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Platelet lysate enhances synovial fluid multipotential stromal cells functions: Implications for therapeutic use

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Abstract

Background aims. Although intra-articular injection of platelet products is increasingly used for joint regenerative approaches, there are few data on their biological effects on joint-resident multipotential stromal cells (MSCs), which are directly exposed to the effects of these therapeutic strategies. Therefore, this study investigated the effect of platelet lysate (PL) on synovial fluid–derived MSCs (SF-MSCs), which *in vivo* have direct access to sites of cartilage injury. *Methods.* SF-MSCs were obtained during knee arthroscopic procedures (N = 7). Colony forming unit–fibroblast (CFU-F), flow-cytometric phenotyping, carboxyfluorescein succinimidyl ester-based immunomodulation for T-cell and trilineage differentiation assays were performed using PL and compared with standard conditions. *Results.* PL-enhanced SF-MSC (PL-MSC) proliferation as CFU-F colonies was 1.4-fold larger, and growing cultures had shorter population-doubling times. PL-MSCs and fetal calf serum (FCS)-MSCs had the same immunophenotype and similar immunomodulation activities. In chondrogenic and osteogenic differentiation assays, PL-MSCs produced 10% more sulfated-glycosaminoglycan (sGAG) and 45% less Ca^{++} compared with FCS-MSCs, respectively. Replacing chondrogenic medium transforming growth factor-β3 with 20% or 50% PL further increased sGAG production of PL-MSCs by 69% and 95%, respectively, compared with complete chondrogenic medium. Also, Dulbecco's Modified Eagle's Medium high glucose (HG-DMEM) plus 50% PL induced more chondrogenesis compared with HG-DMEM plus 10% FCS and was comparable to complete chondrogenic medium. *Conclusions.* This is the first study to assess SF-MSC responses to PL and provides biological support to the hypothesis that PL may be capable of modulating multiple functional aspects of joint resident MSCs with direct access to injured cartilage.

Key Words: *platelet lysate, multipotential stromal cells, synovial fluid, chondrogenesis*

Introduction

Osteoarthritis (OA) is the most common musculoskeletal disease affecting an increasing proportion of the population and is a major cause of global health care expenditures [1]. The OA process can begin in multiple locations within the joint, including the cartilage, bone, ligaments, synovium and meniscus, with eventual whole-joint failure [2]. To date, there are limited therapeutic options for the treatment of joint dysfunction in OA. For early OA emanating in cartilage or "chondrogenic" OA, symptomatic treatments with analgesics, nonsteroid medications, steroid injection and physical therapy, rather than regenerative approaches, are invariably offered [3,4]. In more advanced disease, total knee replacement is generally considered in patients older than 65 years, and >160,000 of these are carried out annually in the United Kingdom alone (according to the National Joint Registry, 2017). However, in younger patients with small chondral lesions, regenerative therapies, including microfracture and autologous chondrocyte implantation, may be used. Although these approaches are associated with good short-term pain relief, they generally lead to formation of fibrocartilaginous tissue that is not capable of withstanding mechanical stress over time [5–7].

There is an urgent need for novel regenerative treatment options for isolated articular cartilage defects before they lead to advanced cartilage loss. One experimental strategy is the use of bone marrow–derived (BM-MSCs) [8,9] or adipose tissue–derived (AT-MSCs) MSCs [10,11], with pre-clinical and clinical results showed promising results. However, because of the expense and time-consuming nature of these procedures, as they require clinical grade facilities and times to expand the cells, there is an increasing interest in

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harnessing the power of endogenous joint resident MSCs in conjunction with scaffolds or growth factors or a combination as part of a one-stage procedure.

One potential source of endogenous MSCs that could play a role in OA joint repair is synovial fluid–derived MSCs (SF-MSCs), which are present in OA, rheumatoid arthritis (RA) and non-arthritic joint fluid [12]. Elevated SF-MSC numbers have been reported in early OA and after meniscal injury, suggesting their potential role in physiological joint repair [13,14]. Furthermore, SF-MSCs are easy to isolate through aspiration and are thought to be derived from the adjacent synovium, which is a tissue rich in MSCs with strong chondrogenic potential [15,16]. Finally, animal model studies showed that synovial origin MSCs facilitated cartilage repair following injection directly to the defect area with or without scaffold [17,18]. Because both the topography and high chondrogenic potential of SF-MSCs indicate their potential role in endogenous joint repair, the question arises as to whether this process could be exploited or further enhanced.

Platelet lysate (PL) is a biological platelet derivative rich in growth factors and cytokines that encourage tissue repair, which is driven by a multitude of growth factors including basic fibroblast-derived growth factor, transforming growth factor beta (TGF-β), platelet-derived growth factor-AA, -AB and -BB [19,20]. Increasingly, PL is being injected directly into OA knee joints with some encouraging results [21], but the mechanisms of its action remain unknown. In 2005, Doucet *et al.* proposed the use of PL as a substitute for fetal calf serum (FCS) for MSC expansion intended for cellular therapy. Subsequent studies have shown the capacity of PL to promote cell proliferation while maintaining immunophenotype and trilineage differentiation of BM-MSCs, AT-MSCs and umbilical cord blood MSCs [20,22–25]. The potential interactions between PL and SF-MSCs have not thus far been explored but could be key toward enhancing the endogenous MSC repair responses.

The aim of this study was therefore to evaluate the impact of PL on SF-MSC immunophenotype, proliferation, immunomodulation and trilineage differentiation compared with FCS containing standard expansion medium, with a particular focus of the capacity of PL to act as chondrogenic inducer. We studied commercial PL (Stemulate; Cook Regentec) because it has a more consistent growth factor content as it is produced in large batches to avoid lot-to-lot variation and is available off the shelf in large quantities.

Methods

Isolation and culture of SF-MSCs

Approval for the study was obtained from the national research ethics committee (Rec reference: 14/YH/0087). Samples were collected after informed written consent from all study participants who were undergoing elective knee diagnostic or therapeutic arthroscopy. No effusion was present at time of arthroscopy, and patients with inflamed synovium were not included because inflammation might have an impact on chondrogenesis [15]. To ensure collection of all SF-MSCs, SF was collected after an initial injection of saline (up to 50 mL) (N = 7 patients). The aspirated fluid was centrifuged at 500 rcf for 5 min, and cells were re-suspended in 10 mL Dulbecco's Modified Eagle's Medium (DMEM) (with no FCS). For expansion, 2 mL of cell suspension were cultured in PL medium containing DMEM (Gibco) supplemented with 5% PL-fibrinogen depleted (Stemulate; PL-MSCs) or standard FCS medium (StemMACS, Miltenyi Biotec; FCS-MSCs), each containing 100 units/mL penicillin and 100 mg/mL streptomycin (all from Gibco). Cells were incubated in a humidified atmosphere at 37°C and 5% CO_2, with a full medium change after 48 h, followed by medium changes twice weekly. Once the cells reached 80% confluence, the adherent cells were harvested with 0.25% trypsin/1 mmol/L ethylenediaminetetraacetic acid (EDTA) solution (Sigma-Aldrich) and passaged at a seeding density of $5–7 \times 10^3$ cells/cm^2. Donor-matched cultures were used in all experiments when they reached passage 3. SF-MSC doubling time was calculated from the number of population doublings up to passage 0 as previously described [26], according to the following formula: days in culture until the end of passage 0/population doubling number up to passage 0. The latter (population doubling number) was calculated as = $\log_2$ (N total cell count at p0/Total CFU-F number).

Characterization of the surface marker expression

The following antibodies were used to compare immunophenotype of PL- and FCS-expanded SF-MSCs: CD90-PE-Cy7, CD45-PE-Cy7 and CD19-PE (BD Biosciences Pharmingen), CD105-PE (AbD Serotec), PE-CD73, CD34-APC and CD14-FITC (Miltenyi Biotec), with appropriate isotype controls. DAPI was used to gate out dead cells. Samples were acquired using a three-laser flow cytometer, LSRII (BD Biosciences) and analyzed by FacsDiva version 8.

CFU-F assay

Freshly obtained SF cells were plated in duplicate 60-mm-diameter petri dishes (Greiner Bio) with either FCS or PL medium. Cells were incubated in a humidified atmosphere at 37°C and 5% CO_2, with a full medium change after 48 h, followed by half medium changes three times a week. Colonies were stained with 1% methylene blue after 2 weeks of culture for

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