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Human platelet lysate promotes Synovial fluid Mesenchymal Stem Cell proliferation and differentiation

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Background

Both synovial and related synovial fluid-derived mesenchymal stem cell (SF-MSC) are highly proliferative and chondrogenic and have direct access to superficial cartilage injuries[1]. Human platelet lysate (PL) is being increasingly used for joint regenerative approaches but the biological basis effect, if any is lacking. We investigated whether combining Human PL with SF-MSCs might enhance MSC activities and represent a novel therapeutic application towards cartilage repair.

Methods and Results

PL stimulates SF-MSC migration:

We first looked at the effect of PL on SF-MSC migration. Using trans-well insert assay we examined whether StemMACS (FCS media) or DMEM with 10% Stemulate (Cook Regentec) PL fibrinogen rich (PL-FR) or fibrinogen depleted (PL-FD) can attract SF-MSCs. After 24 hours there was a significant increase in SF-MSC migration toward 10% PL-FD or PL-FR compared to 10% FCS ($P<0.05$) Figure (1,A). The presence of fibrinogen in the PL had no significant difference on SF-MSC migration ($P>0.5$). However as concentrations of FCS and PL increased in the attracting media, the numbers of migrated cells were significantly decreased ($P<0.05$) Figure (1,B&C).

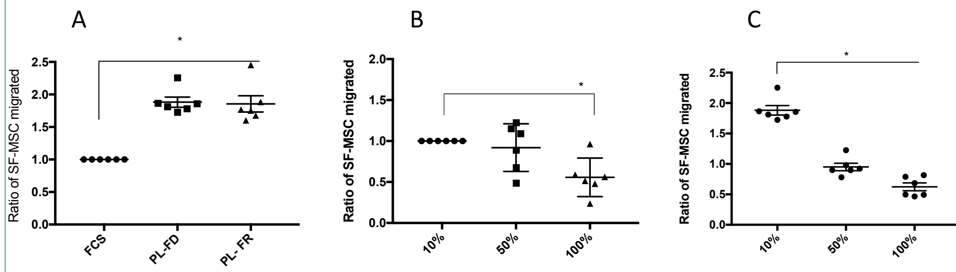

Figure (1): PL stimulates SF-MSC migration using trans-well insert. Cells were serum starved for 24 hours with DMEM containing 0.5% FCS then loaded at the upper chamber. At the lower chamber, DMEM with 10% FCS, 10% PL-FD and 10% PL-FR media was added. Migrated cells were counted under a light microscope. A: ratio of of SF-MSC migrated to 10% FCS,10% PL-FD and 10% PL-FR. B: SF-MSC migrated toward 10%, 50%, and 100% FCS. C: SF-MSC migrated toward 10%, 50%, and 100% PL. (n=6) paired t-test Wilcoxon *p<0.05.

Adhesion and proliferation:

For evaluating the impact of PL on SF-MSC colony-forming unit fibroblasts (CFU-F), freshly isolated SF-MSCs obtained from synovial fluid were plated in FCS media or with DMEM with 10% PL-FR or PL-FD. The results showed there was no significant difference in colony number of SF-MSC plated with FCS media compared to PL-FD and PL-FR ($P>0.5$) Figure (2,A). Moreover, there was no different in the colonies number when cells were plated with PL or FCS media for first 48 hours ($P>0.1$) Figure (2,B). On the other hand, PL significantly increased SF-MSC proliferation, as size of the colonies was significantly bigger compared to FCS ($P<0.05$) Figure (2,C). There was no significant difference in the size of the colonies of SF-MSC seeded with PL-FD or PL-FR ($P>0.1$). At the same time PL significantly shortened doubling time of SF-MSC as compared to FCS ($P< 0.05$) Figure (2,D).

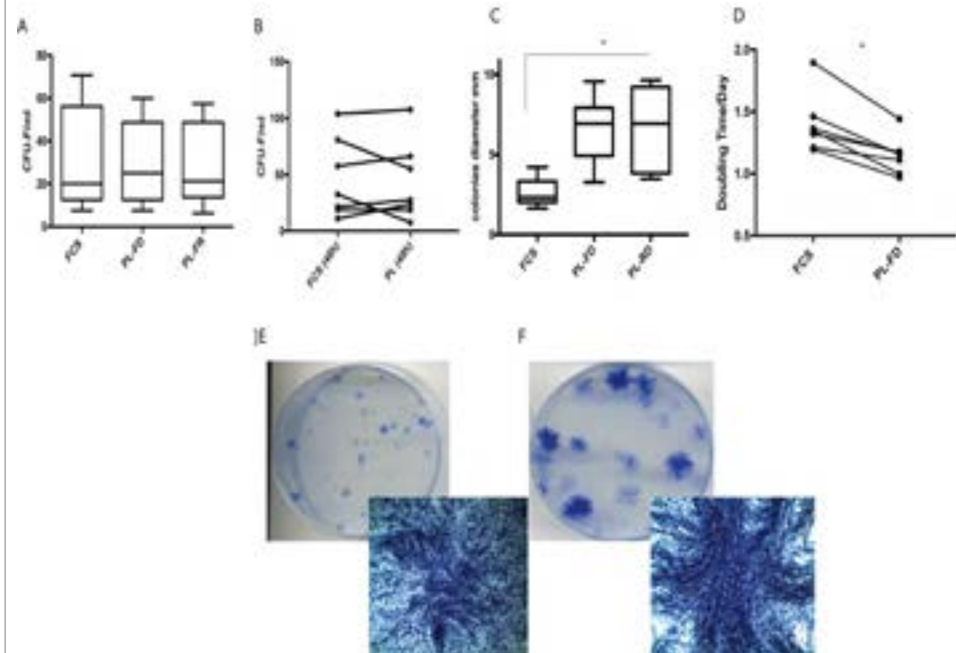

Figure (2): Impact of PL on SF-MSC proliferation A: Average colonies number. B: Impact of PL on SF-MSC adhesion; cells were divided into 2 groups: FCS(48H): cells seeded with FCS media for 48 hours then washed and added to 10% PL media for the next 12 days. PL(48H): cells seeded with 10% PL media for 48 hours then washed and added to FCS media for the next 12 days. C: Average colonies diameter in mm, measured using image J. D: Cultured cells doubling time at p0. E: CFU-F in FCS media, F: CFU-F PL-FD. (n=7) paired t-test Wilcoxon *p<0.05.

The differentiation potential of PL expanded SF-MSCs:

To assess the osteogenic differentiation potential of SF-MSCs expanded in PL (PL-MSC) compared to SF-MSC expanded in FCS (FCS-MSC), cells were cultured in osteogenic media and calcium deposition was assessed at 3 weeks culture [5]. For adipogenic induction we used standard adipoinductive media and cells were double stained with Nile Red and 4',6-diamidino-2-phenylindole (DAPI). For chondrogenic differentiation, chondrogenic media and cells were prepared as previously described [6]. Our results showed SF-MSCs expanded in PL or FCS media had no significant difference in Ca^{++} ($P>0.1$) Figure (3,A). However, there was significate increase in Ca^{++} in both FCS-MSC and PL-MSC when 10% of PL was used as alternative to FCS in the osteogenic media ($P<0.005$) Figure (3,A). Same pattern was shown with adipogenesis; no significant difference between PL and FSC expanded cells and there was significant increase in the adipogenic induction when PL was used instead of FCS in the adipogenic media ($P<0.05$) Figure (3,B).

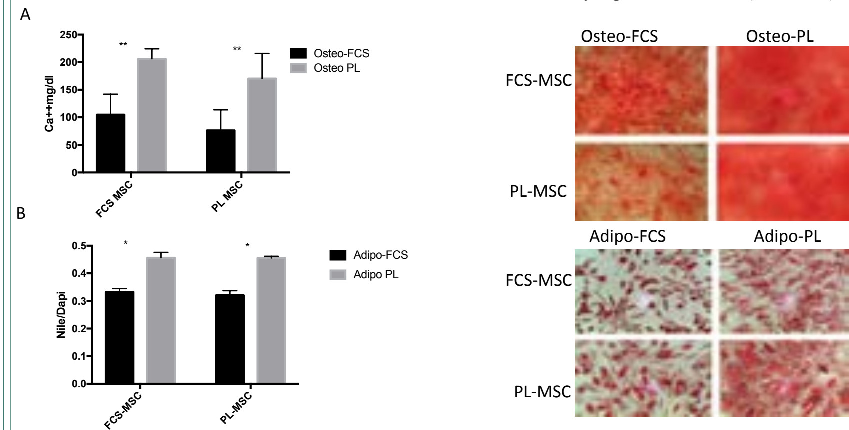

Figure (3): The differentiation potential of SF-MSC. A: Ca^{++} deposition and Alizarin Red red staining after 21 days culture with Osteo-FCS: standard osteogenic media, Osteo-PL: FCS in osteogenic media were replaced with PL. B: Nile Red /DAPI ratio Adipo and oil red staining, Adipo-FCS: standard adipogenic media, PL-Adipo: FCS in adipogenic media were replaced with PL. (n=5) 2 ways ANOVA Tukey post t-test **p<0.005, *p<0.05.

Under standard chondrogenic conditions, there was no significant difference in sGAG production between FCS-MSCs and PL-MSCs Figure (4,A&B). On the other hand replacing TGFβ with 20% PL or 50% PL in the chondroinductive media significantly increase sGAG production compared to standard chondrogenic media ($P<0.005$ and $p<0.0005$) Figure (4,C). Interestingly high glucose media with 50% PL produced sGAG as good as standard chondrogenic media.

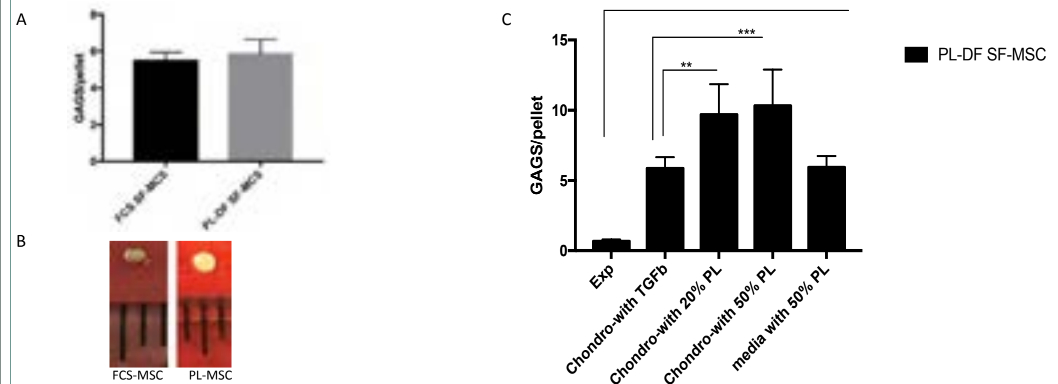

Figure (4):Chondrogenesis and PL . A: sGAG activity of FCS-MSC and PL-MSC with standard chondrogenic media. B: FCS-MSC and PL-MSC pellets after 21 days culture in chondrogenic media. C: sGAG activity of PL-MSC in different chondrogenic media; Exp: high glucose+10% FCS, Chondro-with TGFβ, Chondro-with 20% PL: TGFβ replaced with 20%PL, Chondro-with 50%PL : TGFβ replaced with 50%PL, media with 50%PL: high glucose DMEM with 50%PL. (N=5), 2 ways ANOVA Tukey post t test ****p<0.0001,***p<0.0005**p<0.005, *p<0.05.

Discussion

Our data showed that PL enhanced SF-MSC migration and proliferation. Several studies have also showed that PL induces bone marrow-MSC differentiation [8-12]. In this study we demonstrated that SF-MSC expanded in PL retained their potential to differentiate into osteogenic, adipogenic and chondrogenic lineage and PL can be used as alternative to FCS in osteogenic assay. Interestingly 20% of PL also can be used as alternative to TGFβ to induce chondrogenesis. On the other hand, PL with high glucose media was efficient enough to induce chondrogenesis as good as complete chondrogenic media.

Conclusion

Our research showed a new novel approach that can be applied in management of cartilage damage. PL significantly enhanced migration and proliferation of SF-MSC. Moreover, PL has potential to enhance osteogenesis and adipogenesis. Interestingly, PL with high glucose media alone appears to enhance chondrogenesis. This study provides the first step toward a potential cartilage repair by combining native SF-MSC with PL.

References:

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