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EFFECT OF CHITOSAN MOLECULAR WEIGHT, PERCENTAGE IN SOLUTION AND METHOD OF PRODUCTION TO HUMAN BLOOD CELLS

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Background. Adequate haemostasis after trauma and during surgical operation is a big challenge in modern medicine. About the 40% traumatic and more than 90% of combat deaths took place in pre-hospital settings. And about the 50% from these deaths have been reported due to massive blood loss [Ersoy G, 2007]. Sauaia A. reported 80% of civilian trauma fatalitieswithin the United Statescauses by uncontrollable haemorrhage [Sauaia A., 1995]. Also, haemorrhage in trauma patients is a leading cause resulting reoperation [Hirshberg A, 1993].Topical haemostatic treatment was applied since ancient time. They used herbs, mixture of wax, grease and barley and also animal hides mixed with hot sand to stop bleeding [Hardean E. Achneck, 2010]. Advances in biotechnology have resulted in an explosivegrowth of topical haemostatic agentsin the last two decades.Chitin and chitosan hemostatic dressing are most promising due to effective blood stop and possible additional properties like antibacterial and stimulatory to regeneration.

Both clinical and experimental evaluations of chitosan-based hemostatic dressing suggest their high effectiveness and safety in civil and battlefield application. But still not understanding how does molecular weight influence to haemostatic activities of chitosan-based materials. Also chitosan may be present in different concentration that can change effectiveness and time that need to stop bleeding.

Aim. The aim of this research was to evaluate interaction between human blood cells and various forms of chitosan-based materials with different molecular weight, concentration of chitosan.

Materials.

We prepared solutions from chitosan with molecular weight (MW) 200, 500 and 700 kDa and deacetylation rate 87% in in 1 % acetic acid. Percentage of chitosan in primary solution was 1%, 2%, 3% and 5% respectively. Using these solutions we made following materials with potentials hemostatic activities - Gauze-chitosandressing (G-Ch) with chitosan MW 200 and 500 kDaand chitosan concentration 2%, 3% and 5% and chitosan MW 700 and its concentration 1% and 2%. 200 and 500 kDa MW chitosan used to made freeze-gelation sponges (FG-200 and FG-500). 10 layers of standard cotton gauze (SCG) were used as a control.

3 human subjects volunteered to have 80 mL of blood drawn by a registerednurse at the Medical Institute of SSU. Subjects were healthy adults in age 20, 22, and 24.2.5 ml of blood was immediately placed to 35Becton Dickinson Vacutainers® with 3.6 mg EDTA for complete blood count (CBC) test.

Methods.

The strips of chitosan-based materials and standard cotton gauze with weight 100 mg was placed to Becton Dickinson Vacutainers® and incubated in thermostat in temperature 36 ^{0}C during 10 minutes. All samples were removed and blood transported to the hematology tests. Untreated blood was used as a control.

Results.

During the CBC test we focused on Red Blood Cells (RBC) and Platelets parameters. Current experiment did not show any significant differences between control (untreated blood) and blood that interacted with chitosan-based materials in RBC concentration, haemoglobin level, RBC Distribution Width, and Mean Corpuscular Volume.

Compare the RBC, amount of platelets and its parameters significant changed depending on MW, percentage of chitosan in solution and type of material. All types of G-Ch dressings made from 200 kDa MW chitosan significantly decrease platelets amount in blood in 10 minutes after incubation. Materials made from 2% chitosan solution have strongest effect - platelets level decrease compare the control group in 23.03% ($p \leq 0.001$). Samples from chitosan MW 700 kDa significant decrease platelets level but difference was not more than 8.33% ($p=0.023$). Materials, made from 500 kDa chitosan did not change platelets level except samples from 3% solution that caused significant depression of cell concentration in blood. Both 1% and 2% FG materials did not affect platelets amount.

Our data shown that all types of G-Ch dressing affected Mean Platelet Volume. Materials made from chitosan with MW 500 kDA caused more significant augmentation of platelets volume compare the 200 and 700 kDa samples. Most prominent effect we can see for materials made from 2% solution of 500 kDa MW chitosan – the difference compare the control is 21.63% ($p \leq 0.001$). Platelet Distribution Width changed only in blood samples that interacted with 2% chitosan with MW 200 and 700 kDa. All other samples did not affect this parameter. FG chitosan materials did not change both MPV and PDW.

Conclusion.

The effect of chitosan based materials to blood cells depend on method of production, molecular weight and percentage in chitosan solution. Freeze-gelation materials do not affect blood cells during the 10 minutes of incubation. Gauze-chitosan materials did not cause RBC parameters but significant decreased platelets amount and changed their size. More effective action to blood cells was found in samples made from 200 and 500 kDa 2% chitosan. Effective interaction of chitosan-based materials with platelets may use for haemostatic dressing.