REPORT

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The present report describes the results of a highly reproducible in vitro assay that characterizes the response of an osteoblast-like cell line, MG63, to ZFuze™ vs. PEEK.

Objective:

The objective of this study is to compare the gene and protein expression changes in an osteoblast-like cell line exposed in vitro to ZFuze™ or PEEK.

Materials and Methods:

Culture of MG63 Osteoblast-like Cells: MG63 cells were purchased from ATCC and stored in liquid nitrogen until ready for culture. Upon thawing rapidly at 37ºC, cells were immediately resuspended in culture medium comprised of EMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin. Medium was changed every 48h thereafter. Cells were seeded at $1*10^4$ cells/cm^2 into a T75 culture flask until confluence was reached, at which point they could be passaged or used for experimental applications.

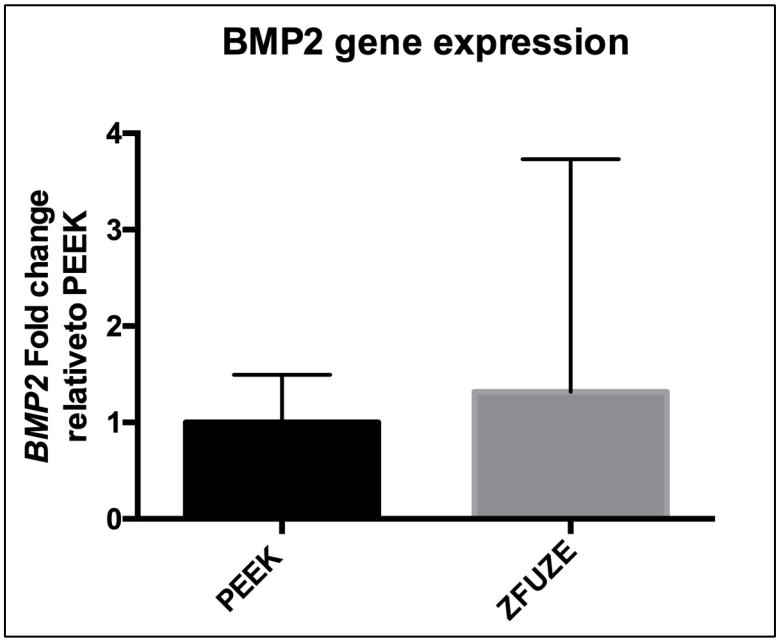
$1*10^4$ cells/cm^2 were plated into 6 well tissue culture plates containing 1.5 cm x 1.5 cm x 1.6 mm test articles composed of either ZFuze™ or PEEK. Upon reaching confluence, medium was changed a final time and the cells were incubated for another 24h. After 24h, the medium in which the cells were incubated for 24h was collected for later ELISAs, or the cells trypsinized for cell counting and subsequently lysed with TRIzol reagent for RNA isolation. A minimum of 3 biologic replicates was performed for each treatment condition.

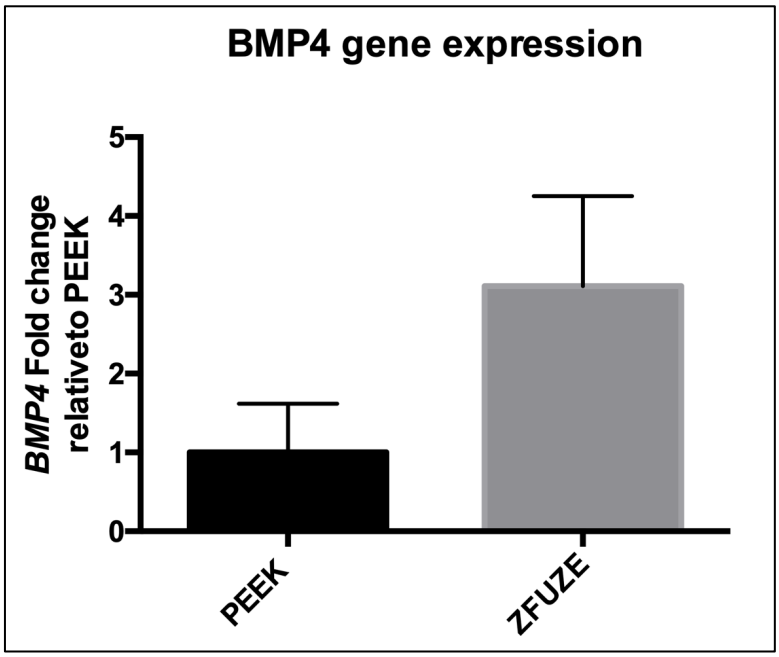
Gene Expression: RNA was isolated using the RNeasy Mini Kit according to the manufacturer's instructions. Isolated RNA concentration was then determined using a NanoDrop spectrophotometer. Reverse transcription of 500 ng of RNA to cDNA was performed via a high-capacity reverse transcriptase kit according to the manufacturer's instructions. SYBR Green gene expression assays were used to determine the relative expression levels of: hprt1 (housekeeper #1), pgk1 (housekeeper #2), bmp2, and bmp4. All results were analyzed by the ΔΔCt method using the two housekeepers, hprt1 and pgk1, to normalize the results.

Enzyme-Linked Immunosorbent Assays: Human BMP-2, BMP-4, and BMP-7 Quantikine ELISA kits (R&D Systems) were used according to manufacturer's instructions. Briefly, media collected followed 24h incubation with the test articles was used undiluted and allowed to incubate for 2h with the ELISA kits, followed by a 2h colorimetric substrate incubation step. The reaction was then stopped and read using a plate reader at 450 nm. A set of manufacturer-provided standards was used to generate a standard concentration curve against which all test article values were compared.

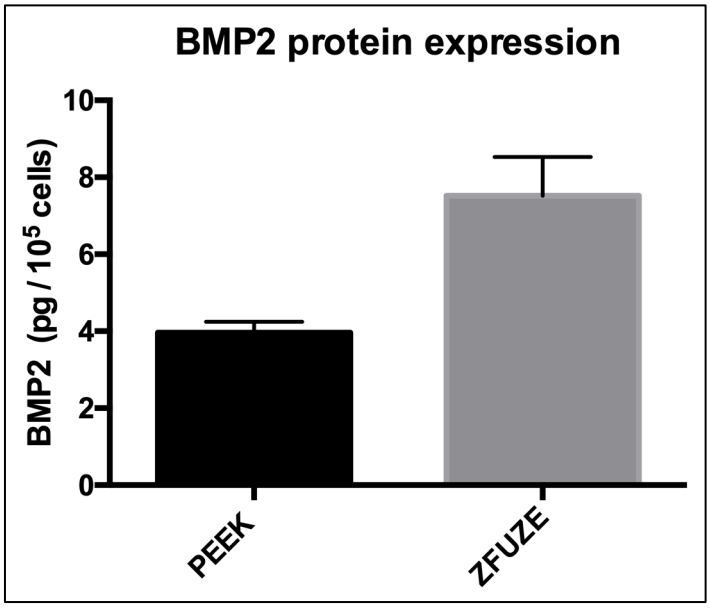
Results:

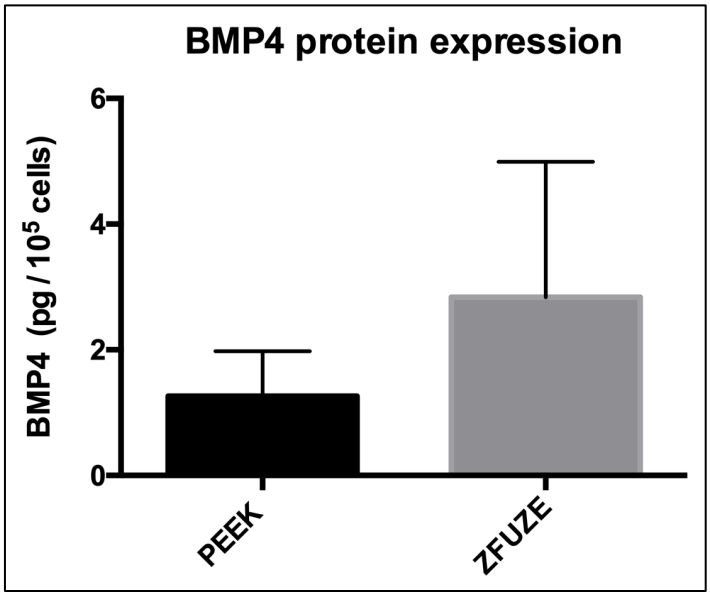
Gene Expression:

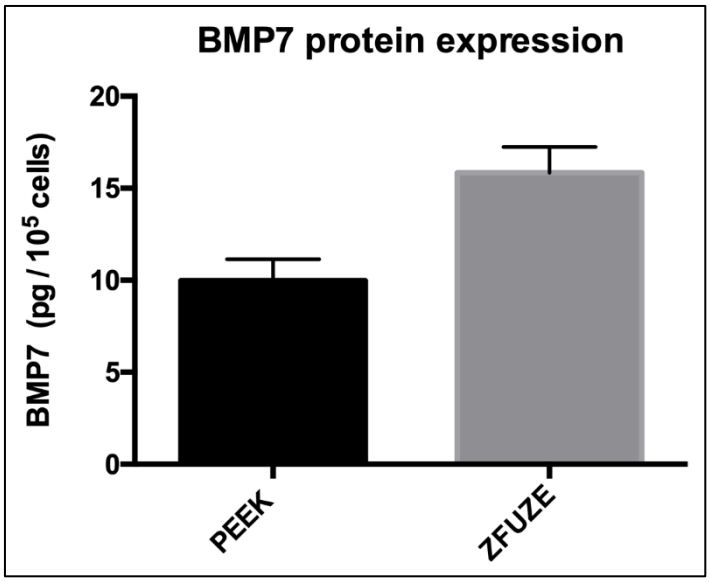




Protein Expression:







Summary:

Gene Expression: ZFuze™ showed greater expression values for *bmp*2 and *bmp*4, markers of osteoblast differentiation, than PEEK.

Protein Expression: ZFuze™ showed greater protein production values for BMP-2, BMP-4, and BMP-7 than PEEK, consistent with a more differentiated state in osteoblast-like cells compared to PEEK.

Conclusion:

Results of the present study suggest that ZFuze™ promotes greater expression of genes and proteins associated with osteogenic differentiation than PEEK.

References:

[1] L. Boulter, O. Govaere, T.G. Bird, S. Radulescu, P. Ramachandran, A. Pellicoro, R.A. Ridgway, S.S. Seo, B. Spee, N. Van Rooijen, O.J. Sansom, J.P. Iredale, S. Lowell, T. Roskams, S.J. Forbes, Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease, Nat Med 18(4) (2012) 572-9.
[2] X.M. Dai, G.R. Ryan, A.J. Hapel, M.G. Dominguez, R.G. Russell, S. Kapp, V. Sylvestre, E.R. Stanley, Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects, Blood 99(1) (2002) 111-20.
[3] S. Gordon, A. Pluddemann, Tissue macrophages: heterogeneity and functions, BMC Biol 15(1) (2017) 53.
[4] D.E. Gyorki, M.L. Asselin-Labat, N. van Rooijen, G.J. Lindeman, J.E. Visvader, Resident macrophages influence stem cell activity in the mammary gland, Breast Cancer Res 11(4) (2009) R62.
[5] S. Niida, M. Kaku, H. Amano, H. Yoshida, H. Kataoka, S. Nishikawa, K. Tanne, N. Maeda, S. Nishikawa, H. Kodama, Vascular endothelial growth factor can substitute for macrophage colony-stimulating factor in the support of osteoclastic bone resorption, J Exp Med 190(2) (1999) 293-8.

[6] B.N. Brown, R. Londono, S. Tottey, L. Zhang, K.A. Kukla, M.T. Wolf, K.A. Daly, J.E. Reing, S.F. Badylak, Macrophage phenotype as a predictor of constructive remodeling following the implantation of biologically derived surgical mesh materials, Acta Biomater 8(3) (2012) 978-87.

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