In Vitro Behavior of Human Osteoblastic Cells Cultured on Titanium Surfaces Modified by Oxidative Nanopatterning

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Statement of Purpose: The biocompatibility of a material depends on cellular response in contact with a surface. Nanoengineered surfaces possess the unique capacity of directly affecting the molecular and cellular events that ultimately determine the overall biological response to an implanted material, such as protein adsorption, cell adhesion and proliferation among others.[1] The versatility of simple chemical oxidative patterning helps to create unique nanotopographical surfaces that influence the behavior of various cell types and modulate the expression of key determinants of cell activity.[2] Thus, the objective of this investigation was to evaluate the biological response of osteoblastic cells from human alveolar bone cultured on titanium surfaces varying the etching parameters such as temperature and composition of H_2SO_4/H_2O_2 solution.

Methods: Titanium discs 12 mm in diameter and 2 mm in thickness were polished, cleaned and dried in air. The surface etching was performed with concentrated sulfuric acid and 30% aqueous hydrogen peroxide using the following solutions: etching at 25°C with 50% $H_2SO_4^{conc}$ and 50% $H_2O_2^{aq}$ for nanotexture; etching at 50°C with 50% $H_2SO_4^{conc}$ and 50% $H_2O_2^{aq}$ for nano + submicrotexture; etching at 50°C with $H_2O_2^{aq}$ for rough microtexture. Polished discs were used as control. Human alveolar bone fragments were obtained from three healthy donors, using the research protocols approved by the local Research Ethics Committee. Osteoblastic cells were obtained by enzymatic digestion using collagenase type II. The cells were cultured in osteogenic medium until subconfluence, which were harvested, subcultured on the sterilized titanium discs at a cell density of 2×10^4 cells *per* disc in 24-well culture plates and divided into control (C), nanotexture (N), nano + submicrotexture (N+S) and rough microtexture (RM) groups. During all culture, cells were incubated at 37ºC in a humidified atmosphere of 5% CO_2 and 95% air. The medium was changed every 3 days. After 7, 10, 14 days of culture, there were evaluated the following parameters: cell proliferation (Countess® Automated Cell Counter, Life Technologies, CA, USA), expressed as number of cells x 10^4/well; cell viability (MTT assay); total protein content, with the utilization of Lowry reagent (Sigma-Aldrich, MO, USA) and expressed as ug protein/mL; alkaline phosphatase (ALP) activity, assayed as the release of thymolphthalein from thymolphthalein monophosphate (Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) and normalized by protein content; finally, detection and quantification of bone-like nodule formation after 28 days with Alizarin red staining. Data were analyzed by SPSS statistical software (SPSS, Chicago, IL, USA), using the Kruskal-Wallis test followed by Mann-Whitney U test. Each individual experiment was performed in quintuplicate (n=5) for determination of averages and standard deviation. The significance level of the data was determined at $p\leq0.05$.

Results: In Figure 1, it can be observed that nanotexture titanium surface induced similar osteoblastic cell proliferation when compared to control group.

	Group / Day	Control	Nanotexture	Nano+ Submicrotexture	Rough Microtexture
Cell Proliferation	7	13±2.8	12.2±3.2	10.8±0.8*	10.6±5.0
	10	12±2.7	10.8±2.4	7±1.6**	7.4±2.0**
	14	14±3.8	14.2±4.1	10.2±1.4*	7.4±3.6**
Cell Viability	7	0.82±0.06	0.67±0.10	0.63±0.12**	0.65±0.14*
	10	0.86±0.06	0.85±0.04	0.85±0.05	0.76±0.23
	14	1,16±0.06	1,12±0.06	1,11±0.06	1,11±0.07
Total Protein Content	7	22,6±0.5	22.9±4.1	22.5±1.6	19.8±0.8*
	10	48,8±3.1	55.1±2.0*	44.5±8.5**	50.6±3.3
	14	64,6±5.1	56.3±1.8*	52.8±3.2	51.06±7.4
ALP	7	2,8±1.3	0.02±2.0	1.5±0.9	1,77±1.5
	10	13,1±6.2	12.5±4.8	8.7±3.8	10.11±3.6
	14	15,1±10.9	16.9±7.3	12±3.4	12.33±6.7
Nodules	28	0,0152±0.001	0.0145±0.001	0.0132±0.001	0.014±0.002

Figure 1. Average and standard deviation of the biochemical parameters evaluated. * $p\leq0.05$ and ** $p\leq0.01$.

Among the etched surfaces, the nanotexture group showed significantly higher cell proliferation in all periods when compared to N+S and RM groups, especially after 10 and 14 days ($p\leq0.01$). Cell viability was similar to all groups in all periods except for N+S and RM, which showed significantly lower viability when compared to control group after 7 days ($p\leq0.05$). The total protein content was significantly increased in osteoblastic cells cultured on nanotexture titanium surface when compared to control and N+S group after 10 days ($p\leq0.05$), with a decrease after 14 days when compared to control ($p\leq0.05$). Alkaline phosphatase activity was higher in cells cultured on nanotexture surface, when compared to the other etched surfaces after 10 days and also with control group after 14 days, although with no statistical difference. The quantification of bone-like nodules showed that there was no significant difference among the studied groups.

Conclusions: it is concluded that the variation of oxidative nanopatterning parameters like temperature and composition of the solution has an impact on biological response of osteoblastic cells, especially when it culminates with the nanotexture surface, suggesting that this nanopattern may provide a better substrate for cell activity and function.

References:

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