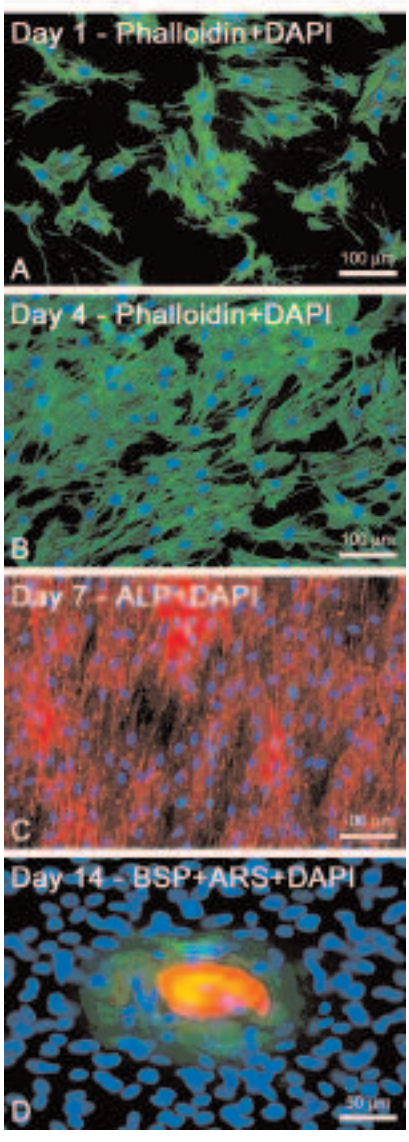
OSTOGENESIS ON TITANIUM: *IN VITRO* EVALUATION OF THE EFFECTS OF DIFFERENTS STIMULATORY METHODS

Adalberto ROSA

Ribeirão Preto School of Dentistry / University of São Paulo (USP)

Human Osteoblastic Cells on Titanium Surfaces



Epifluorescence of human alveolar bone-derived osteoblastic cells grown on Ti surface. Phalloidin labelling indicates actin cytoskeleton (A,B) whereas blue flourescence reveals cell nuclei stained with DAPI (A-D). The development of the osteogenic phenotype is demonstrated by a strong alkaline phosphatase labeling (ALP, red fluorescence) at day 7 (C) and the formation of Alizarin red S (ARS, red fluorescence) stained-/bone sialoprotein (BSP, green fluorescence) labelled- bone-like nodules at day 14 (D)

In oral implantology, one of the biggest challenges has been to increase and/or accelerate osteogenesis on titanium (Ti) surfaces. Regarding the bone-implant interface and possible ways of stimulating osseointegration of Ti, it is possible to act on both sides. In this way, chemical and morphological modifications of implant surfaces and stimulation of bone formation seem to be the most suitable strategies. Therefore, the aim of this study has been to evaluate methods that could contribute to stimulate osteogenesis on Ti surfaces by using osteoblastic cell culture models, and to gain additional information about bone biology. In this project the effect of chemical modifications of Ti surfaces by Ca/P and collagen deposition on *in vitro* osteogenesis were evaluated. Moreover, the role of alkaline phosphatase and biomimetic systems on matrix mineralization, the expression pattern of some noncollagenous proteins, and the effect of a mixture of growth factors, growth hormone, and laser therapy on osteogenesis have been investigated.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

This project allows us to study the behavior of bone-derived cells cultured on Ti during the period necessary for the osteoblastic phenotype to develop. The results showed that the chemical modifications of Ti surfaces stimulate *in vitro* osteogenesis. Human osteoblastic cells are sensitive to the Ca/P modified Ti surface during the transitional stage between the end of the proliferative phase and the onset of the differentiation/matrix maturation phase. Proliferation and differentiation of these cells are enhanced by collagen deposition. It means that both surface modifications could represent useful approaches for producing new Ti implants.

The effect of growth hormone on osteogenesis and on gene expression of osteoblastic markers is donor-age-dependent, being more pronounced on alveolar bone-derived cells from adolescents. Interestingly, its effect on cells derived from bone marrow seems to be somewhat different. It precludes the therapeutic use of growth hormone in combination with Ti implant placement but at the same time opens many interesting opportunities to investigate its role in bone biology. The mixture of growth factors plus proteins similar to the platelet rich plasma affected the development of the osteogenic phenotype both in human and rat cultures, leading to an increase in the number of cells. Despite such increase, the latter express a less differentiated state. The stimulation of osteoblastic cells using laser therapy was evaluated under several different conditions, i.e. laser dose-response, different time of laser exposition and in serum privation condition. None of them seemed to affect cells in any way. The expression of heat shock protein HSP70 is increased after submitting osteoblastic cells to stressful condition represented by thermal treatment. However, it remains to be determined whether Ti could represent a stressful environment capable of increasing HSP70 expression. A method to obtain purified alkaline phosphatase from human bone marrow cells differentiated into osteoblasts was developed. That enzyme can be incorporated into vesicular systems that mimic the matrix vesicles enrolled in the calcification of extracellular matrix. Such approach could be used to increase and/or accelerate the osseointegration of Ti implants.

MAIN PUBLICATIONS

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Adalberto Luiz ROSA

Faculdade de Odontologia de Ribeirão Preto
Universidade de São Paulo (USP)
Departamento de Cirurgia
Avenida do Café, s/n – Campus da USP
14040-904 – Ribeirão Preto, SP – Brasil

+55-16-3602-3980
adalrosa@forp.usp.br