Methods Mol Biol. 2019 Nov 10. doi: 10.1007/7651_2019_265. [Epub ahead of print]

Efficient Labeling of Human Mesenchymal Stem Cells Using Iron Oxide Nanoparticles.

Rawat S[1], Gupta S[1], Bhat M[2], Dinda AK[2], Mohanty S[3].
Author information

Abstract

Stem cells have been used in multiple clinical trials. Tracking these transplanted cells in vivo will provide real-time information on the fate of these cells. Iron oxide labeling is one such uncomplicated noninvasive labeling method. These transformed nanocrystals can be used for varied applications including stem-cell tracking, magnetic resonance imaging, and theranostics. Here we elucidate the protocol for iron oxide nanoparticles synthesis (IONPS) and labeling of mesenchymal stem cells which can be used for imaging and tracking cells to understand their fate in in vivo studies.

Tissue Eng Part A. 2019 Nov 7. doi: 10.1089/ten.TEA.2019.0204. [Epub ahead of print]

Multi-Material Dual Gradient 3D Printing for Osteogenic Differentiation and Spatial Segregation.

Smith BT[1], Bittner SM[2], Watson E[3], Smoak MM[4], Diaz-Gomez LA[5,6], Molina ER[7], Kim YS[8], Hudgins CD[9], Melchiorri AJ[10], Scott DW[11], Grande-Allen KJ[12], Yoo JJ[13], Atala A[14], Fisher JP[15,16], Mikos AG[17].
Author information

Abstract

In this study of 3D printed composite β-tricalcium phosphate (β-TCP)/hydroxyapatite (HA)/poly(ε-caprolactone) (PCL)-based constructs, the effects of vertical compositional ceramic gradients and architectural porosity gradients on the osteogenic differentiation of rabbit bone marrow derived mesenchymal stem cells (MSCs) were investigated. Specifically, three different concentrations of β-TCP (0, 10, and 20 wt%) and three different porosities (33 ± 4 %, 50 ± 4 %, and 65 ± 3 %) were examined to elucidate the contributions of chemical and physical gradients on the biochemical behavior of MSCs and the mineralized matrix production within a 3D culture system. By delaminating the constructs at the gradient transition point, the spatial separation of cellular phenotypes could be specifically evaluated for each construct section. Results indicated that increased concentrations of β-TCP resulted in up-regulation of osteogenic markers including alkaline phosphatase activity and mineralized matrix development. Furthermore, MSCs located within regions of higher porosity displayed a more mature osteogenic phenotype compared to MSCs in lower porosity regions. These results demonstrate that 3D printing can be leveraged to create multiphasic gradient constructs to precisely direct the development and function of MSCs, leading to a phenotypic gradient.

Oncotarget. 2019 Oct 22;10(58):6049-6061. doi: 10.18632/oncotarget.27071. eCollection 2019 Oct 22.

Preclinical analysis of human mesenchymal stem cells: tumor tropism and therapeutic efficiency of local HSV-TK suicide gene therapy in glioblastoma.

Dührsen L[1], Hartfuß S[1], Hirsch D[2], Geiger S[2], Maire CL[1], Sedlacik J[3], Guenther C[2], Westphal M[1], Lamszus K[1], Hermann FG[2], Schmidt NO[1].
Author information

Abstract

Glioblastoma are highly invasive and associated with limited therapeutic options and a grim prognosis. Using stem cells to extend current therapeutic strategies by targeted drug delivery to infiltrated tumors cells is highly attractive. This study analyzes the tumor homing and therapeutic abilities of clinical grade human mesenchymal stem cells (MSCs) in an orthotopic glioblastoma mouse model. Our time course analysis demonstrated that MSCs display a rapid targeted migration to intracerebral U87 glioma xenografts growing in the contralateral hemisphere within the first 48h hours after application as assessed by histology and 7T magnetic resonance imaging. MSCs accumulated predominantly peritumorally but also infiltrated the main tumor mass and targeted distant tumor satellites while no MSCs were found in other regions of the brain. Intratumoral application of MSCs expressing herpes simplex virus thymidine kinase followed by systemic prodrug application of ganciclovir led to a significant tumor growth inhibition of 86% versus the control groups ($p<0.05$), which translated in a significant prolonged survival time ($p<0.05$). This study demonstrates that human MSCs generated according to apceth's GMP process from healthy donors are able to target and provide a significant growth inhibition in a glioblastoma model supporting a potential clinical translation.

ACS Appl Mater Interfaces. 2019 Nov 6. doi: 10.1021/acsami.9b14679. [Epub ahead of print]

Graphene/Si Promoted Osteogenic Differentiation of BMSCs through Light Illumination.

Long X, Wang X, Yao L, Lin S, Zhang J, Weng W, Cheng K, Wang H, Lin J.

Abstract

Graphene (Gr) presents promising applications in regulating the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs). Light illumination is regarded as a spatiotemporally controllable, easily applicable and noninvasive mean to modulate materials' responses. Herein, Gr transferred silicon (Gr/Si) with Schottky junction is utilized to evaluate the visible light-promoted osteogenic differentiation of BMSCs. Under light illumination, light-induced charges, owing to the formation of Schottky junction at the interface of Gr and Si, accumulated on the surface and then changed the surface potential of Gr/Si. Schottky junction and surface potential at the interface of Gr and Si was measured by photovoltaic test and scanning kelvin probe microscopy. ALP activity and Quantitative real-time PCR measurement showed that such variations of surface improved the osteogenic differentiation of BMSCs, and the activation of the voltage-gated calcium channels through surface potential and accumulation of cytosolic Ca2+ could be the reason. Moreover, X-ray photo-electron spectroscopy characterization showed that surface charge could also affect BMSCs differentiation through promotion or inhibition of the osteogenic growth factors adsorption. Such

light-promoted BMSCs osteogenic differentiation on Gr/Si may have many potential in biomedical materials or devices for bone regeneration application.

Sci Rep. 2019 Nov 5;9(1):16031. doi: 10.1038/s41598-019-52442-9.

Impact of humanised isolation and culture conditions on stemness and osteogenic potential of bone marrow derived mesenchymal stromal cells.

Suliman S[1], Ali HRW[2], Karlsen TA[3], Amiaud J[4], Mohamed-Ahmed S[2], Layrolle P[4], Costea DE[5,6,7], Brinchmann JE[3,8], Mustafa K[9].
Author information

Abstract

Therapeutic potential of human bone marrow stromal/stem cells (hBMSC) must be developed using well defined xenogenic-free conditions. hBMSC were isolated from healthy donors (n = 3) using different isolation and expansion methods. Donor I was isolated and expanded by either bone marrow directly seeded and cells expanded in 10% AB human serum (AB) +5 ng/ml fibroblast growth factor-2 (FGF2) [Direct(AB + FGF_{low})] or Ammonium-Chloride-Potassium Lysing Buffer was used before the cells were expanded in 10% AB +5 ng/ml FGF-2 [ACK(AB + FGF_{low})] or Lymphoprep density gradient medium was used before the cells were expanded in 10% AB +5 ng/ml FGF2 [Lympho(AB + FGF_{low})] or bone marrow directly seeded and cells expanded in 10% pooled platelet lysate plasma (PL) + heparin (2 I/U/mL) [Direct(PL)]. Groups for donors II and III were: Direct(AB + FGF_{low}) or 10% AB +10 ng/ml FGF2 [Direct(AB + FGF_{high})] or Direct(PL). HBMSCs were assessed for viability, multi-potency, osteogenic, inflammatory response and replicative senescence in vitro after 1 and 3 weeks. Pre-selected culture conditions, Direct(AB + FGF_{high}) or Direct(PL), were seeded on biphasic calcium phosphate granules and subcutaneously implanted in NOD/SCID mice. After 1 and 11 weeks, explants were analysed for inflammatory and osteogenic response at gene level and histologically. To identify implanted human cells, in situ hybridisation was performed. hBMSC from all conditions showed in vitro multi-lineage potency. hBMSCs expanded in PL expressed stemness markers in vitro at significantly higher levels. Generally, cells expanded in AB + FGF2 conditions expressed higher osteogenic markers after 1 week both in vitro and in vivo. After 11 weeks in vivo, Direct(AB + FGF_{high}) formed mature ectopic bone, compared to immature mineralised tissues formed by Direct(PL) implants. Mouse responses showed a significant upregulation of IL-1α and IL-1β expression in Direct(PL). After 1 week, human cells were observed in both groups and after 11 weeks in Direct(AB + FGF_{high}) only. To conclude, results showed a significant effect of the isolation methods and demonstrated a relatively consistent pattern of efficacy from all donors. A tendency of hBMSC expanded in PL to retain a more stem-like phenotype elucidates their delayed differentiation and different inflammatory expressions.

J Clin Med. 2019 Nov 4;8(11). pii: E1867. doi: 10.3390/jcm8111867.

Secretome and Extracellular Vesicles as New Biological Therapies for Knee Osteoarthritis: A Systematic Review.

D'Arrigo D[1,2], Roffi A[3], Cucchiarini M[4], Moretti M[5,6], Candrian C[7], Filardo G[8,9].

Author information

Abstract

Secretome and extracellular vesicles (EVs) are considered a promising option to exploit mesenchymal stem cells' (MSCs) properties to address knee osteoarthritis (OA). The aim of this systematic review was to analyze both the in vitro and in vivo literature, in order to understand the potential of secretome and EVs as a minimally invasive injective biological approach. A systematic review of the literature was performed on PubMed, Embase, and Web of Science databases up to 31 August 2019. Twenty studies were analyzed; nine in vitro, nine in vitro and in vivo, and two in vivo. The analysis showed an increasing interest in this emerging field, with overall positive findings. Promising in vitro results were documented in terms of enhanced cell proliferation, reduction of inflammation, and down-regulation of catabolic pathways while promoting anabolic processes. The positive in vitro findings were confirmed in vivo, with studies showing positive effects on cartilage, subchondral bone, and synovial tissues in both OA and osteochondral models. However, several aspects remain to be clarified, such as the different effects induced by EVs and secretome, which is the most suitable cell source and production protocol, and the identification of patients who may benefit more from this new biological approach for knee OA treatment.

Int J Mol Sci. 2019 Nov 2;20(21). pii: E5471. doi: 10.3390/ijms20215471.

Impact of the Different Preparation Methods to Obtain Human Adipose-Derived Stromal Vascular Fraction Cells (AD-SVFs) and Human Adipose-Derived Mesenchymal Stem Cells (AD-MSCs): Enzymatic Digestion Versus Mechanical Centrifugation.

Gentile P[1], Calabrese C[2], De Angelis B[3], Pizzicannella J[4], Kothari A[5], Garcovich S[6].

Author information

Abstract

Autologous therapies using adipose-derived stromal vascular fraction (AD-SVFs) and adult adipose-derived mesenchymal stem cells (AD-MSCs) warrant careful preparation of the harvested adipose tissue. Currently, no standardized technique for this preparation exists. Processing quantitative standards (PQSs) define manufacturing quantitative variables (such as time, volume, and pressure). Processing qualitative standards (PQLSs) define the quality of the materials and methods in manufacturing. The purpose of the review was to use PQSs and PQLSs to report the in vivo and in vitro results obtained by different processing kits that use different procedures (enzymatic vs. non-enzymatic) to isolate human AD-SVFs/AD-MSCs. PQSs included the volume of fat tissue harvested and reagents used, the time/gravity of centrifugation, and the time, temperature, and tilt level/speed of incubation and/or centrifugation. PQLSs included the use of a collagenase, a processing time of 30 min, kit weight, transparency of the kit components, the maintenance of a closed sterile processing environment, and the use of a small centrifuge and incubating rocker. Using a kit with the PQSs and PQLSs described in this study enables the isolation of AD-MSCs that meet the consensus quality criteria. As the discovery of new critical quality attributes (CQAs) of AD-MSCs evolve with respect to

purity and potency, adjustments to these benchmark PQSs and PQLs will hopefully isolate AD-MSCs of various CQAs with greater reproducibility, quality, and safety. Confirmatory studies will no doubt need to be completed.

Bioengineering (Basel). 2019 Nov 1;6(4). pii: E101. doi: 10.3390/bioengineering6040101.

Determining Conditions for Successful Culture of Multi-Cellular 3D Tumour Spheroids to Investigate the Effect of Mesenchymal Stem Cells on Breast Cancer Cell Invasiveness.

Brown MJ[1], Bahsoun S[2], Morris MA[3], Akam AEC[4].
Author information

Abstract

Mesenchymal stem cells have been widely implicated in tumour development and metastases. Moving from the use of two-dimensional (2D) models to three-dimensional (3D) to investigate this relationship is critical to facilitate more applicable and relevant research on the tumour microenvironment. We investigated the effects of altering glucose concentration and the source of foetal bovine serum (FBS) on the growth of two breast cancer cell lines (T47D and MDA-MB-231) and human bone marrow-derived mesenchymal stem cells (hBM-MSCs) to determine successful conditions to enable their co-culture in 3D tumour spheroid models. Subsequently, these 3D multi-cellular tumour spheroids were used to investigate the effect of hBM-MSCs on breast cancer cell invasiveness. Findings presented herein show that serum source had a statistically significant effect on two thirds of the growth parameters measured across all three cell lines, whereas glucose only had a statistically significant effect on 6%. It was determined that the optimum growth media composition for the co-culture of 3D hBM-MSCs and breast cancer cell line spheroids was 1 g/L glucose DMEM supplemented with 10% FBS from source A. Subsequent results demonstrated that co-culture of hBM-MSCs and MDA-MB-231 cells dramatically reduced invasiveness of both cell lines ($F_{(1,4)}$ = 71.465, p = 0.001) when embedded into a matrix comprising of growth-factor reduced base membrane extract (BME) and collagen.

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The Crosstalk Between Osteodifferentiating Stem Cells and Endothelial Cells Promotes Angiogenesis and Bone Formation.

Genova T[1,2], Petrillo S[3], Zicola E[4], Roato I[5], Ferracini R[6], Tolosano E[3], Altruda F[3], Carossa S[2], Mussano F[2], Munaron L[1].
Author information

Abstract

The synergistic crosstalk between osteodifferentiating stem cells and endothelial cells (ECs) gained the deserved consideration, shedding light on the role of angiogenesis for bone formation and healing. A deep understanding of the molecular basis underlying the mutual influence of mesenchymal stem

cells (MSCs) and ECs in the osteogenic process may help improve greatly bone regeneration. Here, the authors demonstrated that osteodifferentiating MSCs co-cultured with ECs promote angiogenesis and ECs recruitment. Moreover, through the use of 3D co-culture systems, we showed that ECs are in turn able to further stimulate the osteodifferentiation of MSCs, thus enhancing bone production. These findings highlighted the existence of a virtuous loop between MSCs and ECs that is central to the osteogenic process. Unraveling the molecular mechanisms governing the functional interaction MSCs and ECs holds great potential in the field of regenerative medicine.