# Platelets Response to Hydroxyapatite-Alginate Matrixes

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## INTRODUCTION AND OBJECTIVE

Recently, we studied the role of colloidal solutions with $Mg^{2+}/Zn^{2+}$ - substituted hydroxyapatite (HA) and polyethylene glycol (PEG) in purulent traumatic process, induced by subtoxic doses of cyanides (NaCN) and dinitrophenol (DNP). It was shown, that $Ca^{2+}/Mg^{2+}/Zn^{2+}$-associated HA nanoparticles assisted the normalization of metabolic and oxidizing processes in injured cover tissues and prevented the cell membrane destruction.

It is known, porous HA-alginate (ALG), HA-gelatin composites were investigated as bone replacement material. Data are available also, that addition HA into cellulose derivates - chitosan improved cell attachment (Dorozhkin 2012).

The aim of this study was to estimate human platelets (Pt) interaction with ALG and $Ca^{2+}/Mg^{2+}/Zn^{2+}$ HA-ALG matrixes, modified with biopolymers (BP), such as gelatin (GEL) and cellulose (CL).

## MATERIALS AND METHODS

ALG-BPs matrixes in the thin plate films form and HA-ALG matrixes in bead form are performed and cross-linked by calcium chloride solution.

The film samples, ALG-GEL and ALG-GEL-CL were tested after drying at 22ºC (diameter 30 mm and thickness 0.1 mm).

The HA powder was prepared at Biomed (Russia). A suspension of 10 wt.% HA in distilled water was stirred and a solution of 1.0 wt.% CL was added to produce a gelatinous suspension. The mixture was stirred for a few hours and mixed with ALG solution (3 wt. %) by slow addition of ALG by using more or less ALG in the process. The suspension with different HA/ALG weight ratio was added as drops in calcium chloride solution (5 wt.%) using syringe needle diameter 2.0 mm. The samples and HA/CL-ALG were tested after drying at 22ºC.

The exact Ca/P ratio was measured by X-ray diffraction (XRD) on a sample heated at 900 ºC. FTIR and elemental analysis were used. Microstructures were observed by transmission electron microscopy (TEM) (LEO 912AB OMEGA Carl Zeiss, Germany).

The source of Pt was platelet-rich plasma (PRP), separated from citrate-conserved blood (Pt concentration-500x$10^9$ cells/l). The estimation of human Pt interaction with matrixes (cells/$mm^2$) performed in drop of liquid (20µl), time exposition is 30 min. (short-time study) and in vertical liquid

column (0,5ml), time exposition is 0.5-3 hour (long-time study) at 22 and 37ºC. Pt are studied with method of morphofunctional platelet rate analysis, using vital fluorochrome staining and fluorescencent microscope (Makarov 2012). Morphofunctional PT rate included such parameters as morphofunctional activity (MFA), in points and adhesive PT activity (APA), in points.

## RESULTS AND DISCUSSION

The sample sintered at 900 ºC had Ca/P ratio of 1.67 estimated by elemental analysis. XRD, FTIR indicate that the samples can be related to crystal HA. The results of TEM and XRD analysis indicated that $Ca^{2+}/Mg^{2+}/Zn^{2+}$HA - ALG bead matrixes dried at 22ºC consist of two phases: amorphous and crystalline (Fig.1,a.b). TEM visualized highly porous structure of HA/CL- ALG bead matrixes. Amorphous phase had spherical foam nano- and micro-porous structure (5.0-500 nm) including thin porous HA fibers of slide-like form, about 60 nm length, 6 nm thickness and 48 nm in width (Fig.1, a).. Crystalline phase was revealed in dark field (Fig.1, b).

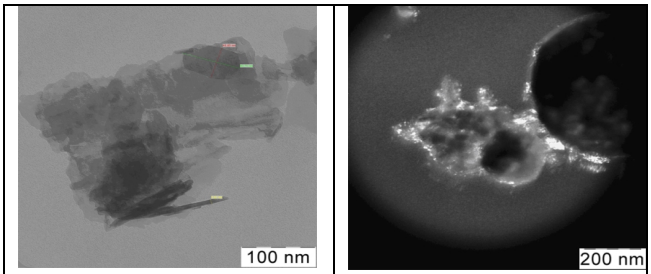


**Figure 1: TEM micrographs of HA/CL-ALG beads structure: a) -amorphous phase, b)– dark field image of crystalline phase**

To compare, Ca HA/ GEL powder sample, prepared as describe earlier (Krylova 2002) had spherical nano-porous (5.0-20 nm) structure. Crystalline phase was revealed consist of HA fibers of needle form about 400 nm length and 40 nm thickness.

Furthermore, important, that the foam porous HA/CL -ALG structure bead matrix was found to give increased resistance to 3D leakage. The average diameter of beads was increased with the increase HA content saving their spherical form after drying. Therefore, it may be proposed, HA matrixes should possess remarkable adhesive properties.

PT of the initial PRP has normal value of morphofuctional parameters - MFA =41 points, APA=40 points.

The analysis of attached PT morphology on surface of ALG-BP films indicate that all PT don't contain granules, 40-45 % of the attached Pt have the dendrite form that is the terminal stage of adhesive PT activation.

The Pt adhesion on ALG-BP film matrixes induced the functional activation of PT in all volume of PRP in vertical liquid column (Fig.3). After 60 min polymer contact all PT were absorbed or inactivated (MFA=17 points; APA=0 points). Long-time PRP exposition with ALG/CL-GEL films in vertical liquid column at 37°C leaded to the considerable decrease of functionally active Pt in all volume PRP, accompanied with their degranulation and formation of numerous trombofibrinous aggregates.

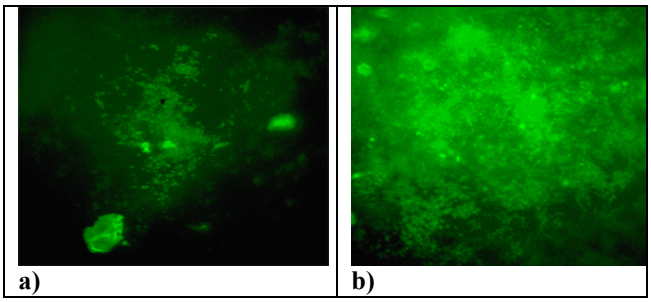


**Figure 2: Pt adhesion on the HA/CL-ALG film at 37ºC (x 400): a) -30 min; b) -60 min of contact**

The Pt activation begins in about 5-10 min contact PRP with ALG-BP film; after 15 min PRP contained 65-70% functionally active cells (cells with granules) were activated; after 30 min – 85-90%; after 60 min – 100%. So, after 1 hours of exposition 5 ml PRP don't content functionally active cells.

It may be concluded, that during PRP contact with ALG-GEL film, the mass adhesion of functionally active PT, 258±23,5 cells/mm$^2$ only at 37ºC occurs, more intensive than on slide glass control. The most intensive PT adhesion is detected on ALG-BP film matrixes with GEL and CL, absorbed 412,8±25,5 cells/mm$^2$ at 22ºC and 625,5±44,8 cells/mm$^2$ at 37ºC, whereas control glass slide compounds 22.5±4.5 and 112±7.2 cells/mm$^2$. It should be noted that at PRP contact with film ALG/CL-GEL, mass adhesion as at 37°C (physiological optimal temperature for PT adhesion) as at 22°C occurs, that is, this matrix has the most adhesive properties. During PRP-contact, the macrostructure of matrixes did not visibly change.

PRP-contact with HA-ALG beads was similar: HA beads caused the mass activation of Pt, accompanied with their degranulation and the formation of a dense trombofibrinous layer in the sample. As a result, functionally active Pt weren't revealed after 30 min of PRP exposition with beads at 37°C. The trombofibrinous layer contacts closely with the surface of beads matrixes.

Significantly, all samples of beads actively adsorbed human leucocytes (LC) on their surface (Fig.3). The quantity of absorbed LC varied from 50 to 250 cells/mm$^2$ in different types of HA/CL-ALG beads, whereas control glass samples had only 5-7 cells/mm$^2$.

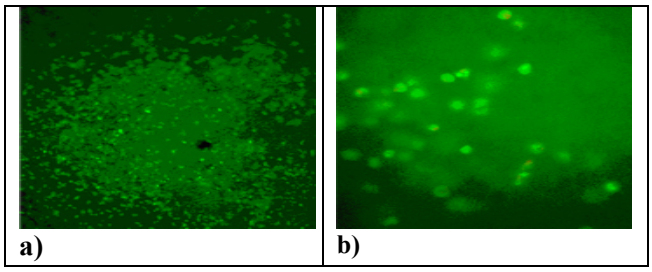


**Figure 3: Vital stained human LC on the surface HA/CL-ALG beads: a) -(x100); b) -(x400)**

Finally, it was concluded that addition of HA modified with CL increases the quantity adhesive LC on beads matrixes (Tabl.1).

**Table 1: The estimation of quantity adhesive LC on HA/CL -ALG beads matrixes**

| Bead diameter, mm | Quantity of LC in PRP, (cells/mm$^2$) | | Quantity of LC in beads, (cells/mm$^2$) |
|---|---|---|---|
| | Before 37°C | After | |
| 1.0 | 6,1 | 0,31* | 100 |
| 1.5 | 2,8 | 0,12* | 200 |
| 2.0 | 6,0 | 0,11* | 212 |
| 3.0 | 5,9 | 0,08* | 222 |

## CONCLUSIONS

ALG-BP film matrixes and $Ca^{2+}/Mg^{2+}/Zn^{2+}$-associated HA bead matrixes can be used as novel absorbtive agents for human PT and LC.

## REFERENCES


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