Osteogenic differentiation of bone marrow mesenchymal cells on Ti surfaces: additive effect of biochemical modification and surface topograhy

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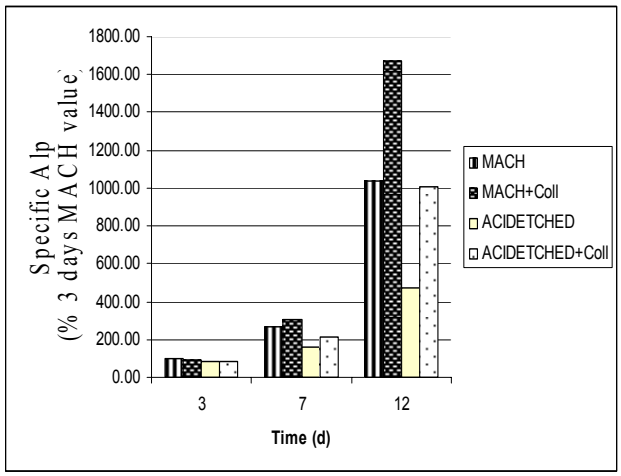
Statement of Purpose: According to Davies the most critical issue in de novo bone formation is the recruitment of osteogenic cells and their migration to the implant surface [1]. Marrow-derived mesenchymal cells (MDMCs) play an especially important role in osteointegration, migrating to the peri-implant wound site and differentiating along the osteogenic pathway, under the effect of a number of factors and transitorily expressed cytokines.
It is generally recognized that new generation bio-active surfaces of bone-contacting devices should be able to stimulate osteogenic differentiation of MDMCs, in order to increase the healing rate and the formation of peri-implant new bone tissue. To this purpose, biochemical modification of titanium implant surfaces by collagen, an extracellular matrix protein that promotes osteoblastic differentiation of bone marrow cells and controls a number of aspects of their progression along the osteogenic pathway, has been investigated by a number of groups [2]. Little is known about the coupling of biochemical and topography stimula on MDMCs differentiation. In this communication we present our results on *in vitro* mesenchymal cell differentiation on smooth Vs rough surfaces, either Ti or Collagen I coated Ti. The scope of the work is to ascertain whether a given topography can dictate the extent of the effect of biochemical modification on MDMCs behavior.

Methods: Experiments were performed on CpTi (grade 2) disks, 6 mm diameter. Surfaces were either machined (mach) or roughened by double acid etching (HF first step, HCl/H_2SO_4, 80C 6 mins, second step) (acidetched). Type I porcine collagen was covalently linked to alginic acid-coated Ti disks, obtained by surface functionalization by allylamine plasma deposition and carbodiimide coupling of alginic acid. Surface chemistry was evaluated by X-ray Photoelectron Spectroscopy (XPS), surface topography and roughness by Stereo-Scanning Electron Microscopy (SEM), using the software MeX 4.2 supplied by Alicona.
Human MDMCs (Cambrex, second passage) were cultured on disks using standard cell culture techniques. Cell growth was performed both in basal and in osteogenic medium (Cambrex). At selected time intervals, cell growth and number was evaluated by MTT test while osteogenic differentiation was evaluated by specific alkaline phosphatase (Alp) measurement (3 replicates). At the end of the experiments, Von Kossa staining experiments were performed to evaluate mineral deposits formation by differentiated cells.

Results/Discussion: XPS analysis confirms that Ti surfaces are clean and that the yield of the collagen coupling reaction is not affected by surface topography. Surface roughness measurement yields data in agreement with literature, roughness is not affected by the surface-linked nanometer-thin collagen coating:
Results of the specific alkaline phosphatase measurements are reported in the graph below (osteogenic medium), expressed as % over the 3 days value of the machined surface. No significant difference between couples of samples are detected at three days, when induction of differentiation is still going on. However, at longer experimental times, cells grown on collagen coated samples show higher specific Alp activity as compared to those grown on the correspondent uncoated surface. Differences over uncoated surfaces are significant at both time points, showing that biochemical stimula from the surface can promote osteogenic differentiation over plain Ti. Interestingly, the collagen effect adds to that of the surface topography, showing that the coating *per se* is not controlling alone the cell behavior, rather it operates in an additive way with surface topography, at least within the range of topographies tested in this work.



Similar results, even if shifted at significantly longer experimental times, were obtained in the basal medium.
Conclusions: In conclusion, present experiments confirm that biochemical modification of Ti surfaces by covalent linking of collagen promotes MDMCs differentiation along the osteogenic pathway. Moreover, data show that the effect due to collagen is coupled to that of surface topography, stressing the need for both topography and biochemical optimization in the design of bioactive surfaces of bone-contacting devices.

References: [1] Davies JD, J Dent Ed., 2003;67:932-949
[2] Morra M, Eur. Cell. Mat. J., 2006;12:1-15