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Colloids Surf B Biointerfaces

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Bone interface modulates drug resistance in breast cancer bone metastasis

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Abstract

Metastatic breast cancer cells on arriving at bone site interact with the bone cells to influence their growth, proliferation, and chemoresistance. There are currently no effective therapeutics available in the clinic for bone metastases. Many existing anti-cancer therapeutics are ineffective at the metastatic bone site due to a lack of accurate models of breast cancer bone metastasis for drug screening. Here, we report the development of an effective in vitro model using osteogenically differentiated human mesenchymal stem cells (MSCs) and human breast cancer cells on 3D nanoclay scaffolds as a testbed for screening drugs. Our results demonstrate that breast cancer cells grown in 3D bone-mimetic scaffolds exhibited altered physiological and biochemical properties, including tumoroids formation, elevated levels of cytokine such as IL-6, and its downstream effector-mediated inhibition of apoptosis and upregulation of multidrug transporters proteins, leading to drug resistance against paclitaxel. Most importantly, Signal Transducer and Activator of Transcription 3 (STAT3), a potential biomarker for chemoresistance in many cancers, was activated in the 3D breast cancer bone metastasis model. Thus, our data suggest that 3D bone-mimetic nanoclay scaffolds-based in vitro tumor model is a promising testbed for screening new therapeutics for breast cancer bone metastasis where bone interface governs drug resistance in breast cancer cells.

Aging (Albany NY)

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Obesity is associated with senescence of mesenchymal stromal cells derived from bone marrow, subcutaneous and visceral fat of young mice

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Free article

Abstract

White adipose tissue (WAT) is distributed in several depots with distinct metabolic and inflammatory functions. In our body there are subcutaneous (sWAT), visceral (vWAT) and bone marrow (bWAT) fat depots. Obesity affects the size, function and inflammatory state of WATs. In particular, obesity may affect the activity of mesenchymal stromal cells (MSCs) present in WAT. MSCs are a heterogeneous population containing stromal cells, progenitor cells, fibroblasts and stem cells that are able to differentiate among adipocytes, chondrocytes, osteocytes and other mesodermal derivatives.In the first study of this kind, we performed a comparison of the effects of obesity on MSCs obtained from sWAT, vWAT and bWAT. Our study showed that obesity affects mainly the biological functions of MSCs obtained from bone marrow and vWAT by decreasing the proliferation rate, reducing the percentage of cells in S phase and triggering senescence. The onset of senescence was confirmed by expression of genes belonging to RB and P53 pathways.Our study revealed that the negative consequences of obesity on body physiology may also be related to impairment in the functions of the stromal compartment present in the several adipose tissues. This finding provides new insights as to the targets that should be considered for an effective treatment of obesity-related diseases.

Acta Biomater

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Engineering the cellular mechanical microenvironment to regulate stem cell chondrogenesis: Insights from a microgel model

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Abstract

Biophysical cues (especially mechanical cues) embedded in cellular microenvironments show a critical impact on stem cell fate. Despite the capability of traditional hydrogels to mimic the feature of extracellular matrix (ECM) and tune their physicochemical properties via diverse approaches, their relatively large size not only induces biased results, but also hinders high-throughput screening and analysis. In this paper, a microgel model is proposed to recapitulate the role of 3D mechanical microenvironment on stem cell behaviors especially chondrogenesis in vitro. The small diameter of microgels brings the high surface area to volume ratio and then the enlarged diffusion area and shortened diffusion distance of soluble molecules, leading to uniform distribution of nutrients and negligible biochemical gradient inside microgels. To construct ECM-like microenvironment with tunable mechanical strength, three gelatin/hyaluronic acid hybrid microgels with low, medium and high crosslinking densities, i.e., Gel-HA(L), Gel-HA(M) and Gel-HA(H), are fabricated in microfluidic devices by Michael addition reaction between thiolated gelatin (Gel-SH) and ethylsulfated hyaluronic acid (HA-VS) with different substitution degrees of vinyl sulfone groups. Our results show that mouse bone marrow mesenchymal stem cell (BMSC) proliferation, distribution and chondrogenesis are all closely dependent on mechanical microenvironments in microgels. Noteworthily, BMSCs show a clear trend of differentiating into hyaline cartilage in Gel-HA(L) and fibrocartilage in Gel-HA(M) and Gel-HA(H). Whole transcriptome RNA sequencing reveals that mechanical microenvironment of microgels affects BMSC differentiation via TGF-β/Smad signaling pathway, Hippo signaling pathway and Integrin/YAP/TAZ signaling pathway. We believe this microgel model provides a new way to further explore the interaction between cells and 3D

microenvironment. STATEMENT OF SIGNIFICANCE: In recent years, hydrogels have been frequently used to construct 3D microenvironment for cells. However, their relatively large size not only brings biased experimental results, but also limits high-throughput screening and analysis. Herein we propose a gelatin/hyaluronic acid microgel model to explore the effects of 3D cellular mechanical microenvironment (biophysical cues) on BMSC behaviors especially chondrogenesis, which can minimize the interference of biochemical gradients. Our results reveal that BMSC differentiation into either hyaline cartilage or fibrocartilage can be regulated via tailoring the mechanical properties of microgels. Whole transcriptome RNA sequencing proves that "TGF-β/Smad signaling pathway", "Hippo signaling pathway" and "Integrins/YAP/ TAZ signaling pathway" are activated or inhibited in this process.

J Control Release

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Genetically-programmed, mesenchymal stromal cell-laden & mechanically strong 3D bioprinted scaffolds for bone repair

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Abstract

Additive manufacturing processes used to create regenerative bone tissue engineered implants are not biocompatible, thereby restricting direct use with stem cells and usually require cell seeding post-fabrication. Combined delivery of stem cells with the controlled release of osteogenic factors, within a mechanically-strong biomaterial combined during manufacturing would replace injectable defect fillers (cements) and allow personalized implants to be rapidly prototyped by 3D bioprinting. Through the use of direct genetic programming via the sustained release of an exogenously delivered transcription factor RUNX2 (delivered as recombinant GET-RUNX2 protein) encapsulated in PLGA

microparticles (MPs), we demonstrate that human mesenchymal stromal (stem) cells (hMSCs) can be directly fabricated into a thermo-sintered 3D bioprintable material and achieve effective osteogenic differentiation. Importantly we observed osteogenic programming of gene expression by released GET-RUNX2 (8.2-, 3.3- and 3.9-fold increases in OSX, RUNX2 and OPN expression, respectively) and calcification (von Kossa staining) in our scaffolds. The developed biodegradable PLGA/PEG paste formulation augments high-density bone development in a defect model (~2.4-fold increase in high density bone volume) and can be used to rapidly prototype clinically-sized hMSC-laden implants within minutes using mild, cytocompatible extrusion bioprinting. The ability to create mechanically strong cancellous 'bone-like' printable implants for tissue repair that contain stem cells and controlled-release of programming factors is innovative, and will facilitate the development of novel localized delivery approaches to direct cellular behaviour for many regenerative medicine applications including those for personalized bone repair.

BMC Biol

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Real-time tracking of stem cell viability, proliferation, and differentiation with autonomous bioluminescence imaging

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Free PMC article

Abstract

Background: Luminescent reporter proteins are vital tools for visualizing cells and cellular activity. Among the current toolbox of bioluminescent systems, only bacterial luciferase has genetically defined luciferase and luciferin synthesis pathways that are functional at the mammalian cell temperature optimum of 37 °C and have the potential for in vivo applications. However, this system is not functional in all cell types, including stem cells, where the ability to monitor continuously and in real-time cellular processes such as differentiation and proliferation would be particularly advantageous.

Results: We report that artificial subdivision of the bacterial luciferin and luciferase pathway subcomponents enables continuous or inducible bioluminescence in pluripotent and mesenchymal stem cells when the luciferin pathway is overexpressed with a 20-30:1 ratio. Ratio-based expression is demonstrated to have minimal effects on phenotype or differentiation while enabling autonomous bioluminescence without requiring external excitation. We used this method to assay the proliferation, viability, and toxicology responses of iPSCs and showed that these assays are comparable in their performance to established colorimetric assays. Furthermore, we used the continuous luminescence to track stem cell progeny post-differentiation. Finally, we show that tissue-specific promoters can be used to report cell fate with this system.

Conclusions: Our findings expand the utility of bacterial luciferase and provide a new tool for stem cell research by providing a method to easily enable continuous, non-invasive bioluminescent monitoring in pluripotent cells.

Sci Rep

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PPARβ/δ-dependent MSC metabolism determines their immunoregulatory properties

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Free PMC article

Abstract

Mesenchymal stem cell (MSC)-based therapy is being increasingly considered a powerful opportunity for several disorders based on MSC immunoregulatory properties. Nonetheless, MSC are versatile and plastic cells that require an efficient control of their features and functions for their optimal use in clinic. Recently, we have shown that PPARβ/δ is pivotal for MSC immunoregulatory and therapeutic functions. However, the role of PPARβ/δ on MSC metabolic activity and the relevance of PPARβ/δ metabolic control on MSC immunosuppressive properties have never been addressed. Here, we demonstrate that PPARβ/δ deficiency forces MSC metabolic adaptation increasing their glycolytic activity required for their immunoregulatory functions on Th1 and Th17 cells. Additionally, we show that the inhibition of the mitochondrial production of ATP in MSC expressing PPARβ/δ, promotes their metabolic switch towards aerobic glycolysis to stably enhance their immunosuppressive capacities significantly. Altogether, these data demonstrate that PPARβ/δ governs the immunoregulatory potential of MSC by dictating their metabolic reprogramming and pave the way for enhancing MSC immunoregulatory properties and counteracting their versatility.

Bioengineering (Basel)

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Bioreactor Parameters for Microcarrier-Based Human MSC Expansion under Xeno-Free Conditions in a Vertical-Wheel System

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Free article

Abstract

Human mesenchymal stem/stromal cells (hMSCs) have been investigated and proven to be a well-tolerated, safe therapy for a variety of indications, as shown by over 900 registered hMSC-based clinical trials. To meet the commercial demand for clinical manufacturing of hMSCs, production requires a scale that can achieve a lot size of ~100B cells, which requires innovative manufacturing technologies such as 3D bioreactors. A robust suspension bioreactor process that can be scaled-up to the relevant scale is therefore crucial. In this study, we developed a fed-batch, microcarrier-based bioreactor process, which enhances media productivity and drives a cost-effective and less labor-intensive hMSC expansion process. We determined parameter settings for various stages of the culture: inoculation, bioreactor culture, and harvest. Addition of a bioreactor feed, using a fed-batch approach, was necessary to replenish the mitogenic factors that were depleted from the media within the first 3 days of culture. Our study resulted in an optimized hMSC culture protocol that consistently achieved hMSC densities between 2×10^5-6×10^5 cells/mL within 5 days with no media exchange, maintaining the final cell population doubling level (PDL) at 16-20. Using multiple hMSC donors, we showed that this process was robust and yielded hMSCs that maintained expansion, phenotypic characteristic, and functional properties. The developed process in a vertical-wheel suspension bioreactor can be scaled to the levels needed to meet commercial demand of hMSCs.