BJR

EDITORIAL

Osteosarcoma cells/cell lines are not appropriate for studies on bone regeneration in vitro

C. Xu,
Y. Liu

From Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, Netherlands.

Cite this article: *Bone Joint Res* 2023;12(5):311–312.

Keywords: Biomaterial, Osteosarcoma cell, Mesenchymal stem cell, Bone tissue engineering

Repairing bone defect, particularly critical-size bone defect, is still a challenge in clinical practice and is more demanding in the elderly population. For bone defect repair, various biomaterials have been developed and used. However, the understanding of the mechanism of biomaterial-induced osteogenesis is still poor. Meanwhile, to enhance these biomaterials' efficiency on osteogenesis, it is vital to reveal the interaction between bone cells and these biomaterials, as well as the molecular cascades in cells after biomaterial implantation. Therefore, abundant in vitro and in vivo studies have been conducted. Currently, in vitro, some osteosarcoma (OS) cells/cell lines such as MG-63, Saos2, and U2OS are used to test the performance of various bone substitutes and to unveil the relevant molecular mechanism of bone regeneration.[1-5] These cells can express some osteogenetic markers (*RUNX2*, *SP7*, *ALP*, etc.) and formulate calcified nodules in extracellular matrix (ECM), which confers the term 'osteoblast-like cells' on them.

The bone microenvironment plays a vital role in the performance of bone cells. For instance, osteoblasts collected from patients with idiopathic osteonecrosis of the femoral head possess lower alkaline phosphatase (ALP) activity and mineralization capacity than osteoblasts from the same skeletal site in age-matched patients with osteoarthritis.[6] But is the use of OS cells/cell lines in these studies appropriate? Theoretically, osteoblasts mainly stem from mesenchymal stem cells (MSCs), including bone marrow-derived MSCs (BMSCs), adipose-derived stem cells (ADSCs), umbilical cord mesenchyma, etc. Thus, to reveal more realistic events after the material implantation in the human body, the optimal cells used for in vitro investigations should be MSCs rather than OS cells/cell lines, as OS cells/cell lines are bone cancer cells and can create a bone tumour microenvironment. It is essential to distinguish how different normal cells are from cancer cells. Hallmarks of cancers include activated invasion and metastasis, accelerated angiogenesis, unstable genome, tumour-promoting inflammation, evasion of growth suppression, and sustained proliferative signalling.[7] Pathological metabolism and unrestrained proliferation of OS cells inevitably affect the results of cytoviability tests such as Alamar Blue, adenosine triphosphate (ATP), and 5-ethynyl-2′-deoxyuridine (EdU) assays, which are usually used to reflect the biocompatibility of biomaterials. Moreover, genome alterations may affect normal osteogenetic differentiation. Compared with normal osteoblasts, OS cells have mutated and altered chromosomes.[8] For example, the alteration at chromosome 1 in OS cells, where the human osteocalcin (OCN) gene locates, may explain why OCN, a crucial marker for osteogenesis in the late phase, is hardly detected in ectocrines of MG-63 cells.[8,9] This may lead to false-negative results of biomaterials' efficiency on bone regeneration. However, there is also a concern about false-positive results. Enhanced invasion and metastasis capacities enable OS cells to adhere to the surface of bone substitutes much more easily than stem cellspro-angiogenesis and the inflammatory tumour microenvironment also promote bone formation. In addition, the different ECM composition between OS cells and normal osteoblasts has been revealed. Pautke et al[10] reported that MG-63, Saos-2, and U2-OS cells demonstrated different proliferation kinetics compared with normal osteoblasts, and the composition of these four cells' ECM was also highly different. In agreement with

Correspondence should be sent to Yuelian Liu; email: y.liu@acta.nl

doi: 10.1302/2046-3758.125.BJR-2023-0088.R1

Bone Joint Res 2023;12(5):311–312.

Pautke et al, Bozycki et al[11] declared that although Saos-2 cells have the most mature osteoblastic labelling profile, they have distinct matrix vesicles responsible for accumulating phosphate and calcium and forming apatites. Hence, it is reasonable to suspect that these differences will misguide those in vitro assays for bone substitutes.

Currently, OS cells/cell lines are widely used because they are immortal, and their osteogenic differentiation capacity will hardly decrease, which is much easier for research. By contrast, MSCs will be senescent and lose their stemness following the culture in vitro. Therefore, fresh primary MSCs will have to be collected from donors or animals periodically, which is more costly and time-consuming than using OS cells. Given the tremendous differences between OS cells and MSCs at the cellular and molecular levels, although results from in vitro studies using OS cells may be consistent with those from in vivo research, we cannot ensure that the performance of OS cells reveals the truth of material-induced bone formation. Since in vitro research on bone regeneration aims to simulate an authentic osteogenesis environment, MSCs, the derivation of osteoblasts in physiological conditions, are ideal candidates for the in vitro study rather than OS cells/cell lines. To date, various MSCs (BMSCs, ADSCs, dental pulp stem cells, etc.)[12-17] have been applied to evaluate the efficiency of those biomaterials on osteogenesis, and MSC treatments even achieved profound success in clinical trials for bone regeneration.[18,19] Due to the accumulated studies, MSCs can now be cultured without sophisticated instruments and unique medium, and commercial MSCs are also available.[20] Thus, MSCs can be cultured and tested in most laboratories worldwide. Therefore, to reveal a more authentic role of bone substitutes in physiological conditions, MSCs present a promising alternative to OS cells, which should not be engaged in these in vitro assessments.

References

1. **Teotia AK**, **Gupta A**, **Raina DB**, **Lidgren L**, **Kumar A**. Gelatin-Modified Bone Substitute with Bioactive Molecules Enhance Cellular Interactions and Bone Regeneration. *ACS Appl Mater Interfaces*. 2016;8(17):10775–10787.
2. **Hassani A**, **Avci ÇB**, **Kerdar SN**, **et al**. Interaction of alginate with nano-hydroxyapatite-collagen using strontium provides suitable osteogenic platform. *J Nanobiotechnology*. 2022;20(1):310.
3. **Savino S**, **Toscano A**, **Purgatorio R**, **et al**. Novel bisphosphonates with antiresorptive effect in bone mineralization and osteoclastogenesis. *Eur J Med Chem*. 2018;158:184–200.
4. **Abdallah D**, **Skafi N**, **Hamade E**, **et al**. Effects of phospholipase D during cultured osteoblast mineralization and bone formation. *J Cell Biochem*. 2019;120(4):5923–5935.
5. **Govindan R**, **El-Sherbiny M**, **Ibraheem KMM**, **et al**. Thyroid-Stimulating Hormone Favors Runx2-Mediated Matrix Mineralization in HOS and SaOS2 Cells: An In Vitro and In Silico Approach. *Molecules*. 2022;27(3):613.
6. **Maestro-Paramio L**, **García-Rey E**, **Bensiamar F**, **Saldaña L**. Osteoblast function in patients with idiopathic osteonecrosis of the femoral head. *Bone & Joint Research*. 2021;10(9):619–628.
7. **Hanahan D**. Hallmarks of Cancer: New Dimensions. *Cancer Discov*. 2022;12(1):31–46.
8. **Ozaki T**, **Neumann T**, **Wai D**, **et al**. Chromosomal alterations in osteosarcoma cell lines revealed by comparative genomic hybridization and multicolor karyotyping. *Cancer Genet Cytogenet*. 2003;140(2):145–152.
9. **Lajeunesse D**, **Frondoza C**, **Schoffield B**, **Sacktor B**. Osteocalcin secretion by the human osteosarcoma cell line MG-63. *J Bone Miner Res*. 1990;5(9):915–922.
10. **Pautke C**, **Schieker M**, **Tischer T**, **et al**. Characterization of osteosarcoma cell lines MG-63, Saos-2 and U-2 OS in comparison to human osteoblasts. *Anticancer Res*. 2004;24(6):3743–3748.
11. **Bozycki L**, **Mroczek J**, **Bessueille L**, **et al**. Annexins A2, A6 and Fetuin-A Affect the Process of Mineralization in Vesicles Derived from Human Osteoblastic hFOB 1.19 and Osteosarcoma Saos-2 Cells. *Int J Mol Sci*. 2021;22(8):3993.
12. **Crane JL**, **Cao X**. Bone marrow mesenchymal stem cells and TGF-β signaling in bone remodeling. *J Clin Invest*. 2014;124(2):466–472.
13. **Lee Y-C**, **Chan Y-H**, **Hsieh S-C**, **Lew W-Z**, **Feng S-W**. Comparing the Osteogenic Potentials and Bone Regeneration Capacities of Bone Marrow and Dental Pulp Mesenchymal Stem Cells in a Rabbit Calvarial Bone Defect Model. *Int J Mol Sci*. 2019;20(20):5015.
14. **Grottkau BE**, **Lin Y**. Osteogenesis of Adipose-Derived Stem Cells. *Bone Res*. 2013;1(2):133–145.
15. **Malec K**, **Góralska J**, **Hubalewska-Mazgaj M**, **et al**. Effects of nanoporous anodic titanium oxide on human adipose derived stem cells. *Int J Nanomedicine*. 2016;11:5349–5360.
16. **Naddeo P**, **Laino L**, **La Noce M**, **et al**. Surface biocompatibility of differently textured titanium implants with mesenchymal stem cells. *Dent Mater*. 2015;31(3):235–243.
17. **Liu H-Y**, **Chiou J-F**, **Wu ATH**, **et al**. The effect of diminished osteogenic signals on reduced osteoporosis recovery in aged mice and the potential therapeutic use of adipose-derived stem cells. *Biomaterials*. 2012;33(26):6105–6112.
18. **Gómez-Barrena E**, **Padilla-Eguiluz N-G**, **López-Marfil M**, **Ruiz de la Reina R**, **REBORNE Consortium**. Volume and location of bone regeneration after autologous expanded mesenchymal stromal cells in hip osteonecrosis : a pilot study. *Bone Joint Res*. 2022;11(12):881–889.
19. **Houdek MT**, **Wyles CC**, **Smith JRH**, **Terzic A**, **Behfar A**, **Sierra RJ**. Hip decompression combined with bone marrow concentrate and platelet-rich plasma for corticosteroid-induced osteonecrosis of the femoral head : mid-term update from a prospective study. *Bone Jt Open*. 2021;2(11):926–931.
20. **Xin W**, **Yuan S**, **Wang B**, **Qian Q**, **Chen Y**. Hsa_circ_0066523 promotes the proliferation and osteogenic differentiation of bone mesenchymal stem cells by repressing PTEN. *Bone Joint Res*. 2021;10(8):526–535.

Author information:
- C. Xu, MSc, PhD Candidate
- Y. Liu, PhD, Head of the Research Group, Associate Professor
  Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), Vrije Universiteit Amsterdam and University of Amsterdam, Amsterdam, Netherlands.

Author contributions:
- C. Xu: Conceptualization, Writing – original draft, Writing – review & editing.
- Y. Liu: Writing – review & editing.

Funding statement:
- The authors received no financial or material support for the research, authorship, and/or publication of this article.

ICMJE COI statement:
- The authors declare no conflict of interest.

Acknowledgements:
- We thank Dr Behrouz Zandieh-Doulabi, Dr Astrid Bakker, and Prof.dr. Jenneke Klein-Nulend for their valuable suggestions.

© 2023 Author(s) et al. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (CC BY-NC-ND 4.0) licence, which permits the copying and redistribution of the work only, and provided the original author and source are credited. See https://creativecommons.org/licenses/by-nc-nd/4.0/