Lectinhistochemistry evaluation of rat's femur implanted with nano-hydroxyapatite and commercial hydroxyapatite

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INTRODUCTION: Hydroxyapatite (HAp) is widely used as biomaterial and a major component of bone. The effects and reparation of a nano-HAp implant in bone tissue is not fully understood in a living body. The study of *in vivo* bone implants using tissue molecular researching tools may help to understand how the bone reacts facing this kind of nano-HA implant. One important molecular pattern of bone is the membrane glycoprotein content, which leads to profile the differences of this content with the use of lectinhistochemistry (LHC). LHC is a technique which uses the lectins' ability of binding oligosaccharides combined to histochemistry tissue staining of membrane specific glycoproteins, thus the molecular approach [1].

METHODS: The nano-HAp powder were manufactured with addition of Mg^{2+} 0.36%wt synthesized by neutralization method, the mean particle size ranged between 75nm to 100nm by TEM. To the *in vivo* assay were used 6 males Wistar rats, 3 formed the test group using nano-HAp implant in form of powder and the other 3 formed the comparison group using commercial HAp (cHAp) powder as implant. The surgery consisted of 2mm hole drilled in the right femur of rats, where the material was implanted, while in the left femur the hole received no implant as control for the repair process, which lasted 4 weeks.

After euthanasia of animals, femur samples of the surgery site were taken and fixed with formalin, decalcified, dehydrated and embedded with paraffin to perform histological slides for both morphological and molecular evaluation. The lectins selected to perform LHC were: PNA, UEA-1, RCA-1, WGA and sWGA (Vector Labs).

RESULTS: The repair process was evaluated morphologically, grossly there was no remarkable feature between them. Microscopically there was a slightly difference between femurs with implanted material and femurs without any implant. The thickness of implanted femurs were slightly bigger, although both femurs were able to successfully close the bone defect performed. The LHC intensity staining pattern is represented in the Table 1. The Fig. 1 shows the staining for WGA and sWGA on cHAp and nano-HAp respectively.

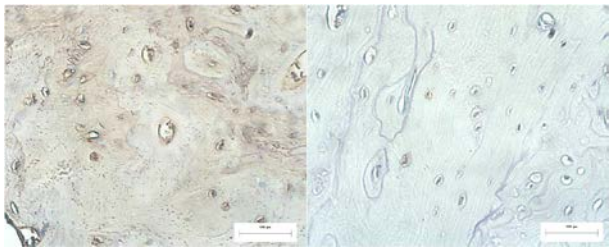

Fig. 1: Left image showing LHC staining pattern for WGA (++) and on the right image the LHC staining pattern for sWGA (+) – Scale bar 100µm

Table 1. LHC pattern of implants in the right femur for both materials and left femur without implants

	cHAp		nano-HAp	
Lectin	**with**	**without**	**with**	**without**
PNA	+	++	+	++
UEA-1	-	-	-	-
RCA-1	++	++	++	+
WGA	+	++	-	+
sWGA	-	-	+	+

DISCUSSION & CONCLUSIONS: The glycoproteins content of cell's membrane shows how the cell is signalling and interaction with the extracellular environment. In order, to understand this bone tissue behaviour when it is facing a implant, LHC is an appropriate tool. Of tested lectins, for PNA and UEA-1 there was no difference between implant groups, although PNA staining were less intense in both implanted materials. A slightly difference were observed with RCA-1 and WGA, but without remarkable significance due the intensity pattern. Nevertheless for sWGA an important feature is present, its absence staining in cHAp group and positive staining in nano-HAp group in both with and without implant, this feature is due the possible migration, dispersion and influence of nano particles in far from the implant site at distant parts of the living body. This must be related to the physical size of the nano particle and its interference throughout the body.

REFERENCES: [1] T.J. Lyons, R.W. Stoddart, S.F. McClure, et al (2007) *J Mol Histol* **38**:13-23.

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