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Low-dose lipopolysaccharide unexpectedly enhances osteogenic differentiation of bone marrow-derived mesenchymal stem cells: a model for low-grade infection

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Background and objective

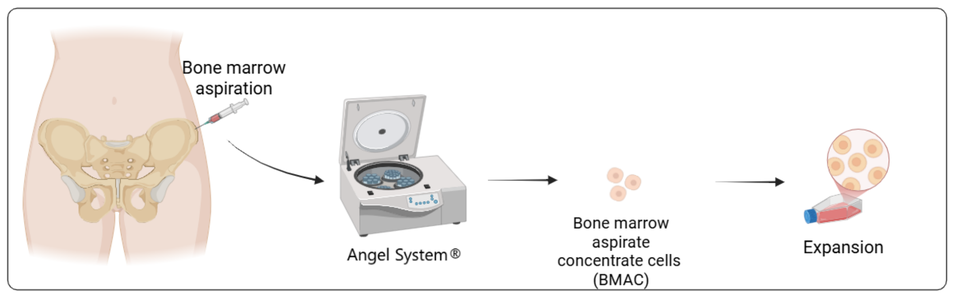
Every year, bone fractures are amongst the most frequent injuries sustained by patients worldwide. Bone tissue is known to have an impressive regenerative capacity when it comes to small defects. However, larger defects require additional medical care. In 5 to 10% of fracture cases, there is partial or complete absence of healing, resulting in what is called a non-union. A non-union can be due to diverse factors such as a lack of mechanical stability, a lack of vascularity, a lack of soft tissue coverage, or the presence of an infection.

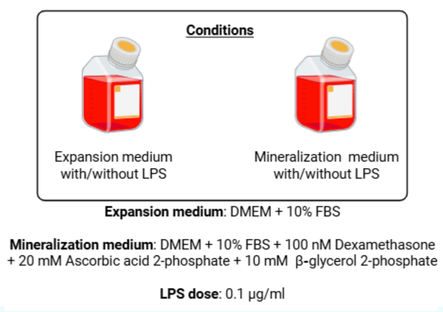
Non-healing bone defects are frequently subject to infections, making them harder to treat and compromising the healing process. A significant concern is the occurrence of low-grade infections that are difficult to detect; the impact of which on fracture healing remains incompletely understood. To understand the influence of bacteria on bone regeneration, we decided to investigate the role of one of the main components of bacteria, lipopolysaccharide (LPS) in our cell culture. LPS, a glycolipid that is a major component of the outer membrane of gram-negative bacteria, is an endotoxin. It thus represents a pro-inflammatory stimulus for the organism and can trigger immune responses. When there is an infection at the fracture site, LPS is believed to have adverse effects on osteogenesis.

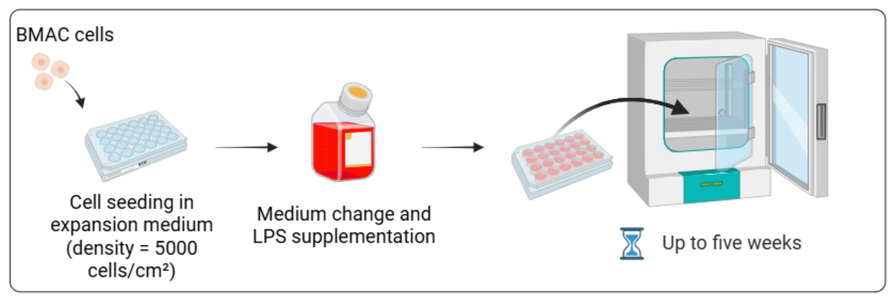
Materials and Methods

The aim of this project is to compare the osteogenic potential of bone marrow derived human mesenchymal stem cells (BM-hMSCs) cultured in 2D in expansion medium and osteogenic medium in the presence and absence of a 0.1 µg/ml dose of LPS. To assess cell viability, osteogenesis and evaluate the influence of LPS on bone regeneration, PrestoBlue® staining and alkaline phosphatase (ALP) activity were measured at days 3, 7, and 14; qPCR was performed at day 14; and Alizarin Red staining was conducted between days 14 and 35, when mineralization became visible to the naked eye.

Bone marrow aspirate concentrate (BMAC) cells were obtained via aspiration of bone marrow in the iliac crest of patients suffering from long bone defects. The cells were first expanded in T75 flasks until confluent enough to be passaged. Those BM-hMSCs were then seeded in wells at a density of 5.000 cells/cm^2 using expansion medium. After 2 days, the medium was replaced with different medium compositions, and the cells were cultured for up to five weeks. The different medium conditions were the following: expansion medium with/without LPS, and mineralization medium with/without LPS. Once the time points were reached, the following assays were performed: PrestoBlue® assay, Alkaline phosphatase activity measurement, and qPCR for osteogenic markers. The osteogenic markers were: COL1A1, ENPP1, ALP, OCN, OPN, BSP, and RUNX2.



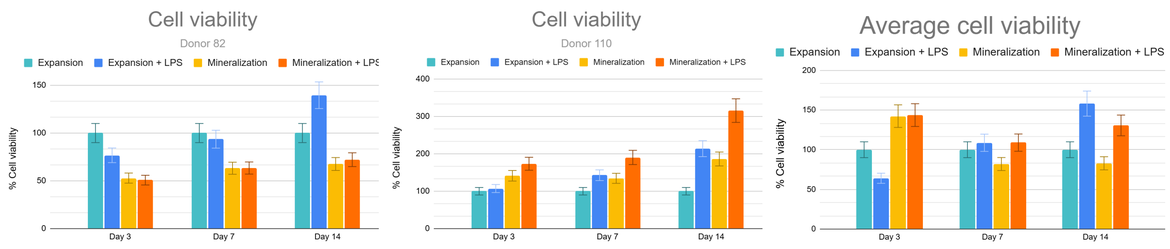




Results

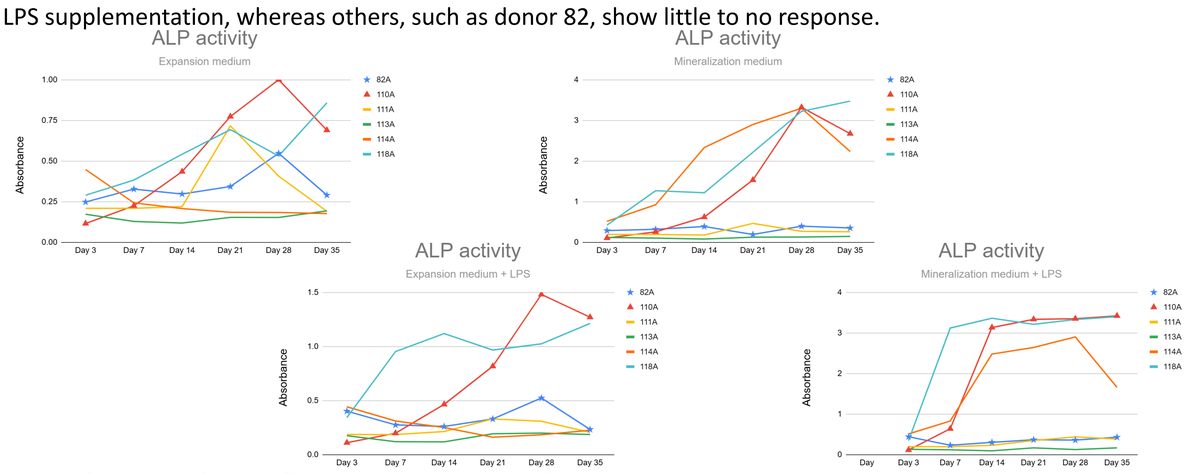
PrestoBlue®

The results of the PrestoBlue® assays indicate that a concentration of 0.1 µg/mL LPS is generally not detrimental to cell viability. While a slight reduction in viability is observed in some donors when the medium is supplemented with LPS, most donors show no decrease. In some cases, viability may even be slightly enhanced.

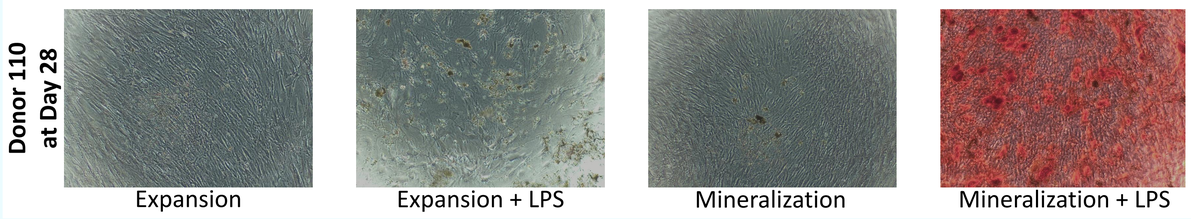


Alkaline phosphatase (ALP) / Alizarin red

The effect of LPS on alkaline phosphatase (ALP) activity, as well as the magnitude of this effect, varies considerably between donors. Some donors, such as donor 110, exhibit increased ALP activity in response to LPS supplementation, whereas others, such as donor 82, show little to no response.



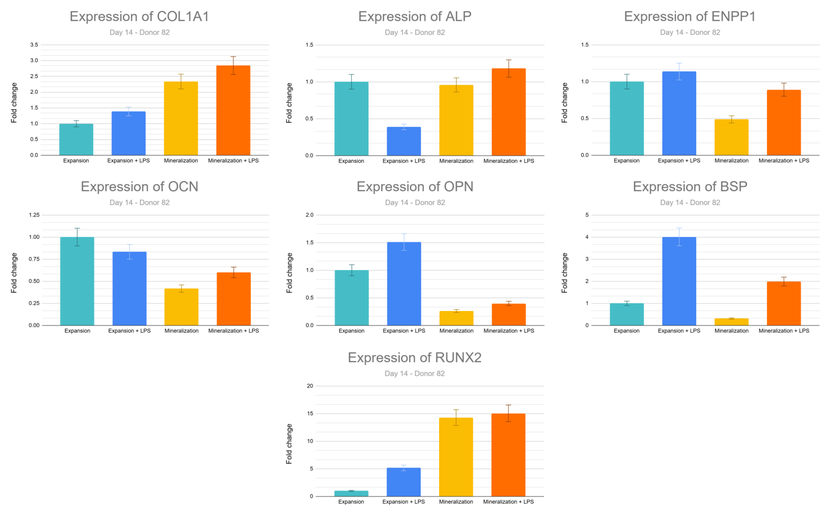
Regarding Alizarin Red staining and quantification, considerable variation is again observed between donors. Some donors display increased mineralization in response to LPS treatment, whereas others show little to no difference in mineralization between LPS-treated and untreated conditions.



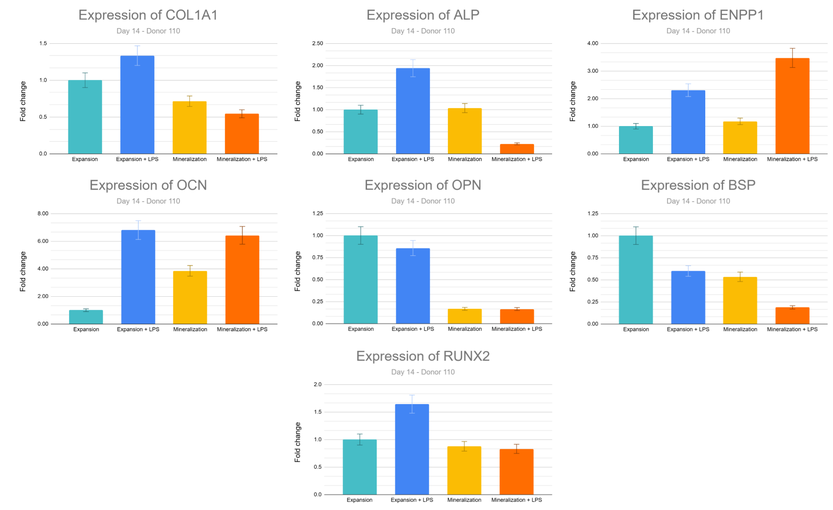
qPCR

Overall, these qPCR results show that osteogenic differentiation is strongly donor-dependent. What we can conclude from these results is that a low LPS dose appears to enhance osteogenesis in some donors, but in others, it only transiently delays the process without inhibiting it.

Donor 82 shows strong osteogenic differentiation, with mineralization clearly increasing RUNX2, ALP, and COL1A1, and this effect is further enhanced by LPS, indicating a pro-osteogenic inflammatory response.



In contrast, donor 110 shows weak mineralization, elevated ENPP1, and impaired differentiation, suggesting that LPS disrupts functional osteogenesis despite some marker increases. However, Alizarin red staining indicates increased mineralization with LPS, contradicting the day 14 qPCR results.



Conclusion and Limitations

These results suggest that a low dose of lipopolysaccharide can have a beneficial effect on osteogenesis in bone marrow aspirate concentrate cells from certain donors. Indeed, some donors show that a low dose of LPS can considerably enhance the osteogenic potential of human mesenchymal stem cells. However, it seems like LPS has very little to no effect on the osteogenic potential of cells from other donors, highlighting strong donor dependency.

The PrestoBlue® results show that such a low dose of LPS has no adverse effect on cell viability, while the qPCR results suggest it influences the expression of osteogenesis-related genes, again indicating donor-dependent responses. Under identical conditions, cells from different donors respond differently to mineralization and inflammation. Some show clear increases in bone-related genes, while others display weaker or less coordinated responses, with inflammation sometimes appearing to impair or delay rather than support differentiation.

It is important to consider that this study assessed the effect of a specific low dose of LPS rather than the impact of bacteria on osteogenesis. This differs significantly from in vivo infection conditions and does not fully reflect biological complexity. The same experiments are currently ongoing with a higher LPS dose (10 µg/mL) to further investigate dose-dependent effects. It should also be considered that qPCR was performed at day 14 and is not fully predictive of the outcome.

In conclusion, a low dose of LPS does not always appear detrimental to osteogenesis. This may have implications for clinical treatment, where complete bacterial eradication is typically performed before continuing fracture management, raising the question of whether this is always necessary given the associated time, cost, and risks.

References

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