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Mesenchymal stromal cells mediated delivery of photoactive nanoparticles inhibits osteosarcoma growth in vitro and in a murine in vivo ectopic model.

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Author information

Abstract

BACKGROUND:

Osteosarcoma (OS) is an aggressive malignant neoplasm that still suffers from poor prognosis in the case of distal metastases or occurrence of multi-drug resistance. It is therefore crucial to find novel therapeutic options able to go beyond these limitations and improve patients' survival. The objective of this study is to exploit the intrinsic properties of mesenchymal stromal cells (MSCs) to migrate and infiltrate the tumor stroma to specifically deliver therapeutic agents directly to cancer cells. In particular, we aimed to test the efficacy of the photoactivation of MSCs loaded with nanoparticles in vitro and in a murine in vivo ectopic osteosarcoma model.

METHODS:

$AlPcS_4$@FNPs were produced by adding tetra-sulfonated aluminum phthalocyanine ($AlPcS_4$) to an aqueous solution of positively charged poly-methyl methacrylate core-shell fluorescent nanoparticles (FNPs). The photodynamic therapy (PDT) effect is achieved by activation of the photosensitizer $AlPcS_4$ in the near-infrared light with an LED source. Human MSCs were isolated from the bone marrow of five donors to account for inter-patients variability and used in this study after being evaluated for their clonogenicity, multipotency and immunophenotypic profile. MSC lines were then tested for the ability to internalize and retain the nanoparticles, along with their migratory properties in vitro. Photoactivation effect was evaluated both in a monolayer (2D) co-culture of $AlPcS_4$@FNPs loaded MSCs with human OS cells (SaOS-2) and in tridimensional (3D) multicellular spheroids ($AlPcS_4$@FNPs loaded MSCs with human OS cells, MG-63). Cell death was assessed by AnnexinV/PI and Live&Dead CalceinAM/EthD staining in 2D, while in the 3D co-culture, the cell killing effect was measured through ATP content, CalceinAM/EthD staining and TEM imaging. We also evaluated the effectiveness of $AlPcS_4$@FNPs loaded MSCs as delivery systems and the ability of the photodynamic treatment to kill cancer cells in a subcutaneous mouse model of OS by bioluminescence imaging (BLI) and histology.

RESULTS:

MSCs internalized $AlPcS_4$@FNPs without losing or altering their motility and viability in vitro. Photoactivation of $AlPcS_4$@FNPs loaded MSCs induced high level of OS cells death in the 2D co-culture. Similarly, in the 3D co-culture (MSCs:OS ratios 1:1 or 1:3), a substantial decrease of both MSCs and OS cells viability was observed. Notably, when increasing the MSCs:OS ratio to 1:7, photoactivation still caused more than 40% cells death. When tested in an in vivo ectopic OS model,

AlPcS4@FNPs loaded MSCs were able to decrease OS growth by 68% after two cycles of photoactivation.

CONCLUSIONS:

Our findings demonstrate that MSCs can deliver functional photosensitizer-decorated nanoparticles in vitro and in vivo and inhibit OS tumor growth. MSCs may be an effective platform for the targeted delivery of therapeutic nanodrugs in a clinical scenario, alone or in combination with other osteosarcoma treatment modalities.

Cytometry A. 2020 Feb 29. doi: 10.1002/cyto.a.23985. [Epub ahead of print]

Quantitative Bioimage Analysis of Passaging Effect on the Migratory Behavior of Human Mesenchymal Stem Cells during Spheroid Formation.

Jiang CF[1], Hsu SH[2], Sun YM[1], Tsai MH[1].

Author information

Abstract

The quality of stem cells obtained through serial subcultivation is the pivotal factor determining the therapeutic effectiveness of regenerative medicine. However, an effective quality monitoring system for cell culture is yet to be established. Detailed parameter studies of the migratory behavior of stem cells at different passages may provide insight into the deterioration of stemness. Thus, this study aimed to evaluate the feasibility of quantitative bioimage analysis for monitoring stem cell quality during in vitro culture and to explore the passaging effects on stem cell migration. An image-based analytical tool using cell tracking, cytometric analyses, and gating with time-lapse microscopy was developed to characterize the migratory behavior of human mesenchymal stem cells (hMSCs) isolated from human adipose tissue (hADAS) and placenta (hPDMC) cultured on chitosan membranes. Quantitative analysis was performed for the single cells and assembled spheroids selected from 15 videos of Passages 3, 5, and 11 for hADAS and those from 12 videos of Passages 7, 11, and 16 for hPDMC. These passages were selected to represent the young, matured, and degenerated stem cells, respectively. Migratory behavior varied with cell passages. The mobility of single hMSCs decreased at degenerated passages. In addition, enhancement of mobility, due to transformation from single cells to spheroids, occurred at each passage. The young hMSCs seemed more likely to move as single cells rather than as aggregates. Once matured, they tended to aggregate with strong 3D spheroid formability and increased mobility. However, the spheroid formability and mobility decreased at late passage. The increase in aggregation rate with passaging may be a compensatory mechanism to enhance the declining mobility of hMSCs through cell coordination. Our findings regarding the passaging effects on stem-cell migratory behavior agree with biochemical reports, suggesting that the developed imaging method is capable of monitoring the cell-culture quality effectively.

Methods Mol Biol. 2020;2126:155-166. doi: 10.1007/978-1-0716-0364-2_14.

Gold Nanoparticles as a Computed Tomography Marker for Stem Cell Tracking.

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Abstract

Stem cell tracking is an essential prerequisite for effective stem cell therapy. Computed tomography (CT) imaging technique is an emerging quantitative tool to detect real time distribution of transplanted cells. Most of CT labels based on the high atomic number (Z) materials have concern over biocompatibility. The present book chapter describes a protocol for the use of biocompatible gold nanoparticles as a CT marker for efficient labeling of mesenchymal stem cells (MSCs) and subsequent cell tracking in rodent models.

Methods Mol Biol. 2020;2126:141-153. doi: 10.1007/978-1-0716-0364-2_13.

Stem Cell Tracking with Nanoparticle-Based Ultrasound Contrast Agents.

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Abstract

Cell therapy is revolutionizing modern medicine. To promote this emerging therapy, the ability to image and track therapeutic cells is critical to monitor the progress of the treatment. Ultrasound imaging is promising in tracking therapeutic cells but suffers from poor contrast against local tissues. Therefore, it is critical to increase the ultrasound contrast of therapeutic cells over local tissue at the injection site. Here, we describe a method to increase the ultrasound intensity of therapeutic cells with nanoparticles to make the injected therapeutic cells more visible.

Methods Mol Biol. 2020;2126:95-106. doi: 10.1007/978-1-0716-0364-2_9.

Magnetic Resonance Imaging of Single Cells.

Kalubowilage M[1], Bossmann SH[2].

Author information

Abstract

This chapter discusses a methodology for simultaneously imaging stem cells and endothelial cells within polysaccharide-based scaffolds for tissue engineering. These scaffolds were then implanted into nude mice. Human mesenchymal stem cells (HMSCs) were labeled with the T_1-marker Gd(III)-DOTAGA-functionalized polysiloxane nanoparticles (GdNPs), whereas endothelial umbilical vein cells (HUVECs) were labeled with citrate-stabilized maghemite nanoparticles (IONPs), which predominantly shorten the T_2-relaxation times of the water molecules in scaffolds and tissue. Dual cell detection was achieved by performing T_1- and T_2-weighted MRI in both tissue scaffolds and in vivo.

Nat Commun. 2020 Feb 28;11(1):1064. doi: 10.1038/s41467-020-14344-7.

Inhalation of lung spheroid cell secretome and exosomes promotes lung repair in pulmonary fibrosis.

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a fatal and incurable form of interstitial lung disease in which persistent injury results in scar tissue formation. As fibrosis thickens, the lung tissue loses the ability to facilitate gas exchange and provide cells with needed oxygen. Currently, IPF has few treatment options and no effective therapies, aside from lung transplant. Here we present a series of studies utilizing lung spheroid cell-secretome (LSC-Sec) and exosomes (LSC-Exo) by inhalation to treat different models of lung injury and fibrosis. Analysis reveals that LSC-Sec and LSC-Exo treatments could attenuate and resolve bleomycin- and silica-induced fibrosis by reestablishing normal alveolar structure and decreasing both collagen accumulation and myofibroblast proliferation. Additionally, LSC-Sec and LSC-Exo exhibit superior therapeutic benefits than their counterparts derived from mesenchymal stem cells in some measures. We showed that an inhalation treatment of secretome and exosome exhibited therapeutic potential for lung regeneration in two experimental models of pulmonary fibrosis.

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Secreted Factors and EV-miRNAs Orchestrate the Healing Capacity of Adipose Mesenchymal Stem Cells for the Treatment of Knee Osteoarthritis.

Ragni E[1], Perucca Orfei C[1], De Luca P[1], Colombini A[1], Viganò M[1], de Girolamo L[1].

Author information

Abstract

Mesenchymal stem cells (MSCs) derived from adipose tissue and used either as expanded cells or minimally manipulated cell preparations showed positive clinical outcomes in regenerative medicine approaches based on tissue restoration and inflammation control, like in osteoarthritis (OA). Recently, MSCs' healing capacity has been ascribed to the large array of soluble factors, including soluble cytokines/chemokines and miRNAs conveyed within extracellular vesicles (EVs). Therefore, in this study, 200 secreted cytokines, chemokines and growth factors via ELISA, together with EV-embedded miRNAs via high-throughput techniques, were scored in adipose-derived MSCs (ASCs) cultivated under inflammatory conditions, mimicking OA synovial fluid. Both factors (through most abundantly expressed TIMP1, TIMP2, PLG and CTSS) and miRNAs (miR-24-3p, miR-222-3p and miR-193b-3p) suggested a strong capacity for ASCs to reduce matrix degradation activities, as those activated in OA cartilage, and switch synovial macrophages, often characterized by an M1 inflammatory polarization, towards an M2 phenotype. Moreover, the crucial importance of selecting the target tissue is discussed, showing how a focused search may greatly improve potency prediction and explain clinical outcomes.

In conclusion, herein presented data shed light about the way ASCs regulate cell homeostasis and regenerative pathways in an OA-resembling environment, therefore suggesting a rationale for the use of MSC-enriched clinical products, such as stromal vascular fraction and microfragmented adipose tissue, in joint pathologies.

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Biomolecules. 2020 Feb 21;10(2). pii: E340. doi: 10.3390/biom10020340.

Molecular Mechanisms Contributing to Mesenchymal Stromal Cell Aging.

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Abstract

Mesenchymal stem/stromal cells (MSCs) are a reservoir for tissue homeostasis and repair that age during organismal aging. Beside the fundamental in vivo role of MSCs, they have also emerged in the last years as extremely promising therapeutic agents for a wide variety of clinical conditions. MSC use

frequently requires in vitro expansion, thus exposing cells to replicative senescence. Aging of MSCs (both in vivo and in vitro) can affect not only their replicative potential, but also their properties, like immunomodulation and secretory profile, thus possibly compromising their therapeutic effect. It is therefore of critical importance to unveil the underlying mechanisms of MSC senescence and to define shared methods to assess MSC aging status. The present review will focus on current scientific knowledge about MSC aging mechanisms, control and effects, including possible anti-aging treatments.

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Nanoengineered Osteoinductive Bioink for 3D Bioprinting Bone Tissue.

Chimene D, Miller L, Cross L, Jaiswal MK, Singh I, Gaharwar AK.

Abstract

Bioprinting is an emerging additive manufacturing approach to the fabrication of patient-specific, implantable three-dimensional (3D) constructs for regenerative medicine. However, developing cell-compatible bioinks with high printability, structural stability, biodegradability, and bioactive characteristics is still a primary challenge for translating 3D bioprinting technology to pre-clinical and clinal models. To overcome this challenge, we develop a nanoengineered ionic covalent entanglement (NICE) bioink formulation for 3D bone bioprinting. The NICE bioinks allow precise control over printability, mechanical properties and degradation characteristics, enabling custom 3D fabrication of mechanically resilient, cellularized structures. We demonstrate cell-induced remodeling of 3D bioprinted scaffolds over 60 days demonstrating deposition of nascent extracellular matrix proteins. Interestingly, the bioprinted constructs induces endochondral differentiation of encapsulated human mesenchymal stem cells (hMSCs) in absence of osteoinducing agents such as dexamethasone or bone morphogenic protein-2 (BMP-2). Using next-generation transcriptome sequencing (RNA-seq) technology, we establish the role of nanosilicates, a bioactive component of NICE bioink, to stimulate endochondral differentiation at transcriptome level. Overall, the osteoinductive bioink have ability to induce formation of osteo-related mineralized extracellular matrix by encapsulated hMSCs in growth factor-free conditions. Furthermore, we demonstrated the ability of NICE bioink to fabricate patient-specific, implantable 3D scaffolds for repair of craniomaxillofacial bone defects. We envision transformation of this NICE bioink technology towards a realistic clinical process for 3D bioprinting patient-specific bone tissue for regenerative medicine.