Degree (MD) in Histology & Cell biology, Faculty of Medicine, University of Alexandria.

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Stem cell therapy for the treatment of severe tissue damage after radiation exposure

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The late adverse effects of pelvic radiotherapy concern 5 to 10% of them, which could be life threatening. However, a clear medical consensus concerning the clinical management of such healthy tissue sequelae does not exist. Our group has demonstrated in preclinical animal models that systemic MSC injection is a promise approach for the medical management of gastrointestinal disorder after irradiation. We have shown that MSC migrate to damaged tissues and restore gut functions after irradiation.

The clinical status of four first patients suffering from severe pelvic side effects resulting from an over-dosage was improved following MSC injection in a compassional situation. A quantity of 2x106 - 6x106 MSC /kg were infused intravenously to the patients. Pain, hemorrhage, frequency of diarrheas and fistulisation as well as the lymphocyte subsets in peripheral blood were evaluated before MSC therapy and during the follow-up. Two patients revealed a substantiated clinical response for pain and hemorrhage after MSC therapy. In one patient pain reappeared after 6 months and again substantially responded on a second MSC infusion. A beginning fistulisation process could be stopped in one patient resulting in a stable remission for more than 3 years of follow-up. The frequency of painful diarrhea diminished from an average of 6/d to 3/d after the first and 2/d after the 2nd MSC injection in one patient. In all patients, prostate cancer remained in stable complete remission. A modulation of the lymphocyte subsets towards a regulatory pattern and diminution of activated T cells accompanies the clinical response in refractory irradiation-induced colitis. No toxicity occurred.

MSC therapy was safe and effective on pain, diarrhea, haemorrhage, inflammation, fibrosis and limited fistulisation. For patients with refractory chronic inflammatory and fistulising bowel diseases, systemic MSC injections represent a safe option for salvage therapy. A clinical phase II trial will start in 2017

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Virtual biopsies of tissues and carcinomas using vibrational optical coherence tomography

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Vibrational optical coherence tomography (VOCT) is a new technique that combines the imaging power of optical coherence tomography with the use of sound to characterize the physical properties of tissues. This technique has been developed to perform "virtual" biopsies and biomechanical measurements on normal and malignant tissues non-invasively and non-destructively. It has been previously reported that cutaneous wound healing and the development of malignant skin lesions are associated with changes in tissue stiffness. VOCT produces images of groups of cells as well as biomechanical information in three dimensions that can distinguish normal from pathological tissue. In addition, the biomechanical properties of the tissue margins can be characterized. The images and the biomechanical data from measurements made on different skin lesions and carcinomas together can help plan surgical interventions and monitor the healing process of skin lesions. VOCT produces images of groups of cells as well as measurement of the tissue resonant frequency in three dimensions which assists in distinguishing normal from pathological tissue.

We have imaged and studied several types of skin lesions including a BCC, SCC Actinic Keratosis and a Nevi using VOCT to evaluate the morphology, stiffness, depth and margins of these structures. While cellular components present in skin and carcinomas have resonant frequencies in the range of 30 to 60 Hz, normal collagen has a resonant frequency in the range greater than 90 Hz. In comparison, fibrotic collagen is shown to have resonant frequencies above 150 Hz as does collagen from skin lesions.

It is concluded that the ratio of the resonant frequency squared to the tissue thickness obtained from VOCT can be used to grade the type of tissue response seen. Further studies are underway to establish the relationship between tissue stiffness and lesion morphology for cellular and fibrotic lesions based on the characteristic ratios of resonant frequency and tissue thickness.

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Polymer based nanostructured membranes obtained via electrospinning

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Introduction: In this work electrospinning (ELS) was used for the production of polymer based nanostructured membranes for biomedical applications. Present work reports the the characterization of polycaprolactone (PCL) based membranes, implemented with a bioactive chitosan derivative (CTL) and antibacterial silver nanoparticles (nAg). Methods: PCL 12%w/V solved in DCM:DMF 7:3 was electrospun for 1h with a custom made ELS device. Parameters: 17kV of potential, flow rate of 0.6mL/h, 27G needle, 15cm of distance. Nanofibers were characterized by means of Scanning Electron Microscopy (SEM) and micro-Computed Tomography. Air-plasma treatment was used to increase the hydrophilicity of the membrane surface and to adsorb CTL and CTL-nAg. Ag was quantified with Inductively Coupled Plasma Mass Spectrometry ICP-MS. Wettability and biocompatibility of membranes were tested.

Results: PCL nanostructured membranes produced with this technique exhibited an average thickness of 215µm and an average fibre diameter of 600nm. CTL adsorption was assessed by means of confocal microscopy using FITC labelled CTL. Contact angle measurements showed limited wettability of PCL membrane (as prepared), and increased hydrophilicity of air-plasma and CTL coated membranes. Biocompatibility test were performed using MG63 cells cultured on membrane surface. CTL-coated membranes were able to support cell adhesion and proliferation. In contrast, both as-prepared membranes and air-plasma treated membranes exhibited limited cell adhesion and proliferation.

Discussion and Conclusions: This work highlighted the hydrophilicity and biocompatibility of ELS nanostructured membranes made of PLC and coated with CTL- nAg. These results are promising for applications in the field of tissue engineering.

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LTP modified vascular graft materials for endothelial cells growth

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Cardiovascular disease (CVD) is the no. 1 killer in the world, and is responsible for >17.3 million deaths every year1. Bypass surgery, using the autologous vein has been one of the most effective treatments for CVD. However, more recently vascular grafts have shown great potential in bypass surgery. Vascular grafts currently employ a number of scaffold materials such as Dacron and ePTFE and treatments that mimic the native vessel wall2-3. These however, does not work well for small diameter grafts (<6 mm) due to intimal hyperplasia and thrombosis. In our study we plan to improve the endothelialization of intimal surface of graft by modifying with low temperature plasma (LTP) to increase the cell attachment/viability and proliferation. The scaffolds were treated with LTP using Harris Plasma Cleaner system with air as the feed gas for 30 sec at 45W (HI setting). X ray photoelectron spectroscopic analyses and contact angle wettability studies confirmed the introduction of oxygenated functionalities on the surface and enhanced hydrophilicity due to the improvement in oxygen content ~1 in the graft surface from LTP air plasma. Scaffolds were also modified with fibronectin and collagen by dipping method. Endothelial cell studies by microscopic and metabolic assays indicated that cell viability increased in LTP treated scaffolds especially when treated with protein. Scaffolds treated with fibronectin or collagen had cells viable for a week compared to untreated samples. MTT results validated the improved number of metabolically active viable cells. Work is under progress to improve cell viability on these scaffolds.

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Development of a chitosan-vaseline gauze dressing with wound- healing promoting properties

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Infectious complications and profound fluid loss can lead to shock or even death after trauma, wound dressings are always needed for better treatment1,2. Although some dressings have been produced, some are not effective in killing bacteria or controlling other situations, while some are useful but at great expense3,4. Our group developed a chitosan-based dressing with potent antimicrobial and improved healing properties. The chitosan-vaseline dressing (CVG) was developed by coating chitosan mixture and vaseline on sterile gauze and subsequent drying. Infrared spectroscopy investigated the miscibility of this system and functional group interaction. The structure of the dressing was revealed by scanning electron microscopy. The cytotoxicity of the material was tested in vitro, which showed no significant difference in the dressing extract groups and the negative control group. The increased water retention rate was in the range of 8-12% after applying CVG for two hours. The CVG also showed good antimicrobial nature against both gram positive and gram negative bacteria. Wound healing and tissue compatibility studies were carried out over a period of 14 days on rat models. It was observed fast healing in the CVG treated wounds, comparing to the control group. These results indicate that vaseline with chitosan based dressing material could be promising candidates for wound dressings.

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Single cell nano-electroporation to laser induced photoporation: Novel approaches for cell therapy and diagnostics

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The ability to precisely deliver of foreign cargo into single living cells is of great interest in cell biology and therapeutics research. Conventional bulk electroporation is widely used but has been known to cause high percentage of cell death and require high voltage sources. Microfluidic electroporation platforms can provide high delivery efficiency with high cell viability through better-controlled electric fields applied to cells. Here we develop micro/nano fabricated single cell electroporation platforms, which is an efficient and fast method for multi-nanolocalized single cell nano-electroporation, where electroporation takes place on a multiple region of individual single cell membrane using ITO nano-electrodes array. The gap between two nano-electrodes are 70 nm with triangle tip diameter of 40 nm, which intense an electric field in a precise region of single cell membrane to deliver biomolecules with high transfection efficiency and high cell viability.

On the other hand we developed photoporation based devices, where nano-second pulse laser is used to interact with metal or metal nanoparticles and form plasmonic nanobubbles, which rapidly grew, coalesced and collapsed to induce an explosion, resulting strong fluid on the cell membrane. Thus plasma membrane can disrupt and form transient membrane pores, allowing the delivery of cargos from outside to inside the cell. Using both of these techniques we successfully deliver dyes, DNA, RNA, QDs and nanoparticles, bacteria in cancer cells as well as stem cell. These new approaches can allow us to analyse different dyes/biomolecules interaction in single living cell with spatial, temporal, and qualitative dosage control, which potentially applicable for medical diagnostics and therapeutic studies.

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Flexible electrical stimulation device with chitosan-based dressing accelerates angiogenesis in diabetic wounds

Xiao-Feng Wang, Qing-Qing Fang, Wan-Yi Zhao and **Wei-Qiang Tan**

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Diabetic wounds are recalcitrant to treatment and still affect millions of people worldwide annually. Vascular lesions caused by hyperglycaemia are known to severely impair wound healing capabilities in diabetic patients, revealing the importance of vessel network establishment promotion for proper repair. Exogenous electrical stimulation (ES) is a promising physical treatment of diabetic chronic wounds, because it could provide a directional vector to stimulate charged cells involved in wound healing by enhancing cellular migration. However, uncertainty remains regarding the best electrical ES parameters for diabetic wounds and the molecular mechanisms involved in promoting wound healing. Moreover, the application of ES is also inconvenient for patients. Here, we show that high voltage monophasic pulse current stimulation is the optimal parameter to improve diabetic wound healing and that surface electrode is better than insertion electrode for this purpose. In vitro experiments showed that monophasic pulse current stimulation enhanced the proliferation and migration capacity of human umbilical vein endothelial cells and promoted growth factors released via the Pi3k/Akt and Erk1/2 pathways. In order to create a more convenient process for the patients and provide an optimal environment for cell migration, we used flexible materials and chitosan (good moisture and antibacterial effects) to create a preliminarily design of a flexible ES device with chitosan-based dressing, which was proven to promote the healing of diabetic wounds through accelerating angiogenesis in vivo. Thus, our work provides favourableness support for the development of a more advanced product that may have clinical application for diabetic chronic wounds in the future.

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