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Osteoblast proliferation on bone biomaterials with different biocompatibility profile

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INTRODUCTION: Several biomaterials have been used as bone substitute including ceramics, metals and polymers. Biomaterials can be classified according to its biocompatibility profile as biotolerated, bioinert and bioactive[1]. This study aimed at evaluating proliferation and viability of osteoblastic cells cultured on biotolerated (polymetylmetacrilate-PMMA), bioinert (titanium-Ti and stainless steel-SS), and bioactive (hidroxyapatite-HA and glass ceramic-45S5) biomaterials.

METHODS: Discs (12mm diameter and 3mm height) were prepared (PMMA, HA, and 45S5) or purchased (Ti and SS). Human bone marrow mesenchymal cells (hBMMC) were obtained from healthy donors, under approved research protocols of Committee of Ethics in Research. hBMMC were cultured in osteogenic medium containing α-MEM supplemented with 10% fetal bovine serum, 50 μg/ml gentamicin, 0.3 μg/ml fungizone, 5 μg/ml ascorbic acid, 7 mM β-glycerophosphate, and dexamethasone 10^{-7} M until subconfluence. Osteoblastic cells from first passage were subcultured at a concentration of $2x10^4$ cells/well on biomaterial discs in 24-well culture plates (n=5). Wells with no discs were used as control. During all the culture period, cells were maintained at 37ºC, 5% CO_2 and 95% air, and the medium were changed every 3 or 4 days. Cells were cultured for 7, 14 and 21 days, enzimatically released and counted. Proliferation was expressed as cell number/well at 7, 14 and 21 days and as doubling time between 7 and 14 days [2]. Cell viability was evaluated using the trypan blue method and expressed as percentage of total cell number. Data were compared by one-way ANOVA and Bonferroni test for multiple comparisons and considered to be statistically significant for $p \leq 0.05$.

RESULTS: Proliferation expressed as cell number/well is showed in Fig. 1. At 7 days, there was difference only between 45S5 and control (p=0.05). At 14 days, there were more cells on 45S5 than on any other biomaterial and control (p<0.0001). At 21 days, cell number ranking was as follows: control=45S5=SS>Ti=HA=PMMA (p<0.0001). Doubling time between 7 and 14 days (Fig. 2) was significantly lower only for cells cultured on 45S5 compared to other biomaterials and control (p=0.03). Cell viability was always higher than 90% in any period and there was no difference among any biomaterial.

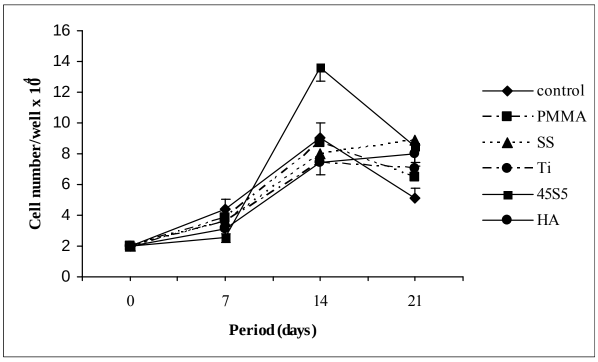

Fig. 1 – Proliferation of osteoblastic cells cultured on biomaterials for 7, 14 and 21 days. Data showed as mean ± SD (n=5).

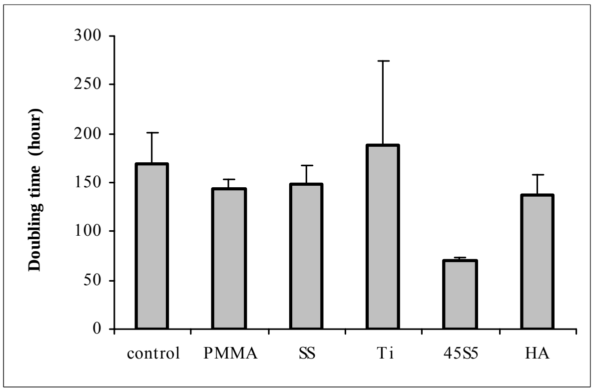

Fig. 2 – Doubling time in hours between 7 and 14 days of osteoblastic cells cultured on biomaterials. Data showed as mean ± SD (n=5).

DISCUSSION & CONCLUSIONS: All tested biomaterials allowed cell proliferation, regardless its biocompatibility profile. For all of them the pattern of proliferation was similar to control that was an increase in cell number from day 0 to day 14 and a decrease thereafter. Among them only 45S5 presented higher proliferation rate as showed by greater cell number at 14 days and lower doubling time. None of them affected cell viability in any period. It remains to be evaluated whether biocompatibility profile affects cell responses related to bone formation.

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