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Validating non-adherence of human mesenchymal stem cells (MSC) on BRAND InertGrade™ 96-well plates can optimize the procedure of testing bone graft substitutes in vitro

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Introduction

For testing cellular parameters of human mesenchymal stem cells (MSC) growing on granules of bone graft substitutes, an experimental procedure was established where cells were seeded on the granules in 24 well plates. After allowing cells to attach to the granules, the granules were transfered with a forceps into 96 well plates to ensure analyzing only cells attached to the bone graft substitutes granules (Seebach et al. 2010). To simplify this procedure, cells should be seeded directly on granules in 96 well plates with no attachment properties for cells to avoid the transfer step. We validated BRAND InertGrade™ 96 well plates for our special purpose: MSC should not attach to the well bottom and/or should be easily removed by washing.

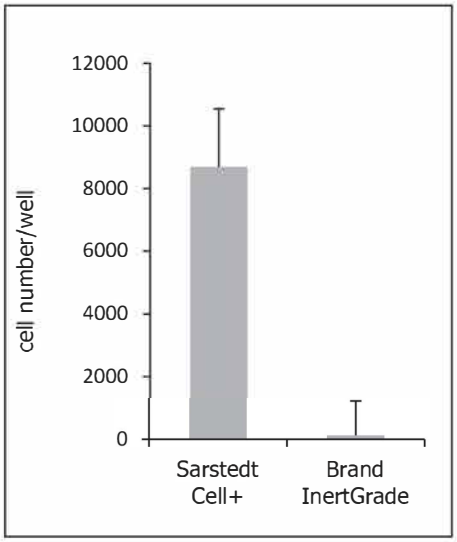


Material & Methods

MSC were isolated and cultured as described earlier (Seebach et al. 2010). For Quantification of remaining cells after washing 7.500 cells/well were seeded into standard cell culture 96 well plates (Sarstedt Cell+) and 96 well plates with low adhering properties (BRAND InertGrade™) (n=3). 24 h after seeding, the wells were washed 3 times with 200 µl warm PBS+/+ and the DNA amount of the remaining cells was quantified using the CyQuant Kit (Molecular Probes Invitrogen) following the instructions of the manufactor. Fluorescence was measured using Stratagene Mx3005p reader (Agilent Technologies). The fluorescence units were converted into total cell numbers using standard curves.

Results

The cell number of the remaining MSC after washing was determined for BRAND InertGrade™ and Sarstedt Cell+ as a standard cell culture plate by CyQuant-Assay (shown as medians; the error bars represent the interquartile ranges).
Only 124 ± 1085 (median ± IQR) MSC/well could be detected on BRAND InertGrade™ 96 well plates compared to standard cell culture plates with 8694 ± 1895 MSC/well.



Conclusion

MSC seeded in InertGrade™ 96 well plates can be washed out effectively. Therefore these plates could be a useful tool to simplify Assays for testing cellular parameters of MSC growing on bone graft substitutes and/or other biomaterials.

References

Seebach C, Schultheiss J, Wilhelm K, Frank J, Henrich D: Comparison of six bone-graft substitutes regarding to cell seeding efficiency, metabolism and growth behavior of human mesenchymal stem cells (MSC) in vitro. Injury 2010; 41(7): 731-8

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