

<https://doi.org/10.7124/bc.000BA3>

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THE EFFECT OF HUMAN PLATELET-DERIVED CELLS ON THE *IN VITRO* PROLIFERATION OF MESENCHYMAL STEM CELLS

Platelet derivatives represent a promising xenobiotic-free alternative to fetal bovine serum (FBS) for the *ex vivo* expansion of bone marrow mesenchymal stem cells (MSCs). The use of FBS is limited due to the risks of zoonotic transmission and xenogeneic immune reactions following transplantation. Platelets contain a significant amount of growth factors in their alpha granules, which are released upon physical disruption of the membrane or physiological activation of the cell. The release method significantly influences the composition and efficacy of the final product; however, comparative studies are lacking. **The aim** – to compare platelet lysate (PL) obtained by freeze-thawing and platelet lysate supernatant rich in growth factors (PR-SRGF), obtained by CaCl_2 activation, in terms of their ability to support the proliferation and clonogenic activity of bone marrow MSCs. **Methods.** Platelet-rich plasma from 15 donors was used to obtain PL (2 freeze-thaw cycles at -80°C , centrifugation at $1600\times g$ for 15 min, filtration through a $45\ \mu\text{m}$ filter) and PR-SRGF ($0.04\ \text{M}\ \text{CaCl}_2$, 40°C , ~60 min until clot formation; centrifugation at $2200\times g$, collection of supernatant). MSCs were isolated from bone marrow aspirates by adhesion to plastic; they were cultured in DMEM medium with 10% FBS or 5%, 7.5%, 10% platelet derivatives at 37°C , 5% CO_2 . Cumulative population doubling (cPD) and colony-forming efficiency (CFE) were assessed at passages P1 and P4. **Results.** PR-SRGF provided a higher rate of MSC proliferation than platelet lysate and bovine serum, from passage 1 to 6 at all concentrations (5%, 7.5%, 10%). At passage 4, the cumulative population doubling (cPD) in PR-SRGF was significantly higher than in platelet lysate at all concentrations ($p < 0.05$ for 5%; $p < 0.01$ for 7.5% and 10%). MSCs cultured in PR-SRGF formed colonies at a significantly higher frequency than in PL, both at P1 and P4 – indicating better support for clonogenic progenitor cells. The preservation of clonogenic activity at late passages indicates that platelet-derived supernatant, rich in growth factors, supports a population of progenitor cells with high proliferative potential more effectively than platelet lysate. **Conclusions.** The method of platelet factor release significantly influences the efficacy of the product. The platelet lysate supernatant, rich in growth factors and obtained by CaCl_2 activation, outperforms platelet lysate in its ability to support the proliferation and clonogenic activity. Further standardisation of production protocols and the establishment of quality criteria are necessary for clinical application.

Keywords: human platelet lysate, platelet-derived supernatant, mesenchymal stem cell culture, freeze-thaw.

Received: 08.06.2026; Accepted: 08.09.2026; Published: 29.09.2026

Citation: Geleta E.V., Gudzenko T.V., Zubov D.O. (2026) The effect of human platelet-derived cells on the *in vitro* proliferation of mesenchymal stem cells. *Biopolymers & Cell*, Special issue(42), 105. <https://doi.org/10.7124/bc.000BA3>
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