Osteoblast-derived giant plasma membrane vesicle: A novel candidate for biomimetic osteogenic differentiation of mesenchymal stem cells

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Directed osteogenic differentiation of human mesenchymal stem cell (hMSC) via the biomimicry of hMSC - osteoblast cross-talk is a fascinating possibility. Here, we hypothesized that osteoblast-derived giant plasma membrane vesicle (GPMV) can partly mimic the role of osteoblast in vitro up on interaction with hMSC, therefore can direct the osteogenic differentiation of hMSC in a biomimetic way. To test the hypothesis, we generated GPMV from human osteoblast (MG-63 cell line) and allowed it to interact with hMSC in vitro. GPMV of different membrane characteristics were generated by using combinations of chemical vesiculant, actin destabilizer, and cholesterol sequestering agent. The GPMVs were characterized in terms of size, number, structural complexity and lipid composition using confocal microscopy, flow cytometry, and NMR spectroscopy. We showed that the GPMV carries cytoplasmic content as a cargo and got fused with the mesenchymal stem cells up on interaction in vitro. Confocal microscopy based image analysis and flow cytometry together showed that the extent of fusion of GPMV with hMSC varies with the membrane property of the GPMV. The hMSCs fused with GPMVs were found viable and metabolically active. We checked the differentiation status of the GPMV treated hMSC by analyzing the cellular expression of different osteogenic markers at mRNA and protein level. RT-PCR showed that GPMVs are capable of inducing higher expression of a number of osteogenic differentiation markers like alkaline phosphatase, collagen type I, Runx2 and osteocalcin in hMSC. A similar result was also obtained at the protein level. Interestingly, here we also noticed a membrane composition dependent variation in osteogenic differentiation properties of GPMVs. The present study opens up a possibility of tuning the course of differentiation of mesenchymal stem cells in a biomimetic way by changing the membrane composition of the GPMV. In summary, the use of osteoblast-derived GPMV for osteogenic differentiation hMSC could be a promising strategy for tissue engineering and regenerative medicine.

References

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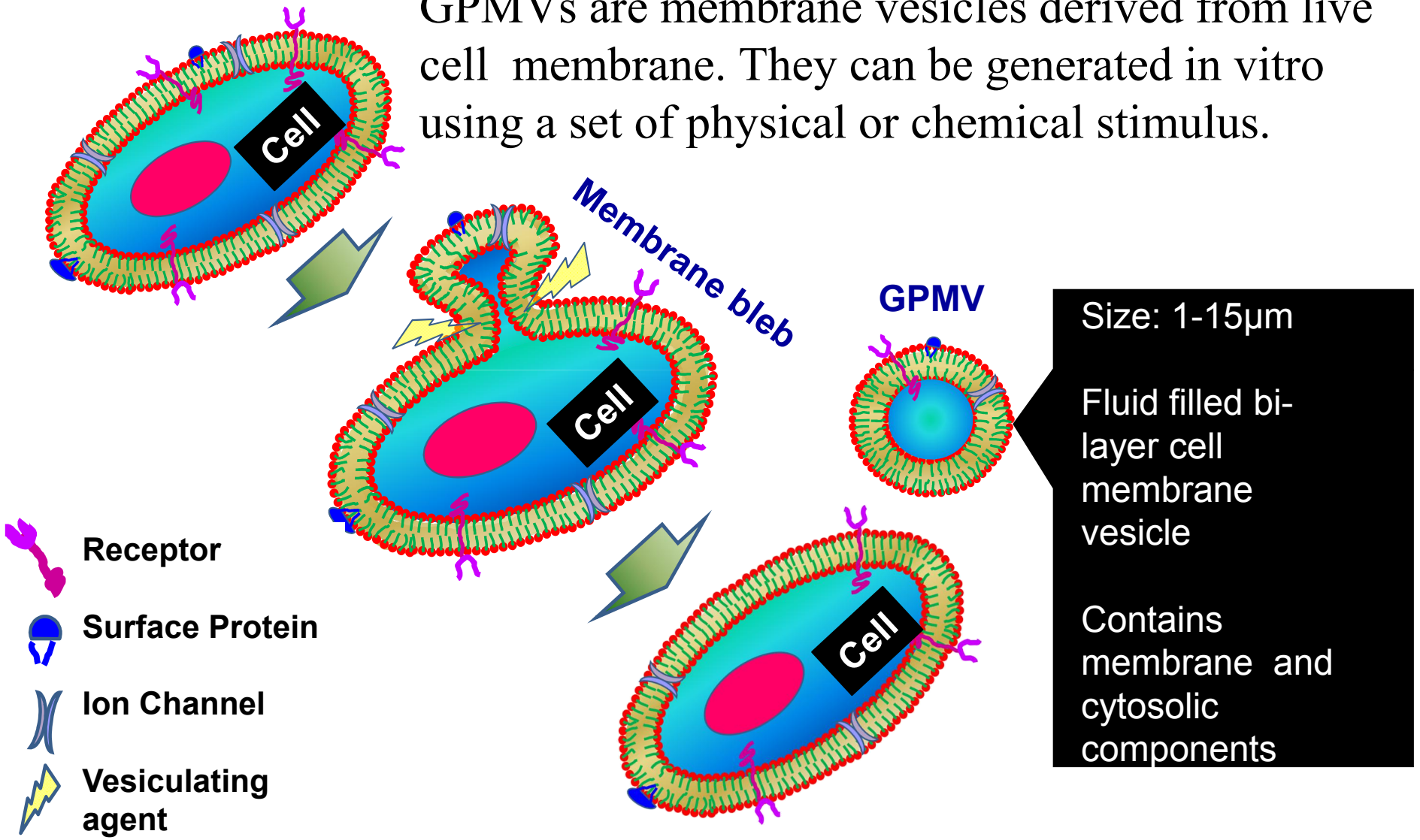
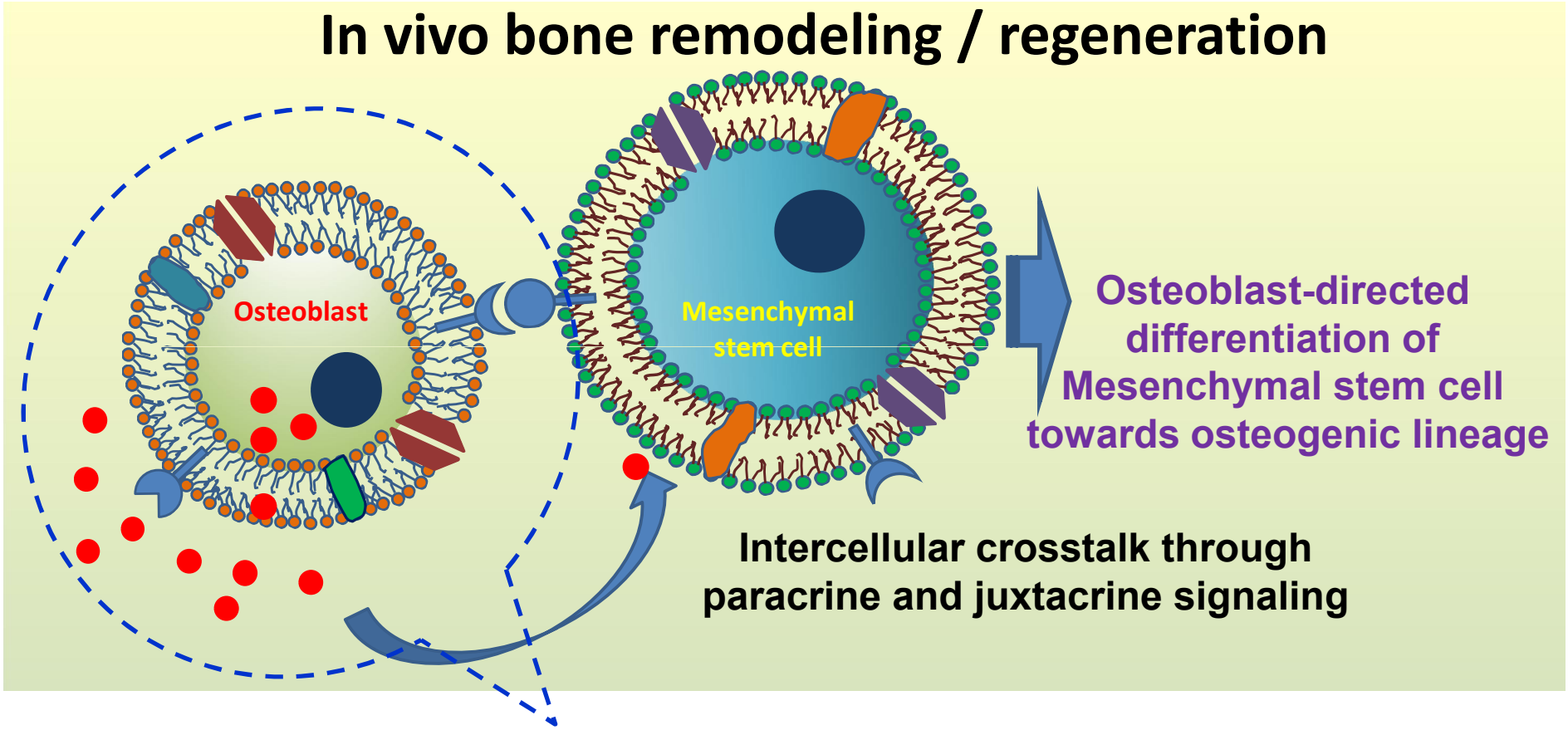
Giant Plasma Membrane Vesicles (GPMV)

GPMVs are membrane vesicles derived from live cell membrane. They can be generated in vitro using a set of physical or chemical stimulus.



GPMV is interesting but is it relevant in regenerative medicine?



Can we mimic this functional role of osteoblast in acellular platform???

3. Can we vary the GPMV composition?

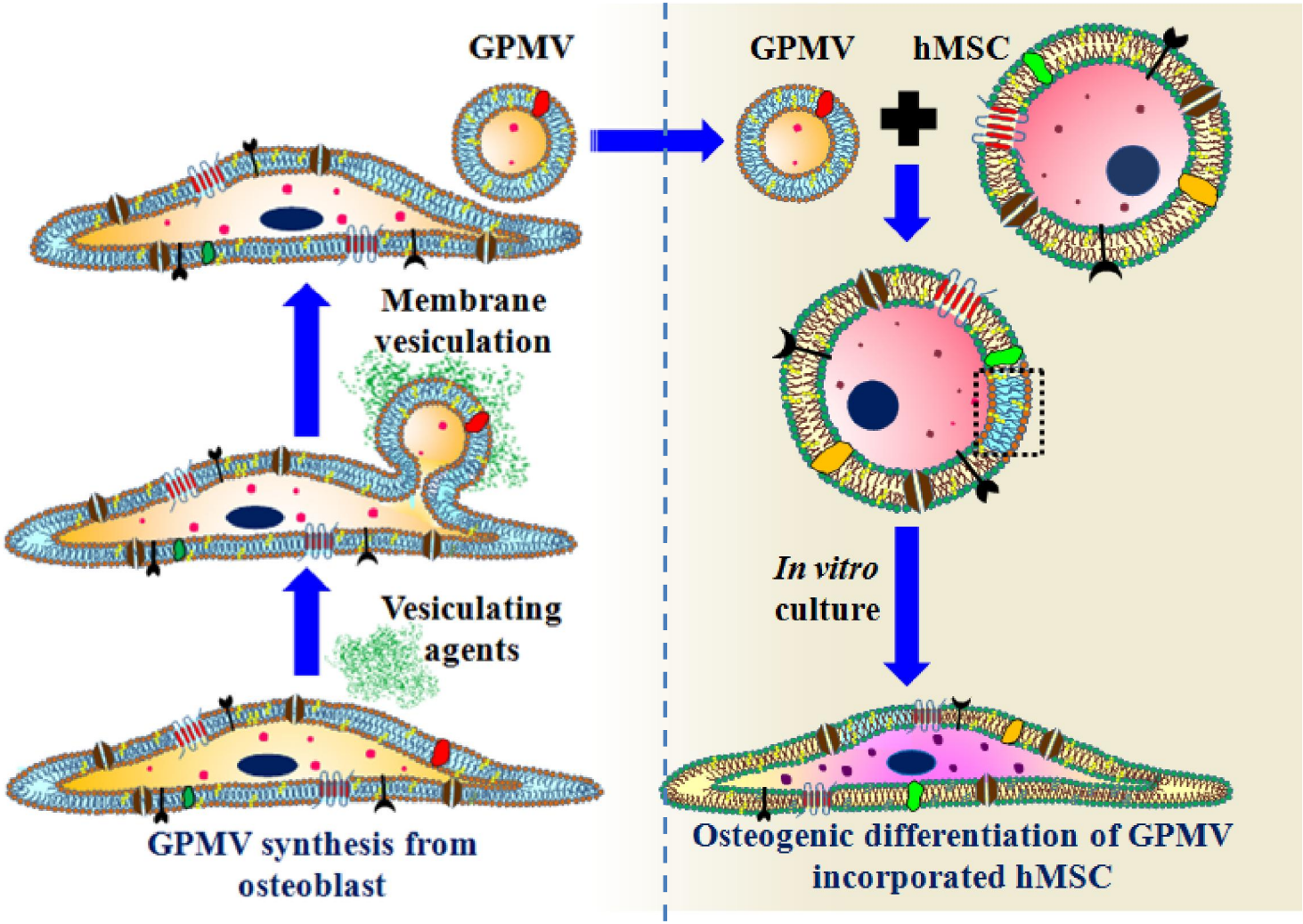
2. Can we tune its production?

1. Can GPMV be generated from osteoblast?

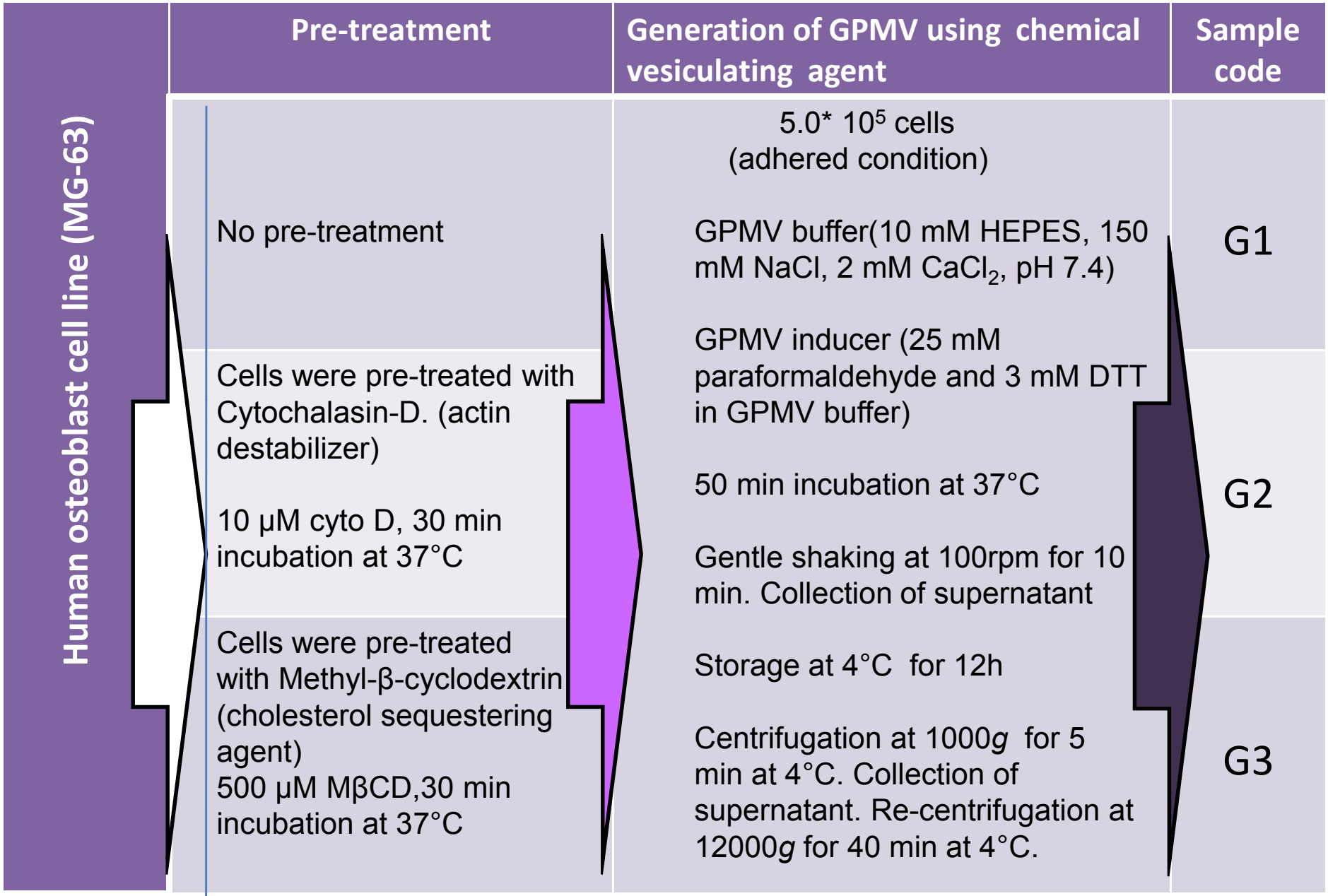
4. Can GPMV be fused with hMSC?

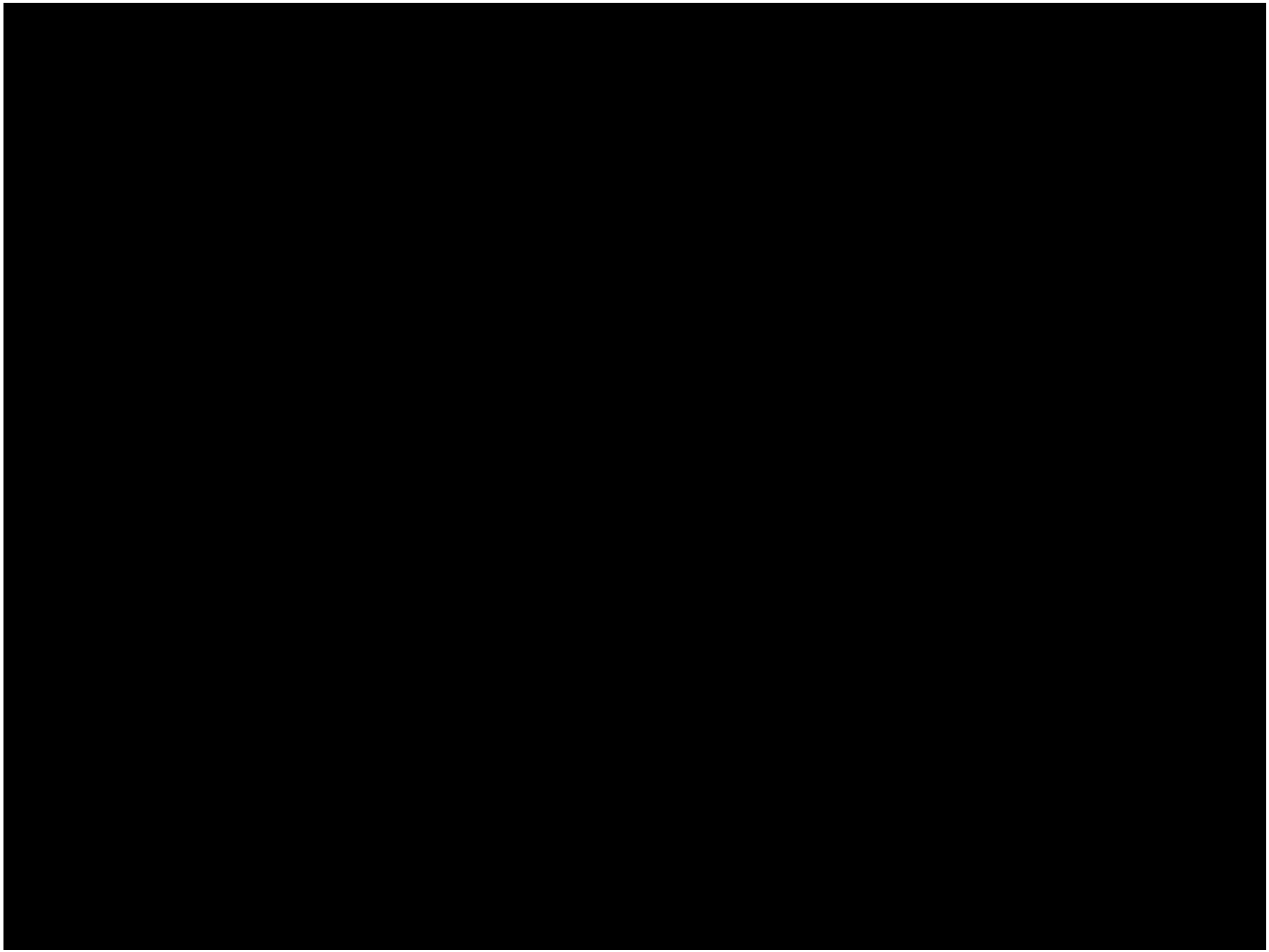
5. After fusion will hMSC survive?

6. Will there be osteogenic differentiation of hMSC?



Synthesis of GPMV from Osteoblast





Summary

- **GPMV can be synthesized from osteoblasts by using chemical vesiculating agent**
- **GPMV generated in the presence of blockers MβCD(methyl-β-cyclo dextrin) and Cyto-D (cytochalasin-D) showed notable difference in production kinetics and composition.**
- **The GPMV can be fused with hMSCs.**
- **GPMV can induce osteogenic differentiation in hMSC**

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THANK YOU