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Three-dimensional culture and characterization of mononuclear cells from human bone marrow

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Abstract

Background aims. The diverse phenotypic changes and clinical and economic disadvantages associated with the monolayer expansion of bone marrow–derived mesenchymal stromal cells (MSCs) have focused attention on the development of one-step intraoperative cells therapies and homing strategies. The mononuclear cell fraction of bone marrow, inclusive of discrete stem cell populations, is not well characterized, and we currently lack suitable cell culture systems in which to culture and investigate the behavior of these cells. *Methods.* Human bone marrow–derived mononuclear cells were cultured within fibrin for 2 weeks with or without fibroblast growth factor-2 supplementation. DNA content and cell viability of enzymatically retrieved cells were determined at days 7 and 14. Cell surface marker profiling and cell cycle analysis were performed by means of multi-color flow cytometry and a 5-ethynyl-2′-deoxyuridine incorporation assay, respectively. *Results.* Total mononuclear cell fractions, isolated from whole human bone marrow, was successfully cultured in fibrin gels for up to 14 days under static conditions. Discrete niche cell populations including MSCs, pericytes and hematopoietic stem cells were maintained in relative quiescence for 7 days in proportions similar to that in freshly isolated cells. Colony-forming unit efficiency of enzymatically retrieved MSCs was significantly higher at day 14 compared to day 0; and in accordance with previously published works, it was fibroblast growth factor-2–dependant. *Conclusions.* Fibrin gels provide a simple, novel system in which to culture and study the complete fraction of bone marrow–derived mononuclear cells and may support the development of improved bone marrow cell–based therapies.

Key Words: *bone marrow, bone marrow niche, cell transplantation, FGF_2, mesenchymal stem cells, mononuclear cells*

Introduction

Bone marrow–derived mesenchymal stromal cells (BMSCs) are typically expanded in monolayer before use in cell-based therapies. Monolayer expansion is known to induce a plethora of phenotypic changes in these cells [1]. Telomere shortening [2,3] and epigenetic modifications [4] result in MSCs losing their ability to self-renew and ultimately the induction of senescence [5–7]. Changes in gene expression result in the differential expression of integrins, extracellular matrix molecules, growth factors and cytokines throughout culture [8,9]. Multipotency reduces with prolonged expansion, resulting in a propensity for BMSCs to differentiate osteogenically [2,10]. Such modifications in cell phenotype raise uncertainty with regard the therapeutic efficacy of these cells. Moreover, monolayer expansion of BMSCs is associated with many clinical and economic disadvantages. Specifically, patients are required to have two surgeries to isolate and transplant the cells, which is both a lengthy and costly process. One approach to overcome the limitations associated with monolayer expansion is to develop a one-step BMSC therapy that can be performed intraoperatively. With the development of clinically approved bone marrow–concentrating devices that can be used within the operating room, this approach has the potential for use in mainstream healthcare. A disadvantage of using a heterogeneous population of mononuclear cells could be that the constitutive MSC population is very small; however, at present, there is little evidence in the literature correlating cell number of clinical outcome. Bone marrow and mononuclear cells (MNC) transplantation has demonstrated modest clinical efficacy for the treatment of osteonecrosis [11], fracture non-union

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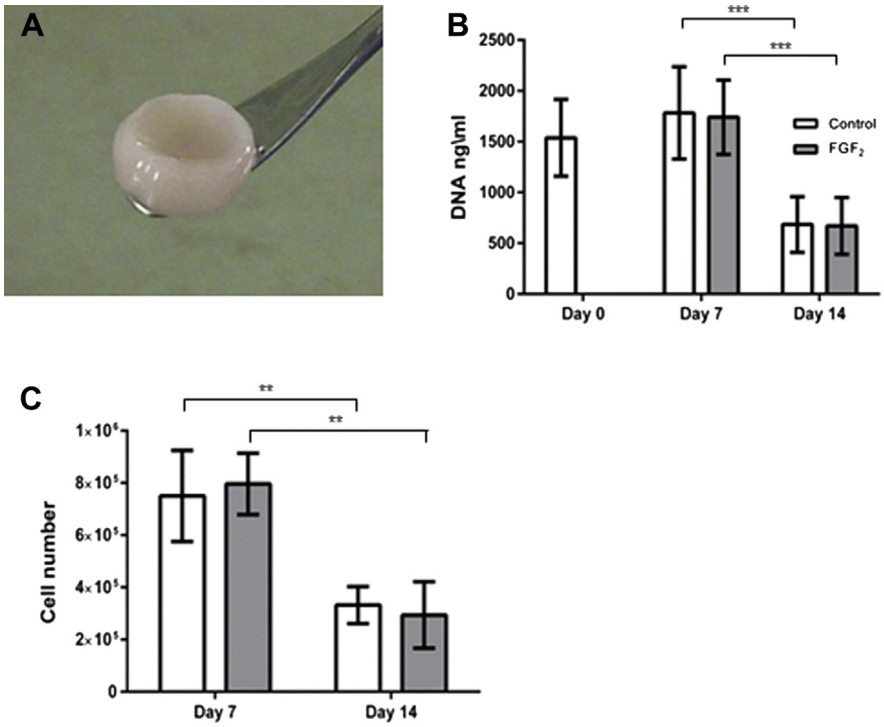

Figure 1. (A) Photograph of a fibrin gel containing MNCs taken after 7 days of culture. (B) DNA content of MNC gels at days 0, 7 and 14 as determined by Picogreen. (C) Number of viable cells retrieved from fibrin after collagenase treatment. Data are presented as mean ± standard deviation; $^{**}P < 0.001$, $^{***}P < 0.0001$.

[12–15] and spinal fusion [16]. Safety but not improvement in clinical outcome has been reported after transplantation after myocardial infarction [17–21]. Thus, it appears that a greater understanding of these cells and fine-tuning is required to harness their therapeutic potential. For example, it may be that growth factor stimulation or gene delivery may improve their regenerative capacity *in vivo*. An alternative approach to intra-operative cell therapy would be to target and mobilize MSCs within the bone marrow to stimulate their innate homing ability to direct intrinsic tissue repair.

The development of one-step MSC/MNC therapies or pharmaceutically driven cell homing strategies both require a better understanding of the behavior of naive MSCs that have not been cultured in monolayer. Thus, there is a need to develop a culture system that retains MSCs in a quiescent state and allows co-culture with other bone marrow cells that would be naturally present. Others have attempted this by culturing MNCs in scaffolds composed of ceramic and hydroxyapatite under perfusion [22,23]. Viable populations of cluster of differentiation (CD)105+ and CD45+ cells were maintained, which subsequently induced ectopic ossification in mice [22]. MNCs have also been cultured successfully in collagen gels, although not quiescently [24]. In the latter study, the proportions of MNC cell types appeared to change during culture, although whether this was due to cell death, cell loss or proliferation of specific cell populations was not clear. Others describe the use of calcium phosphate scaffolds to culture whole, unprocessed bone marrow [25]. Notably, in the aforementioned studies, bone marrow cells were not characterized on initial isolation, making it difficult to determine the effect of *in vitro* culture conditions on the survival and behavior of the constitutive cell populations.

Fibrin gels generated from polymerized fibrinogen and thrombin have been previously used to culture multiple cell types, including MSCs [26], endothelial progenitor cells [27] and cord blood hematopoietic stem cells [28], and as a carrier for bone marrow cell transplantation studies [29,30]. Basic fibroblast growth factor (FGF_2), a potent mitogen of MSCs cultured in monolayer [31,32], appears to be critically important in maintaining an initially non-adherent population of mesenchymal progenitor cells present in freshly isolated MNCs [33]. The aim of this study was to develop a culture system for MNCs—inclusive of naive stem cell populations—that could be later used to maintain and study these cells as close to their *in vivo* phenotype as possible. In this study, we report the use of fibrin to culture MNCs in 3D statically for 7 and 14 days with and without FGF_2 supplementation. Cell surface marker