Microcarrier culture of MSCs with platelet lysate as a serum substitute

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INTRODUCTION

The concerns about the use of animal derived products for the production of therapies in human patients has resulted in the search for alternatives that can replace FBS. One of these possible substitutes is platelet lysate (PL), as platelets are a rich source for various growth factors such as platelet derived growth factor (PDGF) and transforming growth factor beta (TGF-β).

For the production of cells for cell therapy in patients, an upgrade to large scale culture is necessary. Large scale cultures require an efficient process, for which a stirred culture suspension with microcarriers was used.

Unfortunately, large aggregates form when PL is used with microcarriers. Therefore, a pre-treatment of PL to stop the aggregates from forming is essential. Here, we present two methods to use PL in microcarrier culture.

MATERIALS AND METHODS

PL was derived from pooled buffycoats of ten donors. Two types of PL culture medium was prepared: 1) heat-inactivation of PL (HIPL) at 58 C for 30 min followed by centrifugation at 2000*g* for 30 min after which supernatant was used to supplement the culture medium; and 2) pre-gelling of PL (PGPL) by adding PL to culture medium followed by a 90 min incubation period under continuous rotating. The dense cloth that formed was removed. Human MSCs were isolated from bone marrow aspirates and cultured in a monolayer in medium with 5% PL. Culture medium supplemented with 15% FBS was used as a control.

When approximately 80% confluent, cells were harvested for FACS analysis, and seeded for growth curve and into spinner flasks containing Cytodex-1 microcarriers. Cell growth was determined during microcarrier culture. After harvesting the MSCs from the microcarriers the differentiation potential was determined by a standard differentiation assay.

RESULTS

Visual inspection showed that from the first passage an onwards MSCs cultured with HIPL were smaller and more elongated when compared with the cells cultured with FBS or PGPL. From passage 4 and following passages, the MSCs cultured with PGPL became smaller, more condensed and multiple dendrites per cell formed when compared with the MSCs cultured with FBS (Fig 1A).

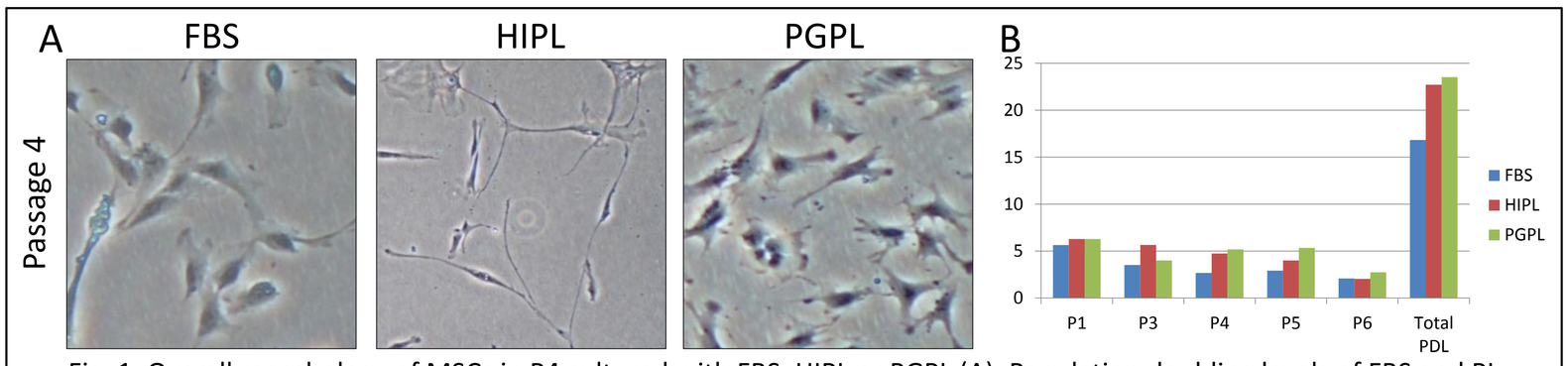
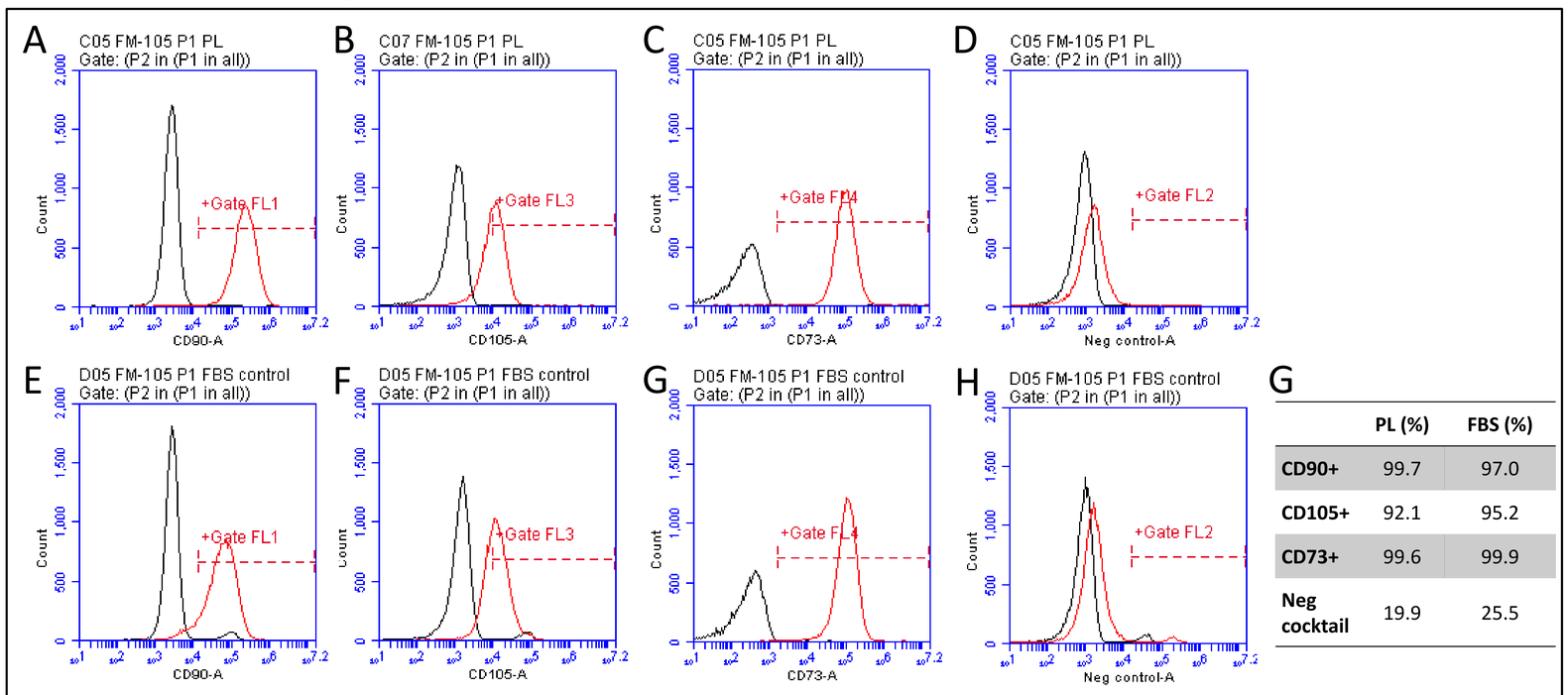


Fig. 1. Overall morphology of MSCs in P4 cultured with FBS, HIPL or PGPL (A). Population doubling levels of FBS and PL cultured MSCs in P1 – P6 (B).

Analysis of cell growth showed that, overall, PGPL resulted in the highest cell numbers (Fig. 1B). Total population doubling level (PDL) was determined for FBS (16.8), HIPL (22.7) and PGPL (23.5). FACS analysis confirmed the presence and absence of specific surface markers (Fig 2).



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	PL (%)	FBS (%)
CD90+	99.7	97.0
CD105+	92.1	95.2
CD73+	99.6	99.9
Neg cocktail	19.9	25.5

Fig. 2. FACS analysis of PGPL cultured MSCs (top row; A-D) and FBS cultured MSCs (bottom row; E-H). Expression of specific MSC markers was similar for both PGPL and FBS cultured MSCs (G).

The potential of PL in a microcarrier culture was assessed using spinner flasks. MSCs were able to attach to and proliferate on Cytodex-1 microcarriers regardless the culture medium used. In all medium types aggregates of >10 microcarriers were observed (Fig. 3A). Cell proliferation on Cytodex-1 microcarriers showed that in the first 10 days of culture the MSCs on FBS had the best proliferation. After 12 days the proliferation rate increased for both PL media, whereas FBS cultured MSC proliferation from this point on decreased. Overall, MSCs cultured with PGPL had the highest cell numbers (Fig. 3B). A standard differentiation assay showed no differences in the differentiation capacity of the MSCs cultured with PGPL, HIPL or FBS supplemented medium.

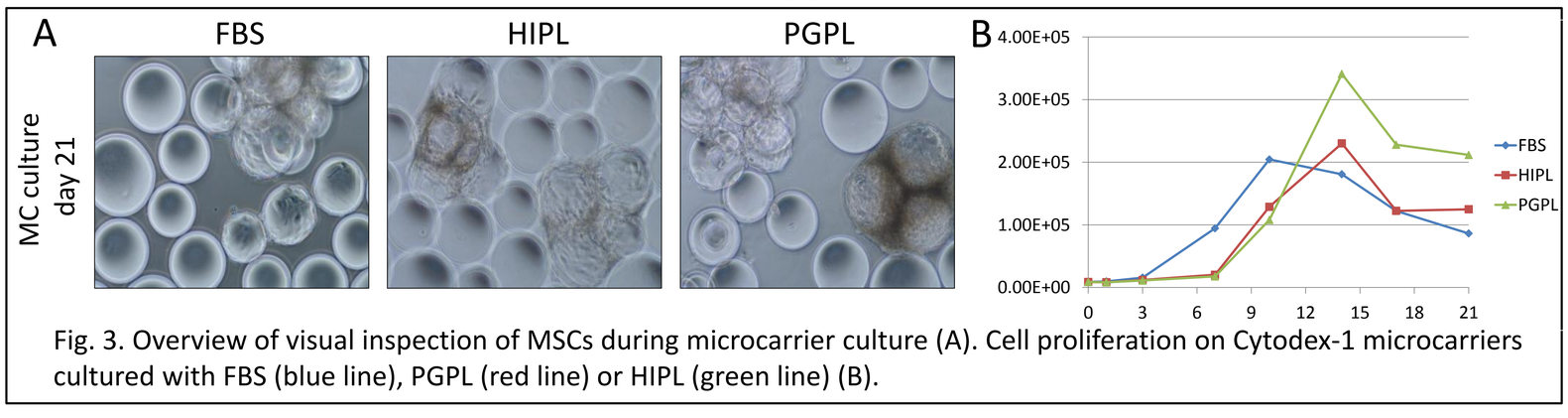

Fig. 3. Overview of visual inspection of MSCs during microcarrier culture (A). Cell proliferation on Cytodex-1 microcarriers cultured with FBS (blue line), PGPL (red line) or HIPL (green line) (B).

CONCLUSION AND DISCUSSION

Based on these results we conclude that with the correct pre-treatment, it is possible to use PL in a microcarrier culture with similar results as FBS. Therefore, PL can be used for the large-scale cultivation of human MSCs for cell therapy.

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