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Efficacy of 3D Culture Priming is Maintained in Human Mesenchymal Stem Cells after Extensive Expansion of the Cells.

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Abstract

The use of non-optimal preparations of mesenchymal stem cells (MSCs), such as extensively expanded cells, might be necessary to obtain the large numbers of cells needed for many clinical applications. We previously demonstrated that minimally expanded (early passage) MSCs can be pre-activated as spheroids to produce potentially therapeutic factors in 3D cultures. Here, we used extensively expanded (late passage) MSCs and studied their 3D-culture activation potential. MSCs were culture-expanded as 2D monolayers, and cells from various passages were activated by 3D culture in hanging drops with either fetal bovine serum (FBS)-containing media or a more clinically-applicable animal product-free (xeno-free) media. Gene expression analyses demonstrated that MSC spheroids prepared from passage 3, 5, and 7 cells were similar to each other but different from 2D MSCs. Furthermore, the expression of notable anti-inflammatory/immune-modulatory factors cyclooxygenase-2 (PTGS2), TNF alpha induced protein 6 (TNFAIP6), and stanniocalcin 1 (STC-1) were up-regulated in all spheroid preparations. This was confirmed by the detection of secreted prostaglandin E2 (PGE-2), tumor necrosis factor-stimulated gene 6 (TSG-6, and STC-1. This study demonstrated that extensively expanded MSCs can be activated in 3D culture through spheroid formation in both FBS-containing and xeno-free media. This work highlights the possibility of activating otherwise less useable MSC preparations through 3D culture generating large numbers of potentially therapeutic MSCs.

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The Potential Role of Quorum Sensing in Clonal Growth and Subsequent Expansion of Bone Marrow Stromal Cell Strains in Culture.

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Abstract

Clonal development (clonogenicity) is an inherent property of a subset of postnatal bone marrow (BM) adherent stromal mesenchymal stem cells (MSCs) from which a multipotent progeny develops in culture. Our data suggest that clonogenicity and BM-MSC expansion are two distinct biological events.

This hypothesis is based on the following observations: (1) the beginning of clonal growth is a property strictly dependent on serum and independent of the social context, (2) the expansion of individual clone is influenced by events deriving from a social context during initial growth, (3) clonogenic cells grown in a social context in presence of serum can emancipate themselves to generate a secondary different progeny, and (4) the ability of socially generated clones to develop an inherent potential for further growth suggests that quorum sensing may operate in BM-MSC cultures and determine the potential growth of clonal strains.

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Minimally Manipulated Mesenchymal Stem Cells for the Treatment of Knee Osteoarthritis: A Systematic Review of Clinical Evidence.

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Abstract

BACKGROUND:

The use of laboratory-expanded mesenchymal stem cells (MSCs) is subject to several restrictions, resulting in "minimal manipulation" methods becoming the current most popular strategy to increase the use of MSCs in an orthopaedic practice. The aim of the present systematic review is to assess the clinical applications of "minimally" manipulated MSCs, either as bone marrow aspirate concentrate (BMAC) or as stromal vascular fraction (SVF), in the treatment of knee osteoarthritis (OA).

METHODS:

A systematic review of three databases (PubMed, ScienceDirect, and Google Scholar) was performed using the following keywords: "Knee Osteoarthritis" with "(Bone marrow aspirate) OR (bone marrow concentrate)" or with "(adipose-derived mesenchymal stem cells) OR (adipose derived stromal cells) OR (stromal vascular fraction) OR (SVF)" as either keywords or MeSH terms. The reference lists of all retrieved articles were further reviewed for identification of potentially relevant studies.

RESULTS:

Twenty-three papers were included in the final analysis (10 on BMAC and 13 on SVF). Of these, only 4 were randomized controlled trials (RCTs). Bias risk evaluation, performed using a modified Coleman score, revealed an overall poor quality of the studies. In terms of clinical application, despite the

apparent safety of minimally manipulated MSCs and the short-term positive clinical outcomes associated with their use, clinicians reported different preparation and administration methods, ranging from single intra-articular injections to intraosseous applications to administration in combination with other surgical procedures.

CONCLUSIONS:

The available literature is undermined by both the lack of high-quality studies and the varied clinical settings and different protocols reported in the few RCTs presently published. This prevents any recommendation on the use of either product in a clinical practice. Nevertheless, the use of minimally manipulated MSCs (in the form of BMAC or SVF) has been shown to be safe and have some short-term beneficial effects.

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Co-culture of the bone and bone marrow: a novel way to obtain mesenchymal stem cells with enhanced osteogenic ability for fracture healing in SD rats.

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Abstract

BACKGROUND:

Mesenchymal stem cells (MSCs) have great potential for the repair and regeneration of bone fracture, but their optimal origins remain controversial.

METHODS:

Bone marrow-MSCs (BM-MSCs) and bone-bone marrow-MSCs (B-BM-MSCs) were isolated from 12 SD rats, and the morphology, MSC-associated markers, and proliferative capacity of these cells were compared using an inverted microscope, flow cytometry, and CCK-8 assays, respectively. After 14 days of osteoblastic induction, osteoblast phenotypes were detected by ALP and calcium nodule staining, and the expression of BMP-2 and TGF-β1 was observed by western blotting. Then, the rat tibia fracture model was established with 3 groups (n = 6 per group), the control, BM-MSC, and B-BM-MSC groups. Computed tomography (CT) imaging was performed to evaluate fracture healing at weeks 2, 4, and 6. Finally, the fractured bones were removed at weeks 4 and 6, and HE staining was performed to evaluate fracture healing.

RESULTS:

Although the 2 types of MSCs shared the same cellular morphology and MSC-associated markers, B-BM-MSCs had a higher proliferative rate than BM-MSCs from day 9 to day 12 ($p < 0.05$), and the expression levels of ALP and calcium were obviously higher in B-BM-MSCs than in BM-MSCs after osteogenic induction ($p < 0.01$ and $p < 0.001$, respectively). Western blot results showed that the expression levels of BMP-2 and TGF-β1 in B-BM-MSCs were higher than in BM-MSCs before and after osteogenic induction ($p < 0.01$). In the animal experiments, CT imaging and gross observation showed

that B-BM-MSCs had a greater capacity than BM-MSCs to promote fracture healing, as the Lane-Sandhu scores of B-BM-MSCs at weeks 4 and 6 after operation (3.00 ± 0.81 and 9.67 ± 0.94, respectively) were higher than those of BM-MSCs (1.33 ± 0.47 and 6.67 ± 1.25, respectively; both $p < 0.05$). The HE staining results further supported this conclusion.

CONCLUSIONS:

Taken together, our study results proved that MSCs obtained by co-culturing the bone and bone marrow from SD rats had better proliferative, osteogenic differentiation, and fracture healing capacities than BM-MSCs, perhaps suggesting a novel way to obtain MSCs for bone tissue repair.