Covalently-linked Hyaluronan promotes bone formation around Ti implants in a rabbit model

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Statement of Purpose: Interactions at the bone-implant interface are recognized as the key to osteointegration. The immobilization to Ti surfaces of critical organic components of bone to affect tissue response is presently actively investigated. Most studies are based on surface modification by peptides or proteins, while recent literature suggests a role also for glycosaminoglycans (GAGs) [1].

Hyaluronan (HA) is a linear polysaccharide consisting of the repeating disaccharide unit N-acetyl-D-glucosamine - D-glucuronate. Concerning bone regeneration, considerable HA is synthesized in the early stages of callus formation during the repair of fractured long bones. Moreover, it is known that large amounts of HA are secreted when implants of decalcified bone remineralize as bone [2].

Based on the quoted knowledge, it was speculated that the presentation of a HA-rich interfacial environment by covalent linking of HA to Ti implant surfaces could stimulate the early onset of peri-implant healing and bone regeneration mechanisms. The scope of this work was the *in vivo* evaluation of the effect of covalent linking of HA to Ti implant surfaces in a 4 weeks rabbit model.

Methods: Periodate-treated HA (Mw 800kDa) was covalently linked by cyanoborohydride chemistry to amino groups introduced on the Ti surface by allylamine plasma deposition (samples coded HATi, while control untreated samples are coded Ti). Surface topography and chemistry of the samples (12 x 2 mm screws) were characterized by Scanning Electron Microscopy (SEM) and X-ray Photolectron Spectroscopy (XPS).

In vivo testing of EtO sterilized fixtures was performed both in the cortical bone of femur middiaphyses (5 animals, 2 implants in each femur) and in the trabecular bone of the lateral aspects of distal femurs (5 animals, 1 implant in each femur). Animals were sacrificed at 4 weeks. Evaluation was performed by hystomorphometry (cortical and trabecular bone) and measurement of peri-implant bone microhardness and push out force (cortical only). Statistical analysis was performed using SPSS v.12.1 software (SPSS Inc., Chicago, Illinois, USA), by non parametric Wilcoxon signed-rank test, followed by Monte Carlo methods to compute one-sided probability. Data are reported as median, SEM at a significant level of $p < 0.05$ (one-tailed).

Results/Discussion: Surface analysis by XPS shows that a few nanometer thick HA overlayer is successfully linked to the aminated surface by the quoted coupling reaction. As expected, surface topography of the screw is not affected by the surface modification process, within the resolution of SEM microscopy. As to *in vivo* findings the table below shows the obtained results (n = 5) (AI: Affinity Index ; BI: Bone Ingrowth, F_{max} :maximum push-out force, BMI%: bone maturation index, calculated by dividing the microhardness of the bone regrown at the interface by the microhardness of the bone away from the interface).

Bone	Parameter		Screw	
			Ti	HA Ti
Trabecular	AI (%)	Median	22.5	69.0**
		SEM	5.8	5.8
	BI (%)	Median	30.3	56.3*
		SEM	5.1	3.4
Cortical	AI (%)	Median	55.0	69.7*
		SEM	5.2	2.9
	BI (%)	Median	84.5	91.0*
		SEM	3.3	0.7
Cortical	F_{max} (N)	Median	185.3	232.2*
		SEM	10.7	18.4
Cortical	BMI%	Median	79.1	90.6*
		SEM	1.0	1.0

Wilcoxon signed rank test: *, $p < 0.05$; **, $p < 0.01$.

Data show, for all measured parameters, significant enhancement of peri-implant healing and bone regeneration around Ti implants bearing a covalently linked HA layer. In cortical bone, the comparison of hystomorphometric and microhardness findings confirms that there is not only more bone around HATi implants, but it is also more mature. Histological findings and the very significant enhancement of histomorphometry parameters in the marrow-rich trabecular bone, suggests indeed a stimulation of healing mechansims by the interfacial HA layer, according to pathways reported in the literature.

Conclusions: In conclusion, results show, by a number of different parameters and in both major bone macroarchitectures, that biochemical modification of Ti surfaces by covalent linking of HA stimulates bone regeneration at the implant interface, in a 4 weeks rabbit model. These results are clearly relevant in the development of bone-contacting devices. Based on these experiments it is not possible to conclude whether the observed results are specific for HA, or if other covalently-linked GAGs induce similar effects. The role of covalently linked sulphated and non-sulphated GAGs, and the evaluation of the effect of HA molecular weight, are currently investigated

References: [1] Rammelt S, Biomaterials, 2006;27, 5561-5572

[2] Bernard GW, in: Redefining Hyaluronan, Abatangelo and Weigel Eds., Elsevier, 2000, pp. 215-231