

Serum-free Osteogenic Differentiation of a Mesenchymal Stem Cell Line

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Background

- One main source of variability in cell culture can be the media used, generally supplemented with animal serum.
- Serum-free alternatives mitigate variability, but for Mesenchymal Stem Cells (MSCs) it can be difficult to find a good serum-free alternative.
- This work aims to compare a commercially available serum-free media to different serum-containing media, for the osteogenic differentiation of an immortalized MSC cell line.

Methods

- hTERT-MSC Y201 cell line [1] was cultured for 21 days in 3 different media (table 1), following 3 supplementation profiles (table 2). Cells were analysed at day 7, 14 and 21. Fig. 1 details culturing conditions.
- Cells analysed by optical microscopy and cell metabolic activity measured by resazurin reduction assay.

Table 1 – Media composition

BM3	DMEM (GIBCO) + 10% FBS (GIBCO)
CD1	StemMACS™ MSC Expansion Media Kit XF, human (Miltenyi Biotec), serum-free and xeno-free
HSM	Human Mesenchymal-XF Expansion Medium (Merck), human-serum

Table 2 – Supplementation profile

EM	No supplements
OM1	1 st media change – AA2P 2 nd media change – βGP, Dex
OM2	1 st media change – AA2P, βGP, Dex
AA2P	L-Ascorbic Acid 2-phosphate (5 mg/ml)
βGP	Beta-glycerophosphate (0.5 M)
Dex	Dexamethasone (10 μM)

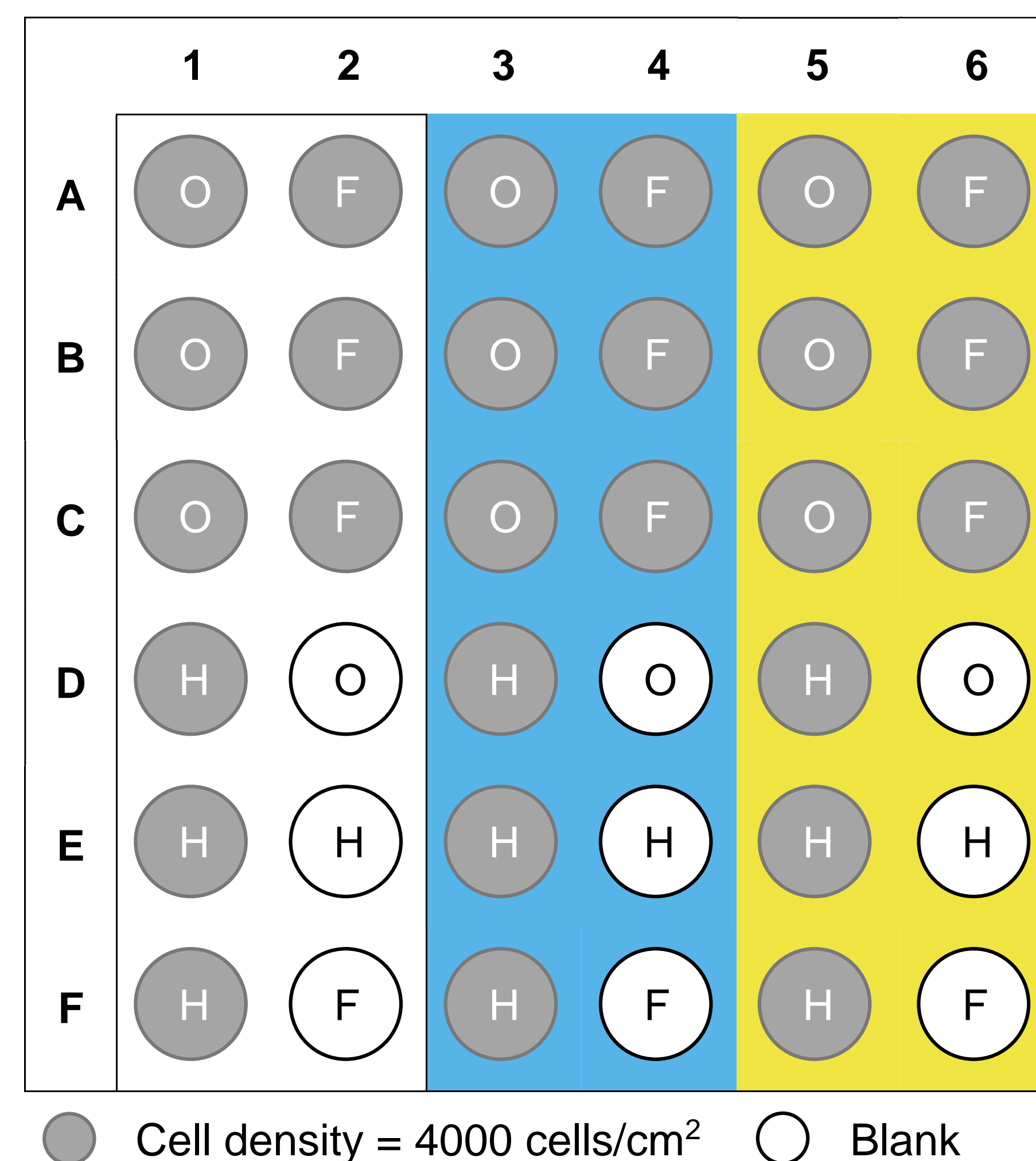


Fig. 1 – Culture conditions: O – 1 full media change/week; H – 3 half media changes/week; F – 3 full media changes/week.

Results

- Y201 metabolic activity in CD1 following conditions O and H is comparable to results in BM3 and CD1, regardless of supplementation profile (Fig. 2).
- Optical microscopy suggests Y201 cells mineralize faster in CD1, following condition O and supplementation profile OM2. Fig. 3 illustrates results for all three media following condition O at day 21.

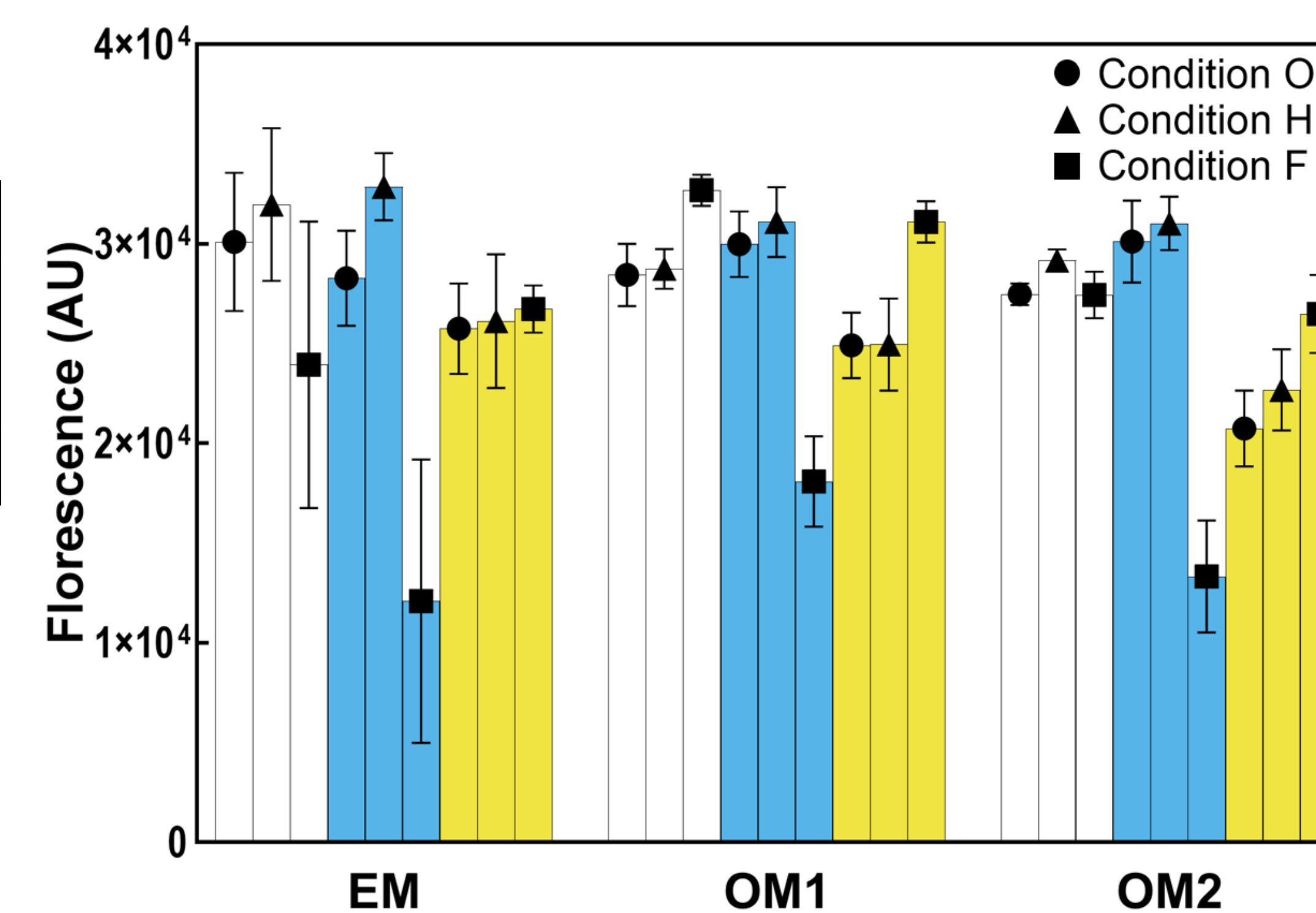


Fig. 2 – Metabolic activity of Y201 cells on day 21.

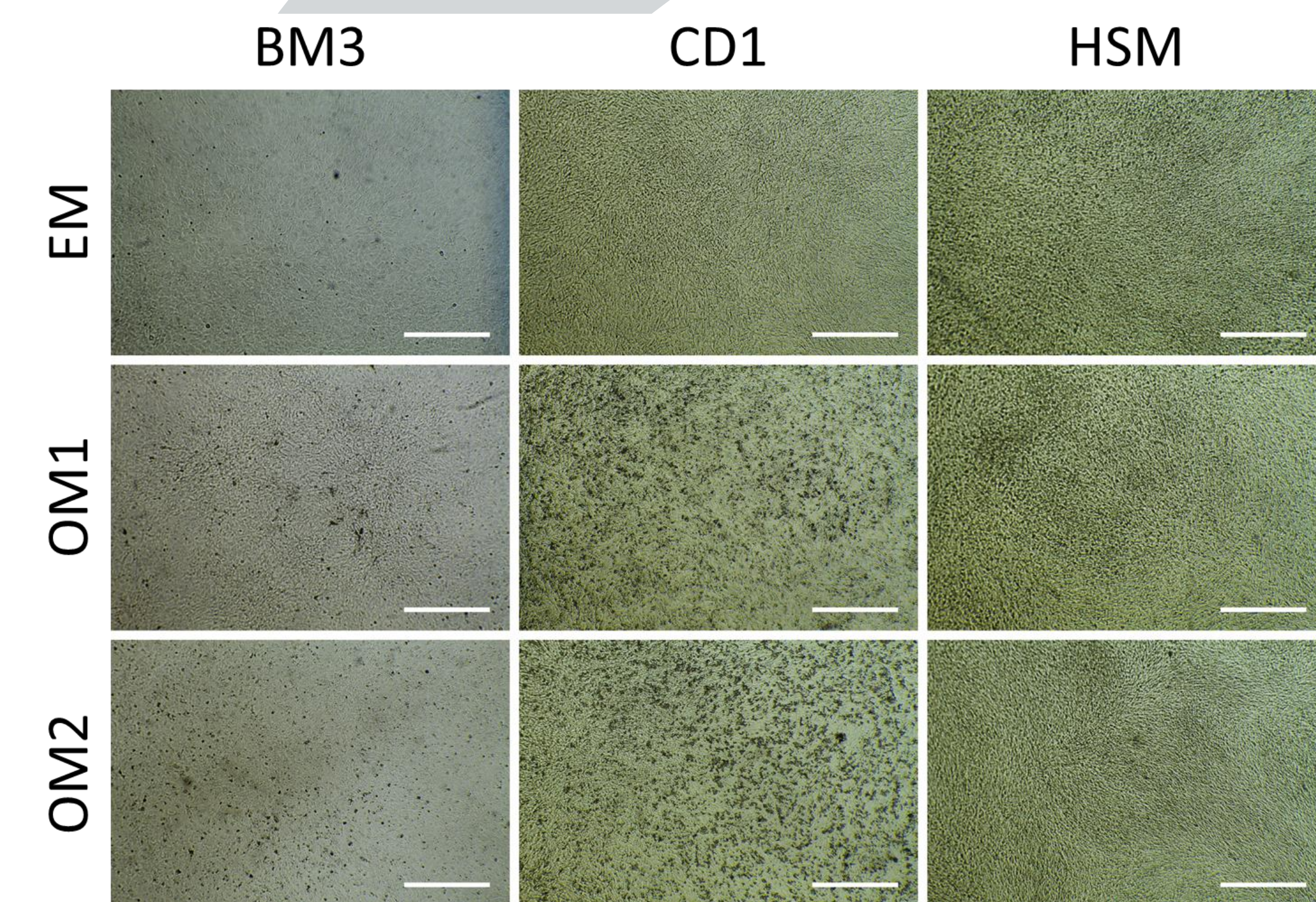


Fig. 3 – Y201 cell at day 21, following condition O. Scale bar = 500 μm.

Discussion and Conclusions

- Current results suggest CD1 outperforms BM3 and HSM for the mineralization of Y201. Regardless of media, condition O (1 media change/week) following supplementation profile OM2 seems to provide the best results.
- Overall, it is shown that serum-free media can be an effective alternative to serum-containing media for the osteogenic differentiation of hTERT-MSCs Y201.

Acknowledgements

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References

[1] S. James, et al., Stem Cell Reports 4(6) (2015) 1004-1015