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# Creating Structured Hydrogel Microenvironments for Regulating Stem Cell Differentiation

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**Free article**

## Abstract

The development of distinct biomimetic microenvironments for regulating stem cell behavior and bioengineering human tissues and disease models requires a solid understanding of cell-substrate interactions, adhesion, and its role in directing cell behavior, and other physico-chemical cues that drive cell behavior. In the past decade, innovative developments in chemistry, materials science, microfabrication, and associated technologies have given us the ability to manipulate the stem cell microenvironment with greater precision and, further, to monitor effector impacts on stem cells, both spatially and temporally. The influence of biomaterials and the 3D microenvironment's physical and biochemical properties on mesenchymal stem cell proliferation, differentiation, and matrix production are the focus of this review chapter. Mechanisms and materials, principally hydrogel and hydrogel composites for bone and cartilage repair that create "cell-supportive" and "instructive" biomaterials, are emphasized. We begin by providing an overview of stem cells, their unique properties, and their challenges in regenerative medicine. An overview of current fabrication strategies for creating instructive substrates is then reviewed with a focused discussion of selected fabrication methods with an emphasis on bioprinting as a critical tool in creating novel stem cell-based biomaterials. We conclude with a critical assessment of the current state of the field and offer our view on the promises and potential pitfalls of the approaches discussed.

**Keywords:** biomaterials; biopolymers; differentiation; microenvironments; polyelectrolytes; stem cells; substrates; therapeutics.

Int J Mol Sci
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# Stable Reference Genes for qPCR Analysis in BM-MSCs Undergoing Osteogenic Differentiation within 3D Hyaluronan-Based Hydrogels

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## Abstract

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) enables the monitoring of changes in cell phenotype via the high-throughput screening of numerous genes. RT-qPCR is a fundamental approach in numerous research fields, including biomaterials, yet little attention has been given to the potential impact of 3D versus monolayer (2D) cell culture and to the requirement for a constant validation of the multiple steps of gene expression analysis. The aim of this study is to use high-quality RNA to identify the most suitable reference genes for RT-qPCR analysis during the osteogenic differentiation of human bone marrow mesenchymal stem/stromal cells (BM-MSCs). BM-MSCs are cultured under osteogenic conditions for 28 days in 2D or within hyaluronic acid hydrogels (3D). RNA is subject to quality controls and is then used to identify the most stable reference genes using geNorm, NormFinder, and the ΔCq method. The effect of the reverse transcriptase is investigated, as well as the expression of osteogenic-related markers. This study shows marked differences in the stability of reference genes between 2D (*RPLP0/GAPDH*) and 3D (*OAZ1/PPIA*) culture, suggesting that it is critical to choose appropriate reference genes for 3D osteogenic cell cultures. Thus, a thorough validation

under specific experimental settings is essential to obtain meaningful gene expression results.

**Keywords:** OAZ1; PPIA; RPLP0; RT-qPCR; biomaterials; endogenous control; hyaluronic acid; mesenchymal stem cells; osteogenesis; tissue engineering.

Clin Cancer Res
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# Mesenchymal Stem Cells Successfully Deliver Oncolytic Virotherapy to Diffuse Intrinsic Pontine Glioma

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## Abstract

**Purpose:** Diffuse intrinsic pontine glioma (DIPG) is among the deadliest of pediatric brain tumors. Radiation therapy is the standard of care treatment for DIPG, but offers only transient relief of symptoms for DIPG patients without providing significant survival benefit. Oncolytic virotherapy (OV) is an anti-cancer treatment that has been investigated for treating various types of brain tumors.

**Experimental design:** Here, we have explored the use of mesenchymal stem cells (MSC) for OV delivery and evaluated treatment efficacy using preclinical models of DIPG. The survivin promoter drives the conditional replication of OV used in our studies. The efficiency of OV entry into the cells is mediated by fiber modification with seven lysine residues (CRAd.S.pK7). Patients' samples and cell lines were analyzed for the expression of viral entry proteins and survivin. The ability of MSCs to deliver OV to DIPG was studied in the context of a low dose of irradiation.

**Results:** Our results show that DIPG cells and tumors exhibit robust expression of cell surface proteins and survivin that enable efficient OV entry and replication in DIPG cells. MSCs loaded with OV disseminate within a tumor and release OV throughout the DIPG brainstem xenografts in mice. Administration of OV-loaded MSCs with radiotherapy to mice bearing brainstem DIPG xenografts results in more prolonged survival relative to that conferred by either therapy alone ($p<0.01$).

**Conclusions:** Our study supports oncolytic virus CRAd.S.pK7 encapsulated within MSCs as a therapeutic strategy that merits further investigation and potential translation for DIPG treatment.

Stem Cells Transl Med

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# Mesenchymal stromal cells in Human Immunodeficiency Virus-infected patients with discordant immune response: Early results of a phase I/II clinical trial

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## Abstract

Between 15% and 30% of HIV-infected subjects fail to increase their $CD4^+$ T-cell counts despite continuous viral suppression (immunological nonresponders [INRs]). These subjects have a higher morbidity and mortality rate, but there are no effective treatments to reverse this situation so far. This study used data from an interrupted phase I/II clinical

trial to evaluate safety and immune recovery after INRs were given four infusions, at baseline and at weeks 4, 8, and 20, with human allogeneic mesenchymal stromal cells from adipose tissue (Ad-MSCs). Based on the study design, the first 5 out of 15 INRs recruited received unblinded Ad-MSC infusions. They had a median $CD4^+$ nadir count of 16/μL (range, 2-180) and $CD4^+$ count of 253 cells per microliter (171-412) at baseline after 109 (54-237) months on antiretroviral treatment and 69 (52-91) months of continuous undetectable plasma HIV-RNA. After a year of follow-up, an independent committee recommended the suspension of the study because no increase of $CD4^+$ T-cell counts or $CD4^+$ /$CD8^+$ ratios was observed. There were also no significant changes in the phenotype of different immunological lymphocyte subsets, percentages of natural killer cells, regulatory T cells, and dendritic cells, the inflammatory parameters analyzed, and cellular associated HIV-DNA in peripheral blood mononuclear cells. Furthermore, three subjects suffered venous thrombosis events directly related to the Ad-MSC infusions in the arms where the infusions were performed. Although the current study is based on a small sample of participants, the findings suggest that allogeneic Ad-MSC infusions are not effective to improve immune recovery in INR patients or to reduce immune activation or inflammation. ClinicalTrials.gov identifier: NCT0229004. EudraCT number: 2014-000307-26.

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# HS-5 and HS-27A Stromal Cell Lines to Study Bone Marrow Mesenchymal Stromal Cell-Mediated Support to Cancer Development

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# Abstract

In this study, we compared the overall gene and pathway expression profiles of HS-5 and HS-27A stromal cell lines with those of primary bone marrow MSCs to verify if they can be considered a reliable alternative tool for evaluating the contribution of MSCs in tumor development and immunomodulation. Indeed, due to their easier manipulation *in vitro* as compared to primary MSC cultures, several published studies took advantage of stromal cell lines to assess the biological mechanisms mediated by stromal cells in influencing tumor biology and immune responses. However, the process carried out to obtain immortalized cell lines could profoundly alter gene expression profile, and consequently their biological characteristics, leading to debatable results. Here, we evaluated the still undisclosed similarities and differences between HS-5, HS-27A cell lines and primary bone marrow MSCs in the context of tumor development and immunomodulation. Furthermore, we assessed by standardized immunological assays the capability of the cell lines to reproduce the general mechanisms of MSC immunoregulation. We found that only HS-5 cell line could be suitable to reproduce not only the MSC capacity to influence tumor biology, but also to evaluate the molecular mechanisms underlying tumor immune escape mediated by stroma cells. However, HS-5 pre-treatment with inflammatory cytokines, that normally enhances the immunosuppressive activity of primary MSCs, did not reproduce the same MSCs behavior, highlighting the necessity to accurately set up *in vitro* assays when HS-5 cell line is used instead of its primary counterpart.