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Interaction of head and neck squamous cell carcinoma cells and mesenchymal stem cells under hypoxia and normoxia

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Free PMC article

Abstract

Mesenchymal stem cells (MSCs) exhibit strong tropism towards tumor tissue. While MSCs generally surround tumors, they can also infiltrate tumors and thereby influence their proliferation. Interactions between MSCs and tumor cells are usually tested under normoxia, but the majority of solid tumors, including head and neck squamous cell carcinoma (HNSCC), are also characterized by hypoxic areas. Hence, the present study aimed to assess the interaction between MSCs and tumor cells under hypoxic conditions. MSCs were cultivated under normoxia and hypoxia, and conditioned media were used to cultivate the HNSCC cell line FaDu. The cell cycle distribution and viability of MSCs and the proliferation of FaDu cells were analyzed under normoxia and hypoxia, and changes in cytokine levels in the conditioned media were evaluated. No cell cycle changes were observed for MSCs after 24 h of cultivation under hypoxia, but the cell viability had declined. Hypoxia also led to a decrease in the proliferation of FaDu cells; however, FaDu cells proliferated faster after 48 h under hypoxia compared with normoxic conditions. This

effect was reversed after incubation under normoxia for 72 h and hypoxia for 72 h. While these changes constituted a trend, these differences were not statistically significant. A cytokine assay showed an increase in interleukin (IL)-6 in the hypoxic medium. Overall, the results indicated that there was an interaction between MSCs and tumor cells. The presence or absence of oxygen seemed to influence the functionality of MSCs and their protumorigenic properties, in which IL-6 was identified as a potential mediator. Since MSCs are a component of the tumor stroma, further *in vitro* and *in vivo* studies are needed to investigate this interaction in order to develop novel approaches for tumor therapy.

Biosens Bioelectron

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Magnetic sensor based on image processing for dynamically tracking magnetic moment of single magnetic mesenchymal stem cell

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Abstract

Developing an economical and universal method to measure the magnetic moments of magnetic mesenchymal stem cells (MSCs) labelled with superparamagnetic iron oxide (SPIO) nanoparticles is crucial for cell tracking. In this study, we used a gradient magnetic field created by a nickel needle to track the motion of cells. A simple and quantifiable magnetic sensor was employed to evaluate the magnetic properties of single viable MSCs. We measured the magnetic moments of microbeads and MSCs using the proposed method and compared the results with magnetic moments measured using a superconducting quantum interference device and with iron contents measured using an inductively coupled plasma spectrometer, respectively. The correlation coefficients indicated satisfactory agreement in both cases, thus confirming the accuracy of the system. By labelling MSCs with SPIOs, we implemented a miniature magnetic sensor to measure the magnetic moments of single magnetic MSCs quantitatively using an image-processing

algorithm. Existing methods for the measurement of magnetic moments at the micro/nanoscale have various limitations. Our system realised the measurement of single viable cells, thereby providing a theoretical foundation for the labelling and tracking of MSCs with SPIO nanoparticles. Additionally, the proposed system is both economical and universal.

Stem Cell Res Ther

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Influence of platelet storage time on human platelet lysates and platelet lysate-expanded mesenchymal stromal cells for bone tissue engineering

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Abstract

Background: Human platelet lysate (HPL) is emerging as the preferred xeno-free supplement for the expansion of mesenchymal stromal cells (MSCs) for bone tissue engineering (BTE) applications. Due to a growing demand, the need for standardization and scaling-up of HPL has been highlighted. However, the optimal storage time of the source material, i.e., outdated platelet concentrates (PCs), remains to be determined. The present study aimed to determine the optimal storage time of PCs in terms of the cytokine content and biological efficacy of HPL.

Methods: Donor-matched bone marrow (BMSCs) and adipose-derived MSCs (ASCs) expanded in HPL or fetal bovine serum (FBS) were characterized based on in vitro proliferation, immunophenotype, and multi-lineage differentiation. Osteogenic differentiation was assessed at early (gene expression), intermediate [alkaline phosphatase

(ALP) activity], and terminal stages (mineralization). Using a multiplex immunoassay, the cytokine contents of HPLs produced from PCs stored for 1-9 months were screened and a preliminary threshold of 4 months was identified. Next, HPLs were produced from PCs stored for controlled durations of 0, 1, 2, 3, and 4 months, and their efficacy was compared in terms of cytokine content and BMSCs' proliferation and osteogenic differentiation.

Results: BMSCs and ASCs in both HPL and FBS demonstrated a characteristic immunophenotype and multi-lineage differentiation; osteogenic differentiation of BMSCs and ASCs was significantly enhanced in HPL vs. FBS. Multiplex network analysis of HPL revealed several interacting growth factors, chemokines, and inflammatory cytokines. Notably, stem cell growth factor (SCGF) was detected in high concentrations. A majority of cytokines were elevated in HPLs produced from PCs stored for ≤ 4 months vs. > 4 months. However, no further differences in PC storage times between 0 and 4 months were identified in terms of HPLs' cytokine content or their effects on the proliferation, ALP activity, and mineralization of BMSCs from multiple donors.

Conclusions: MSCs expanded in HPL demonstrate enhanced osteogenic differentiation, albeit with considerable donor variation. HPLs produced from outdated PCs stored for up to 4 months efficiently supported the proliferation and osteogenic differentiation of MSCs. These findings may facilitate the standardization and scaling-up of HPL from outdated PCs for BTE applications.

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Xeno-Free Spheroids of Human Gingiva-Derived Progenitor Cells for Bone Tissue Engineering

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Abstract

Gingiva has been identified as a minimally invasive source of multipotent progenitor cells (GPCs) for use in bone tissue engineering (BTE). To facilitate clinical translation, it is important to characterize GPCs in xeno-free cultures. Recent evidence indicates several advantages of three-dimensional (3D) spheroid cultures of mesenchymal stromal cells (MSCs) over conventional 2D monolayers. The present study aimed to characterize human GPCs in xeno-free 2D cultures, and to test their osteogenic potential in 3D cultures, in comparison to bone marrow MSCs (BMSCs). Primary GPCs and BMSCs were expanded in human platelet lysate (HPL) or fetal bovine serum (FBS) and characterized based on *in vitro* proliferation, immunophenotype and multi-lineage differentiation. Next, 3D spheroids of GPCs and BMSCs were formed via self-assembly and cultured in HPL. Expression of stemness- (SOX2, OCT4, NANOG) and osteogenesis-related markers (BMP2, RUNX2, OPN, OCN) was assessed at gene and protein levels in 3D and 2D cultures. The cytokine profile of 3D and 2D GPCs and BMSCs was assessed via a multiplex immunoassay. Monolayer GPCs in both HPL and FBS demonstrated a characteristic MSC-like immunophenotype and multi-lineage differentiation; osteogenic differentiation of GPCs was enhanced in HPL vs. FBS. $CD271^+$ GPCs in HPL spontaneously acquired a neuronal phenotype and strongly expressed neuronal/glial markers. 3D spheroids of GPCs and BMSCs with high cell viability were formed in HPL media. Expression of stemness- and osteogenesis-related genes was significantly upregulated in 3D vs. 2D GPCs/BMSCs; the latter was independent of osteogenic induction. Synthesis of SOX2, BMP2 and OCN was confirmed via immunostaining, and *in vitro* mineralization via Alizarin red staining. Finally, secretion of several growth factors and chemokines was enhanced in GPC/BMSC spheroids, while that of pro-inflammatory cytokines was reduced, compared to monolayers. In summary, monolayer GPCs expanded in HPL demonstrate enhanced osteogenic differentiation potential, comparable to that of BMSCs. Xeno-free spheroid culture further enhances stemness- and osteogenesis-related gene expression, and cytokine secretion in GPCs, comparable to that of BMSCs.