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**Article:**
Use of platelet lysate for bone regeneration - are we ready for clinical translation?

Altaie A et al. Platelet lysate for bone regeneration

Ala Altaie, Heather Owston, Elena Jones

Ala Altaie, Heather Owston, Elena Jones, Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, St. James’s University Hospital, Leeds LS9 7TF, United Kingdom

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Abstract

Current techniques to improve bone regeneration following trauma or tumour resection involve the use of autograft bone or its substitutes supplemented with osteoinductive growth factors and/or osteogenic cells such as mesenchymal stem cells (MSCs). Although MSCs are most commonly grown in media containing fetal calf serum, human platelet lysate (PL) offers an effective alternative. Bone marrow (BM)-derived MSCs grown in PL-containing media display faster proliferation whilst maintaining good osteogenic differentiation capacity. Limited pre-clinical investigations using PL-expanded MSCs seeded onto osteoconductive scaffolds indicate good potential of such constructs to repair bone in vivo. In an alternative approach, nude PL-coated scaffolds without seeded MSCs have been proposed as novel regenerative medicine devices. Even though methods to coat scaffolds with PL vary, in vitro studies suggest that PL allows for MSC adhesion, migration and differentiation inside these scaffolds. Increased new bone formation and vascularisation in comparison to uncoated scaffolds has also been observed in vivo. This review outlines the state-of-the-art research in the field of PL for ex vivo MSC expansion and in vivo bone regeneration. To minimise inconsistency between the studies, further work is required towards standardisation of PL preparation in terms of the starting material, platelet concentration, leukocyte depletion, and the method of platelet lysis. PL quality control procedures and its “potency” assessment are urgently needed, which could include measurements of key growth and attachment factors important for MSC maintenance and differentiation. Furthermore, different PL formulations could be tailor-made for specific bone repair indications. Such measures would undoubtedly speed up clinical translation of PL-based treatments for bone regeneration.

Key words: Platelet lysate; Bone regeneration; Mesenchymal stem cells

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Core tip: Human platelet lysate (PL) offers an exciting opportunity for expanding mesenchymal stem cells (MSCs), as well as bone regeneration as a scaffold coating. In this review we describe the state-of-the-art research in the area of bone regeneration utilising PL and MSCs and emphasise the need for standardisation of PL preparation and its quality control in order to progress further in this exciting area of research. Different PL formulations could be tailor-made for specific scaffolds and bone repair indications, both in autologous and allogenic settings. More preclinical and clinical work is needed to progress this research into clinical translation.

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INTRODUCTION
Bone regeneration following trauma, tumor resection or as a result of chronic or degenerative bone disease represents a serious clinical problem. The use of autograft bone is limited by its volume and accessibility and is known to be associated with donor site morbidity and pain[1]. New strategies involve the use of allograft, xenograft or synthetic scaffolds, often combined with patient's own stem cells or growth factors. Multipotential stromal cells, also termed mesenchymal stem cells (MSCs) reside in the bone marrow (BM) and other connective tissues in humans and are quite abundant in vivo making them the preferred choice for use in bone regeneration[2]. In the past, MSCs were derived from patient's own BM and seeded on osteoconductive scaffolds following ex vivo culture expansion, these clinical studies yielded promising results and good long-term safety profiles[3, 4]. However, in most of these studies MSCs were grown in media containing fetal calf or fetal bovine serum (FCS/FBS), a source of bioactive molecules required for MSC attachment and proliferation. Although FCS is a well known supplement for MSC expansion, its major drawback is a possibility for triggering immunological responses in the recipient against xenogenic antigens present in FCS. In this review we will describe the current knowledge on the use of a potential alternative to FCS, human platelet lysate (PL), for ex vivo MSC expansion and in vivo bone regeneration, highlighting various steps in PL manufacture and their possible impact of PL bioactivity.

THE USE AND BENEFITS OF HUMAN PLATELET-RICH PLASMA AND PL AS FCS REPLACEMENTS
As mentioned above, there is an urgent need for an alternative to FCS as a medium supplement. The straightforward substitute is human serum (HS) and successful expansion of MSCs with the use of autologous serum has been achieved in several independent studies[5, 6]. On the other hand, the reports on the efficacy of allogenic HS have been contradictory[7, 8]. One important consideration is the age of the donor, as serum from elderly individuals seems inadequate to support MSC proliferation or differentiation[9, 10].

An alternative to HS is platelet-rich plasma (PRP), which was shown to enhance
MSC proliferation in *ex-vivo* culture as early as 2006\(^\text{11}\). PRP is commonly produced from whole blood collected using EDTA as an anticoagulant. A low-speed centrifugation (10 min at 230–270 g) is performed first, followed by high-speed centrifugation (10 min at 2300 g or higher). The supernatant is called platelet-poor-plasma (PPP). Subsequently, 9/10\(^{th}\) of the supernatant (PPP) volume is removed, and the platelet pellet is resuspended in 1/10\(^{th}\) PPP volume, which is then referred to as PRP\(^\text{12}\). To activate platelets and hence maximise the release of growth factors from platelets’ alpha granules into the PRP, thrombin is added, the product is called tPRP\(^\text{13}\).

PRP as a supplement for MSC culture has several drawbacks. Firstly, not all growth and attachment factors are released from platelets without their activation\(^\text{14, 15}\). Secondly, the presence of fibrinogen and the formation of fibrin clots after thrombin stimulation are suggested to be the reasons for the partial loss of platelet derived growth factor BB (PDGF-BB) content. A substantial amount of released PDGF-BB was found to be trapped in fibrin glue, so the maximum amount of PDGF-BB could not be obtained\(^\text{13}\). Thirdly, the storage temperature of the PRP should be no less than 4°C. If the PRP is frozen at -20°C or -80°C, it could no longer be referred to as PRP as freezing releases growth factors and cytokines from the platelets. Yet some early studies have referred to platelet concentrates which have been frozen as PRP\(^\text{11}\).

In many ways, these drawbacks can be mitigated with the use of PL instead of PRP. Shish and Burnouf summarises the main production methods of PL materials for stem cells expansion and regenerative medicine\(^\text{12}\). During PL manufacture platelets are lysed, and sometimes activated prior to their lysis, in order to release the maximum amount of bioactive molecules and reduce batch-to-batch variation. Filtering is performed to remove cellular debris and WBC contamination is minimised by leukodepletion steps. In order to use pooled PL, the blood group type being used for PL preparation is important to avoid immunogenic reaction \(^\text{16}\). Although MSCs have been shown not to absorb immunogenic ABO antigens from culture media containing FCS or human substitutes such as AB serum and PRP, MSC could absorb these antigens from the fresh AB plasma used for washing and cell infusion\(^\text{17}\). In order to prevent contact of MSCs to ABO blood group antigens,
platelet concentrates derived from blood group O are combined with plasma from blood group AB\cite{12, 15, 18}. Recent studies have shown that MSC population doublings and expansion kinetics were significantly enhanced in PL-supplemented BM MSC cultures compared with cultures supplemented with FCS, HS, or tPRP\cite{18, 19}. Clinically relevant numbers of MSCs could be obtained within the first to second passage in PL-supplemented cultures. This rapid increase in MSC yields could be due to increased MSC attachment (evident as increased colony numbers) as well as their faster proliferation (evident as bigger colony sizes), illustrated in Figure 1. Several other studies have shown that media supplemented with PL shortened MSC expansion time, while preserving MSC phenotype and differentiation capacities\cite{20-24}. BM MSC grown with 100 mL/L PL efficiently differentiated into the adipogenic, osteogenic and chondrogenic lineages\cite{25-28}. Furthermore, PL was shown to be superior to FCS for BM MSC adipogenesis differentiation\cite{26, 27}. There is also evidence that PL soluble factors can regulate osteogenesis via RUNX2- and Osx-independent pathways\cite{25, 29}. The effect of PL on MSC immunoregulation capacity remains to be further explored. One study suggested that the fibrinogen depletion step was necessary in PL manufacture in order to prepare clinical-grade MSCs intended for immunomodulation\cite{30}. In another study, inhibition of T cell proliferation was observed equally in MSCs cultured in PL and FCS\cite{18}. The other advantage of PL is that it can be prepared from expired platelet concentrates (older than 5 days) that are no longer suitable for use in blood transfusion. The PL produced from expired platelets has been shown to have the same impact on MSC growth and osteogenic differentiation as PL from fresh platelets in one study\cite{28}. Freezing and thawing seems to be the most effective process to release growth factors in platelet samples, because both WBCs and platelets are destroyed by this procedure and growth factors are not stable in plasma\cite{31}.

**GROWTH AND ATTACHMENT FACTORS PRESENT IN PL**

Contained within the alpha granules of platelets are a broad spectrum of growth factors that have been shown to enhance MSC proliferation. These include PDGF-
AA, PDGF-AB/BB, transforming growth factor-β1 (TGF-β1) (Table 1), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) with low batch-to-batch variability, and most of these growth factors are stable for up to 14 days\[^{32, 33}\]. The higher level of these growth factors and cytokines in PL compared to FCS and HS could in part explain why PL-expanded MSCs grow faster, however, only a few studies have presented comparisons of the growth factor levels in PL compared to FCS \[^{15, 26, 34}\]. Interestingly, PDGF-BB was not detected in FCS\[^{26, 34}\]. When PDGF-BB was low in the growth medium, BM MSC cell yield was found to be poorer in comparison to a higher PDGF-BB level media\[^{27}\]. Also, the inhibition of PDGF-BB in medium supplemented with 100 mL/L PL significantly decreased MSC proliferation\[^{19, 33}\]. However, the addition of the equivalent concentration of recombinant PDGF-BB to the media did not significantly enhance MSC proliferation\[^{33}\]. Therefore, it is possible that PDGF-BB could be the main but not the only factor responsible for enhanced MSC proliferation and other growth factors, for example TGF-β1 and bFGF are involved.

The presence of the chemokines and attachment factors in the MSC growing media is equally important. In relation to chemokines and soluble adhesion molecules, PL has been shown to contain chemokine (C-C) ligand 5 (CCL5/RANTES), CXCL1, CXCL2 and CXCL3 and, in particular, very high concentrations of soluble cluster of differentiation 40 ligand (sCD40L), soluble vascular cell adhesion molecule (sVCAM), and soluble intracellular adhesion molecule (sICAM)\[^{33}\]. The attachment factors are important for adhesion of MSCs to plastic surfaces whereas chemokines ensure MSC migration and equal distribution across these surfaces.

**IN VIVO BONE FORMATION USING PL EXPANDED MSCS IN PRE-CLINICAL INVESTIGATIONS**

As mentioned above, PL provides several advantages over FCS for MSC expansion, however, PL preparation methods remain varied. Table 1 summarises the most commonly used methods to obtain PL including those used for pre-clinical investigations\[^{25, 35-38}\]. Most of the studies shown in Table 1 prepared PL via apheresis in a closed system using centrifugation to separate cells by weight (specific
gravity), or filtration to separate cells by size, or used a combination of both, with the resulting platelet count of $1 \times 10^9$ platelet/mL. Such platelet concentrates have a larger volume (typically 200–300 mL), and their total platelet content is about 6–8 fold higher of a platelet concentrate from a single whole-blood donor\cite{12}. Although it is known that the apheresis procedure results in low WBC count, precise levels of WBCs present in the platelet concentrate were not reported in these studies. Alternatively, two studies used the buffy coat procedure\cite{15, 18} to obtain a platelet concentrate and then combined it with inline filtration to reduce WBC contamination.

Only a few pre-clinical studies investigated in vivo bone generation using PL-expanded BM MSCs (Table 2). All of these studies have reported some success in new bone formation in vivo. Notably there were differences in scaffolds and animal models, making direct comparisons of bone repair outcomes difficult (Table 2). Only two of the five selected studies succeeded to present the results of in vivo bone formation on a scaffold seeded with MSCs grown fully with PL without any exposure to FCS\cite{25, 35}. Other studies introduced FCS at different stages of the study - either for MSC expansion purposes\cite{36, 37} or for in vitro seeding of MSCs onto the scaffold\cite{37}. To fully eliminate FCS-exposure for clinical translation, it is important to use PL from the beginning and throughout all stages of investigation. Pre-coating scaffolds with PL is likely to be advantageous for MSC attachment. With the use of a natural xenograft bone scaffold, it is possible to achieve good attachment and proliferation of solely PL-expanded BM MSCs by 3 hours incubation of the BM MSC in DMEM supplemented with 50 mL/L PL media, as illustrated on Figure 2.

**IN VITRO AND IN VIVO BONE FORMATION USING PL COATED SCAFFOLDS**

Seeding exogenous MSCs onto a scaffold prior to implantation may not be necessary for repairing some types of bone defects. In this scenario, the combination of PL with a ‘nude’ bone scaffold could be an attractive answer to target homing, attachment and differentiation of neighbouring endogenous MSCs to the repair site. The initial in vitro studies investigating this varied in scaffold type and the method of combining PL with the scaffold. The simplest method involved overnight incubation
of a hydroxyapatite/beta-tricalcium phosphate (HA/β-TCP) scaffold immersed in PL\textsuperscript{37}. Methods utilising polymer based scaffolds involved layer by layer assembly \textsuperscript{39} or combining PL loaded nanoparticles with a polymer powder\textsuperscript{40}. Of note, ‘soft’ PL loaded scaffolds have also been explored for use in wound healing\textsuperscript{41} and cartilage regeneration\textsuperscript{42}, where methods included overnight incubation of polymer-fibrin scaffolds with a fibrinogen and PL solution or mixing PL with a polymer solution to create a hydrogel, respectively. Freeze-thaw cycles, using at least two rounds were the most common method of preparing PL from PRP.

A concern remains whether PL proteins could block the scaffolds’ pores, thus preventing cell infiltration into scaffold. However, a similar scaffold pore size and 3D structure was reported after the addition of PL in two studies\textsuperscript{40,42}. Growth factor release from PL loaded scaffolds varied between studies, where 50ml/L growth factor release after 1 day\textsuperscript{40} or 2 days\textsuperscript{41} was reported, with 91ml/L release by 7 days\textsuperscript{41}. Importantly, the bioactivity of growth factors from loaded scaffolds was comparable to PL controls up to 7 days\textsuperscript{41}. When MSCs were seeded onto the scaffolds (all expanded in 100 mL/L FCS), \textit{in vitro} cell adhesion and migration appeared to be improved by the addition of PL\textsuperscript{37,40,42}. Furthermore, the presence of PL appeared to induce faster osteogenic differentiation\textsuperscript{40}. When a non-osteoconductive polymer scaffold was used, Ca\textsuperscript{2+} was deposited throughout the scaffold indicating that MSCs differentiated into osteoblasts\textsuperscript{39}.

Some studies progressed to investigate the PL coated scaffolds and membranes \textit{in vivo}. In one study a ‘healing impaired’ diabetic mouse model for full thickness skin wounds was used. At 15 days post injury the PL loaded scaffold group had significantly smaller open wound measurements in comparison to controls \textsuperscript{41}. Further histological evaluation showed higher levels of collagen deposition\textsuperscript{41}. In another aforementioned study (Table 2), HA/β-TCP scaffolds, PL loaded or unloaded seeded with MSCs were inserted subcutaneously into SCID mice for 7 weeks\textsuperscript{37}. Evidence of new bone formation and vascularisation was seen in PL coated compared to uncoated scaffold controls\textsuperscript{37}. PL coated scaffolds without prior MSC seeding were used as controls; however, this data was not described in the paper. In the future this type of data could provide an exciting insight into whether
pre-seeding with MSCs is needed or if the PL coating is enough to encourage \textit{in vivo} bone formation.

Based on the available literature it can be concluded that there are two types of PL incorporation into a scaffold, either simply by overnight incubation of a pre-fabricated scaffold with PL or through addition of PL into the scaffold manufacturing process. The former of which has \textit{in vivo} evidence that it may improve bone regeneration. However, the optimal technique of PL incorporation onto a scaffold is likely to depend on the nature of the scaffold itself (chemistry, porosity, its basic architecture) as well as its intended clinical use. For example, PL-loaded scaffold intended for use as ‘hard’ bone void filler, should aim to achieve faster MSC differentiation. On the other hand, PL loaded onto a ‘soft’ guided bone regeneration device (a polymer or natural membrane) should speed up MSC homing and proliferation rather than to induce their differentiation. These disparate objectives could be achieved by fine-tuning PL formulations and using different methods of PL incorporation into the desired scaffold.

For orthopaedic applications, ideally ‘off-the-shelf’ PL-loaded and packaged scaffolds could be made available to surgeons in the operating theatre, however currently no data exists on long-term storage, sterility and stability of PL-loaded scaffolds. Alternatively, commercially available scaffolds could be loaded with autologous PL during surgery. Although theoretically possible, it remains unclear whether PL preparation and loading can be achieved to fit into intra-operative time frames, so far, only overnight incubation has been evaluated pre-clinically. Further investigation into speeding up the production of autologous PL for use in surgery, as well as whether allogenic ‘bottled’ PL can be produced consistency in larger volumes to a controlled standard is needed for this area to progress.

\textbf{FUTURE CONSIDERATIONS FOR THE USE OF PL IN BONE REPAIR}

One of the advantages of PL is its relative ease of preparation (Figure 3A). Nevertheless, there are a few points that should be considered if PL is used for clinical translation for bone repair (Figure 3B). The blood collection method, WBC reduction step, platelet concentration method, freeze and thaw temperatures, and addition of the anticoagulation factors to each of these steps might affect the growth
and attachment factors levels in PL that in turn influence MSC growth and differentiation.

The first step of the PL preparation is the collection of the platelet concentrate either by apheresis, buffy coat or PRP methods. Buffy coat and PRP methods are procedures to obtain platelet concentrate from whole blood by two step centrifugation, for buffy coat first with low speed spin and second with high speed spin. Conversely, the PRP method starts with high speed spin followed by a low speed spin (Figure 3A). However, study variations are expected as apheresis leads to a high platelet count and lower WBC contamination compared to the buffy coat and PRP methods\cite{31, 43, 44}. The buffy coat method tends to reduce the WBC contamination, compared to PRP\cite{44}. The leucocyte reduction step is vital as WBC-derived molecules could influence MSC expansion\cite{45}. Upon WBC lysis, matrix metalloproteinases (MMPs) and free oxygen radicals may be released that could lead to MSC detachment and apoptosis\cite{46}. Some studies tend to apply extra centrifugation steps to reduce WBCs in the platelet concentrates but a degree of WBC contamination can still be seen, which also impacts the growth factors’ levels in PL\cite{31, 47}. Therefore, it is imperative to include rigorous leucocyte depletion steps in PL preparation procedure, as centrifugation steps remain insufficient. To release growth factors from the platelet concentrates, mechanical bursts of freeze and thaw cycles are implemented \cite{18, 25, 26, 31, 34, 37}. Most studies used -80°C as the freeze point and 37°C as the thaw point. However, the freeze and thaw temperatures seem to affect the yields of the growth factors in PL. In order to obtain the highest growth factors levels, one study recommended the -196°C/4°C cycle, as PDGF-BB levels in PL from PRP using this cycle were significantly higher than from PRP using -80°C/4°C cycle\cite{27}.

Since PL contains fibrinogen and coagulation factors, adding anticoagulants is vital. Heparin is usually added to PL-media before use. However, the concentration of heparin should not exceed 0.61 IU/mL when using unfractionated heparin or 0.024 mg/mL for low-molecular-weight heparin\cite{48}. This is because higher concentrations of heparin may negatively affect MSC proliferation, colony formation and differentiation\cite{48}. Additionally, available commercial heparin, which is manufactured primarily from porcine sources, has been shown to cause adverse reactions to human tissues, despite being approved for human use\cite{30, 49, 50}. These concerns are avoided with the use of the human origin heparin or by preparing PL from serum instead of plasma\cite{27}.
ARE WE READY FOR CLINICAL TRANSLATION?

PL represents a new and exciting opportunity for bone defect repair, either as a cell culture supplement for MSC expansion or as a coating agent for osteoconductive scaffolds and guided bone regeneration devices. PLs contain a multitude of bioactive factors that act synergistically to facilitate MSC attachment, proliferation and differentiation. Still, the exact amounts and ratios between these bioactive molecules remain to be established in order to achieve the desired and timely therapeutic effect on bone repair. For this, a consorted scientific and industrial effort is needed to establish standard protocols and quality control procedures for PL production that are tailor-made for intended therapeutic use. This can be split into two long term areas of investigation, primarily, large scale production of allogenic PL and secondly, fast small scale production of autologous PL exclusively for use in acute surgery.

Additionally, the time scale of growth factor release from scaffolds needs to be detailed to further understand the potential effect on bone regeneration. In order to have standard PL, a minimal quality control criteria is needed. For example, a minimum of 3-4 fold increase in the platelet concentration compared to the start point, the levels of PDGF-BB and the degree of WBC contamination, could be three factors that can serve as initial quality control criteria of standard PL. Finally, while the existing pre-clinical evidence on the use of PL-expanded MSCs or PL-coated scaffolds is encouraging, clinical studies investigating the benefits of PL-based products for improving bone regeneration are still lacking. Such evidence is definitely required to progress further in this field.
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<table>
<thead>
<tr>
<th>Studies</th>
<th>Platelet collection</th>
<th>Leucocyte reduction</th>
<th>Freeze &amp; thaw temperature</th>
<th>Anti-coagulation factors</th>
<th>PDGF Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>[15]</td>
<td>Buffy coat</td>
<td>Inline filtration</td>
<td>-30°C/37°C</td>
<td>100 IU/mL H</td>
<td>(AA) 7424 ± 1043 pg/mL* (BB) 4655 ± 1353 pg/mL (AB) 40458 ± 1791 pg/mL</td>
</tr>
<tr>
<td>[18]</td>
<td>Buffy coat</td>
<td>Inline filtration</td>
<td>-30°C/37°C</td>
<td>2 IU/mL H</td>
<td>Higher than FCS</td>
</tr>
<tr>
<td>[38]</td>
<td>Apheresis</td>
<td>NR</td>
<td>-20°C/37°C</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>[25]</td>
<td>Apheresis</td>
<td>NR</td>
<td>-80°C/NR</td>
<td>2 IU/mL H</td>
<td>NR</td>
</tr>
<tr>
<td>[36]</td>
<td>NR</td>
<td>NR</td>
<td>-80°C/NR</td>
<td>10 IU/mL H</td>
<td>NR</td>
</tr>
<tr>
<td>[51]</td>
<td>Apheresis</td>
<td>NR</td>
<td>-80°C/37°C</td>
<td>2 IU/mL H</td>
<td>(AB) 530 ± 60 pg/mL</td>
</tr>
<tr>
<td>[37]</td>
<td>Apheresis</td>
<td>NR</td>
<td>-80°C/NR</td>
<td>NR</td>
<td>(AA) 19596 pg/mL (BB) 22329 pg/mL</td>
</tr>
</tbody>
</table>

H – heparin; bold+highlight – pre clinical investigation; * - measured in media contain 10ml/L PL; NR – not reported; PDGF – platelet derived growth factor; AA, AB and BB refer to different isotypes of PDGF
Table 2: Summary of pre-clinical studies with PL-expanded BM MSCs aimed at bone regeneration

<table>
<thead>
<tr>
<th>Studies</th>
<th>Animal model</th>
<th>MSC source</th>
<th>MSC expansion media (Supplements)</th>
<th>Construct type</th>
<th>Scaffold pre-coating before loading with MSCs</th>
<th>Construct in vitro culture before implantation (supplements)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[38]</td>
<td>Mouse</td>
<td>Iliac crest BM</td>
<td>50 mL/L PL</td>
<td>BCP + 2x10^5 MSCs</td>
<td>A</td>
<td>10^8 M DEX</td>
</tr>
<tr>
<td>[36]</td>
<td>Sheep</td>
<td>Iliac crest BM</td>
<td>200 mL/L FCS</td>
<td>Rat-tail-derived collagen + 4x10^7 MSCs</td>
<td>PL</td>
<td>NR</td>
</tr>
<tr>
<td>[37]</td>
<td>Mouse</td>
<td>Iliac crest BM</td>
<td>100 mL/L FCS</td>
<td>HA(65%)+ β-TCP(35%)+ 3x10^5 MSCs</td>
<td>PL for 24 hours</td>
<td>100 mL/L FCS</td>
</tr>
<tr>
<td>[25]</td>
<td>Mouse</td>
<td>Iliac crest BM</td>
<td>50 mL/L PL</td>
<td>HA(65%)+ β-TCP (35%)+ MSCs*</td>
<td>PL for 24 hours</td>
<td>50 mL/L PL</td>
</tr>
<tr>
<td>[35]</td>
<td>Mouse</td>
<td>Iliac crest BM</td>
<td>50 mL/L PL</td>
<td>BCP+ 4x10^6 MSCs</td>
<td>A</td>
<td>80 mL/L PL</td>
</tr>
</tbody>
</table>

BCP – biphasic calcium phosphate, HA+β-TCP – hydroxyapatite/beta-tricalcium phosphate
A – absent, DEX- dexamethasone, NR – not reported, * MSC number not reported. Bold+ highlight – no FCS was used at any stage of investigation.
Figure 1: The enhancement of MSC colony formation in PL-cultured BM aspirates from a single patient. Day-14 colonies formed from 200 μL of fresh BM aspirate seeded on plastic culture dish with either 100 mL/L FCS (A) or 100 mL/L PL (B). Higher magnification (40X) of individual colonies grown in 100 mL/L FCS (C) or 100 mL/L PL (D). Quantified analysis of the average size of the colonies in pixels (E).
The average number of colonies (F). Colony number and size were analysed using NIS Elements BR Nikon software.

Figure 2: The attachment of PL-expanded BM MSCs on Orthoss scaffold. BM MSCs at passage 2 were incubated with the scaffold by continuous mixing at 37°C and 5% CO₂ for 3 hours with DMEM+50 mL/L PL. The scaffold was then washed and incubated for 10 days with DMEM+50 mL/L PL. SEM image illustrate MSCs on the scaffold (A). Higher magnification demonstrates MSC morphology on the scaffold surface (B). MSC cells, Orthoss scaffold.
Figure 3. Summary of the current methods used for PL preparation. Step by step guide of the PL preparation methods (A). Suggested stages to standardise in PL preparation (B).
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