

**In vitro osteogenesis on chemically modified (ASD-AK) titanium surface**AL. Rosa<sup>1</sup>, RL. Franco<sup>1</sup>, MM. Beloti<sup>1</sup>, R. Chiesa<sup>2</sup><sup>1</sup> Faculty of Dentistry of Ribeirão Preto, University of São Paulo-Brazil. <sup>2</sup> Department of Chemistry, Materials and Chemical Engineering "G Natta" Politecnico di Milano, Milano-Italy.

**INTRODUCTION:** Titanium (Ti) has been widely used as bone biomaterial in dentistry and medicine. Several modifications of Ti surface were described aiming to improve its biocompatibility. A chemical modification (ASD-AK) consisted of anodic spark discharge using consecutively solutions containing phosphorous ions, calcium ions, and alkali etching was described [1]. ASD-AK produces Ti surfaces containing Ca and P that allows higher cell proliferation rate compared to untreated Ti [2]. However, it remains to be showed whether ASD-AK improves bone response on Ti surface. The aim of this study was to compare in vitro osteogenesis on ASD-AK and on untreated Ti.

**METHODS:** ASD-AK and untreated Ti coupons (10x10x1 mm) were prepared as described elsewhere[1]. Human alveolar bone fragments (explants) were obtained from healthy donors, under approved research protocols of Committee of Ethics in Research. Osteoblastic cells were obtained from these explants by enzymatic digestion and cultured in  $\alpha$ -MEM supplemented with 10% fetal bovine serum, 50  $\mu$ g/ml gentamicin, 0.3  $\mu$ g/ml fungizone, 5  $\mu$ g/ml ascorbic acid, 7 mM  $\beta$ -glycerophosphate, and dexamethasone  $10^{-7}$  M until subconfluence. Cells from first passage were subcultured at a concentration of  $2 \times 10^4$  cells/well on ASD-AK and untreated Ti in 24-well culture plates. During all the culture period, cells were maintained at 37°C, 5% CO<sub>2</sub> and 95% air, and the medium were changed every 3 or 4 days. For attachment evaluation, cells were incubated for 24h, enzymatically released and counted. Data were expressed as percentage of seeded number. For proliferation, cells were cultured for 1 and 10 days, enzymatically released and counted. Data were expressed as doubling time in hours, with higher values meaning lower proliferation. At 10 days, cell viability was evaluated using the trypan blue method and expressed as percentage of total cell number. At 14 days, alkaline phosphatase (ALP) activity was measured using a commercial kit (Labtest) and expressed as  $\mu$ mol thymolphthalein/h/mg protein. Bone-like nodule formation was stained by alizarin red at 21 days. Average area of the nodules was measured using an image analyzer and the amount of bone-like

formation was expressed as percentage of sample area. Data were compared by Student t-test.

**RESULTS:** All data are shown in Table 1. For cell attachment (p=0.9), viability (p=0.35), and bone-like nodule formation (p=0.12) there was no statistically significant difference between ASD-AK Ti and untreated Ti. Cell proliferation (p=0.02) and ALP activity (p=0.02) were both affected by treatments, being higher in untreated Ti.

*Table 1. Attachment (% of seeded number), doubling time (hours), viability (% total cell number), ALP activity ( $\mu$ mol thymolphthalein/h/mg protein), and bone-like nodule formation(% of sample area) of osteoblastic cells derived from human alveolar bone cultured on ASD-AK and untreated Ti. Data were expressed as mean  $\pm$  standard deviation (n=5).*

	ASD-AK Ti	Untreated Ti
Attachment	18.4 $\pm$ 10.85	18.3 $\pm$ 13.57
Doubling time	337.4 $\pm$ 185.52	96.3 $\pm$ 11.38
Viability	100 $\pm$ 0	98.5 $\pm$ 3.44
ALP activity	16.9 $\pm$ 1.62	26.4 $\pm$ 5.18
Bone-like nodule formation	4.3 $\pm$ 5.52	6.4 $\pm$ 1.26

**DISCUSSION & CONCLUSIONS:** In this study both initial and final events of in vitro osteogenesis were evaluated. Initial events were evaluated by cell attachment and proliferation and final ones by ALP activity and bone-like nodule formation. Cell attachment was not affected by any Ti surfaces but cells cultured on ASD-AK Ti presented a slower proliferation rate that could not be explained by differences in their viability. Also, ALP activity was lower for cells cultured on ASD-AK Ti but it did not affect bone-like nodule formation. It was observed that both Ti surfaces were capable of sustaining in vitro osteogenesis despite some differences between them. These results failed to show that ASD-AK treatment could stimulate in vitro osteogenesis on Ti surface, suggesting that ASD-AK treatment does not affect bone biocompatibility of Ti.

**REFERENCES:** <sup>1</sup> Sandrini et al (2003) *J Appl Biomech Biomater*; 1:33-42. <sup>2</sup> Giordano et al (2004) *Appl Biomech Biomater*; 2: 35-44.

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