Oral Biology
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Lecture - 30
Biocompatible Assays

Ok welcome to the course. Today Hemocompatibility experiment is going to experimented by myself Dr. Chitra, Assistant Professor, Department of Biomaterials, Saveetha Dental college. Today we are going to visualize about hemocompatibility. So, before going into details we just discuss the glimpse of Biocompatibility and importance of this study.

So, what is the need to analyze biocompatibility? Recently lot many materials are emerging towards therapeutic as well as diagnostic application and variety of materials are evolving day by day towards the development of medical society. Biocompatibility means the materials which are used for biomedical application should not cause any adverse effect to the living tissue or biological components.

Biocompatible material should not produce any toxic or immunological response when exposed into biological environment. Hence, the material scientists should design and fabricate a compatible material for biomedical applications. Now, we move on to hemocompatibility. Hemocompatibility is one of the primary assessment to analyze the biocompatibility properties of the material, when in contact with blood cells or blood environment.

To analyze their erythrocyte compatibility with nano or micron size particulates, the particulates are exposed into red blood cell corpuscles with the presence of phosphate buffer saline for appropriate incubation to exactly scrutinize the rupture rate of red blood corpuscles. So, we are going to expose our; so we are going to expose particulates in RBC at particular time, then we quantify the rupture rate from that solution.

That means, we have to take a quantified particulates in a Eppendorf microcentrifuge tube and sonicated for 5 to 10 minutes, then after sonication we introduce blood sample into that tube, at that time particulates interact with blood cells that stimulates the rupture rate of RBC membranes.

Hence erythrocyte rupture rate is mainly based on the osmotic pressure of the buffer environment. Generally, lysis of RBCs is mainly with respect to the hypertonicity of phosphate buffer saline. So, finally, after the exposure of particulates in erythrocyte, the rupture rate was observed at the wavelength of 540 nanometer because the hemoglobin red color was observed at that particular wavelength.

There are mainly 5 steps for this assessment: 1st step is blood sample collection from healthy volunteer, then 2nd step sample preparation for the assessment, then 3rd step exposure of sample to the RBCs, then after incubation for 1 hour then finally, reading was captured at 540 nanometer.

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We have to collect the blood from healthy volunteer or we can procure from authenticated laboratories to avoid the coagulation we used EDTA as an anticoagulant 1.5 mg EDTA per ml of blood.

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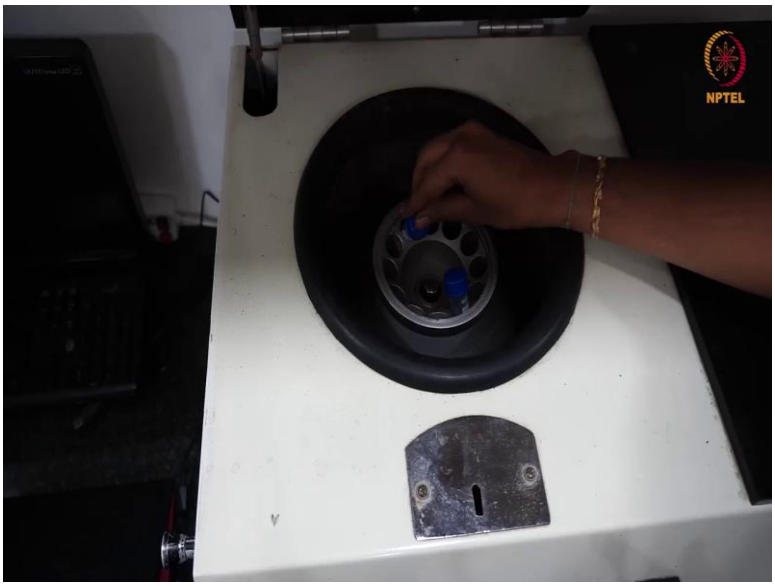

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We have to centrifuge the blood for isolation of RBCs, 10000 RPM for 10 minutes. Then we have to decant the plasma and white blood cells, we can again wash the blood two to three times with PBS to pure to purely collect the RBCs without plasma and WBCs. Now, blood sample is prepared, then we have to prepare the nanoparticles.

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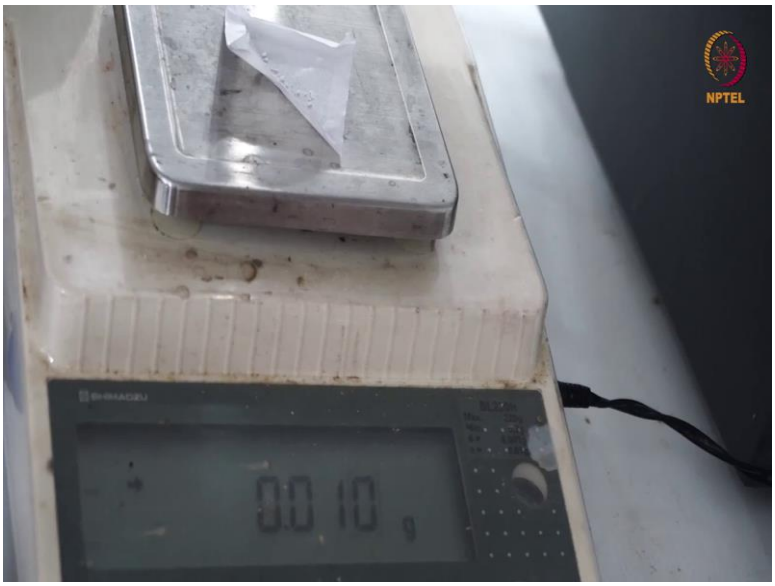

Now, I am going to weigh 10 mg of my material to analyze the compatible properties. So, we have to tare initially to reduce the weight of this butter sheet. Now, I am weighing 10 mg of my bioactive sample. So now, I weighed mg of sample, then I have to collect this bioactive particulates in a micro centrifuge tube.

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Similarly, I have to prepare 3 tube for the replicates. Similarly, I have to prepare 3 tubes for the triplicates ok. For the assessment we have to treat test material with blood.

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So, for negative control, I am taking PBS 950 moles of PbS in every tubes with triplicates. For negative control RBC alone with the PbS, that mean complete protection of RBCs without any hindrances, sorry without any hindrances.

Here I have taken bioactive glasses as a test material. Here I have choosed 10 mg sample, 10 mg per ml, but that you have to optimize based on your requirement for that you have to do repeat analysis by trial and error, then you have to quantify how much concentration of sample is compatible. For positive control I am taking dd water 950 mole of dd water. So, this dd water completely rupture the RBCs. So, we have taken this ruptured RBCs as a positive control.

Now, I weighed bioactive glasses 10 mg of sampling in 950 mole of PbS. I am going to sonicate this thing to complete dispersion of particles. Now, I am analyzing biocompatibility with bioactive glasses, I have taken 10 mg of sample in 950 mole of PbS. Now, I am going to sonicate this for complete dispersion of particulates.

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Now, the sonication is completed. The particulates have completely dispersed in phosphate buffer saline. Here we have positive control, this is dd water, then we have negative control, phosphate buffer saline. In this we have dispersed bioactive nano particulates in phosphate buffer saline. So, this is my positive control, this is my negative control and this is my test material.

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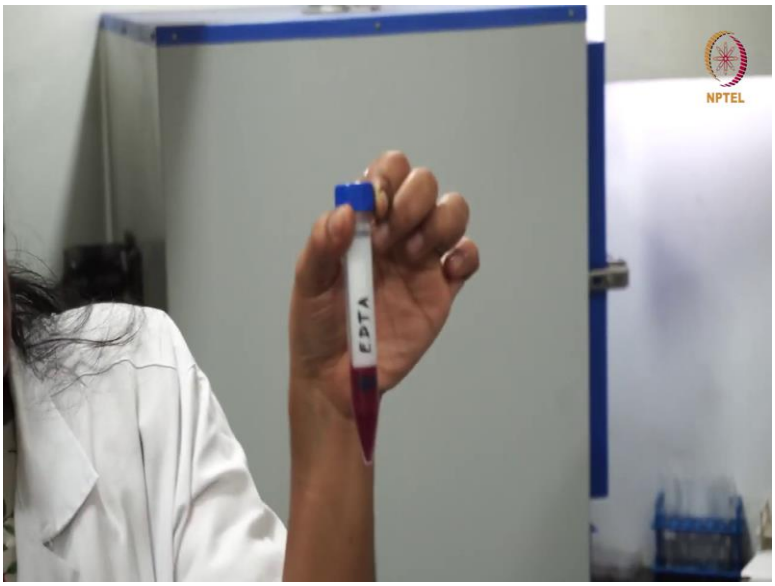

In first step we have collected the blood sample, then we process that blood sample. Now, the blood sample is prepared. In the second step, we have weighed bio active nanoparticles and dispersed in VP, dispersed using sonicated, now the particles is also ready.

In the third step I am going to put 50 mole of RBCs in these tubes; that means, in positive negative as well as test materials.

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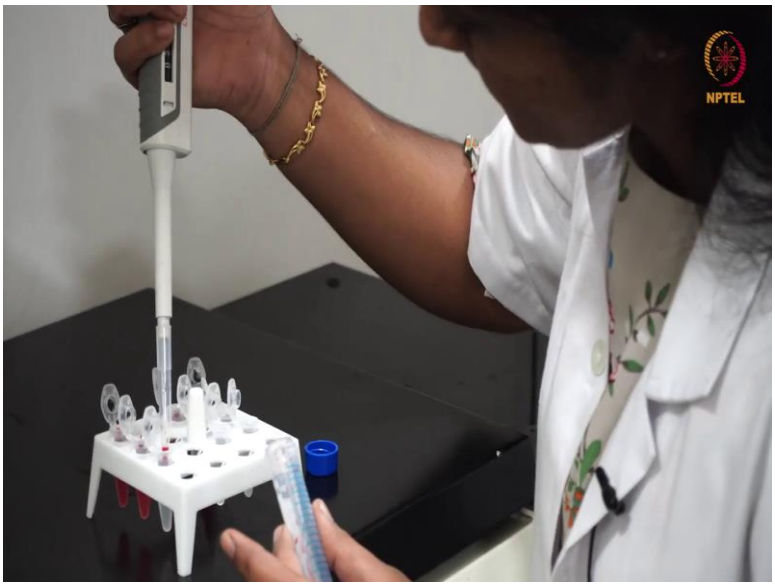

50 mole of erythrocytes is exposed into positive control, negative control as well as with the test material with the triplicates. In this kind of assessments it may be possible for mechanical damage or variations by handling. So, generally we used with the triplicates or n number of tubes usually we do.

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Now, already we have completely dispersed the particulates in PbS with sonicator, then we introduced erythrocyte in to this tube. Now, we have to incubate this for 1 hour then we analyze the compatibility of erythrocyte.

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After 1 hour incubation, we are going to centrifuge the blood sample for the centrifugation. Now, compatible RBCs are settled down, damage RBCs turned into red color.

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We fixed 10000 RPM for centrifugation in 4 degree centigrade for 5 minutes. After centrifugation we again check the blood samples.

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Now, this is positive control each and every RBCs are damaged. This is our negative control, RBCs are completely settled in a protective way. This is with the triplicates and this is our test material treated RBCs.

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Here, the material is very compatible to the RBCs compared to positive control, a material showed good biocompatible properties with erythrocytes.

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Now, after incubation we have collected 3 positives, 3 negatives, 3 test sample bioactive glasses was used.

Now, we have to quantify the erythrocyte rupture rate in UV visible spectrometer at the wavelength of 540 nanometer ok. After this step finally, we have to take one, we have to take the solution and analyze the absorbance; that means, optical density in UV visible

spectrophotometer at 540 nanometer. That will gives the reading of the solution, the absorbance that will give the absorption of the solution with that we have to plot a graph. Hemolysis versus test concentration that formula we have to analyze the results.

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$$Hemolysis\ (\%) = \frac{Sample\ absorbance - Negative\ control}{Positive\ control - Negative\ control}\ X\ 100 \ldots\ldots\ldots\ldots (Eq.1)$$

Formula is OD of the test material minus negative control divided by positive control minus negative control into 100.

This is the formula for the quantification of RBC rupture rate. With this formula we can develop a graph, we can plot a graph hemolysis versus material concentration. In your x axis you will get hemolysis percentage, sorry in your y axis you will get hemolysis percentage and in your x axis you will get concentration of material or the materials which you are used.

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After incubation, after centrifugation now we are going to analyze the optical density of the solution with the help of a photo spectrometer. UV visible photo spectrometer to quantify the optical density value of the solution.

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This is QWET, initially we wash this with double distilled water, then we have to include test specimens or test solutions.

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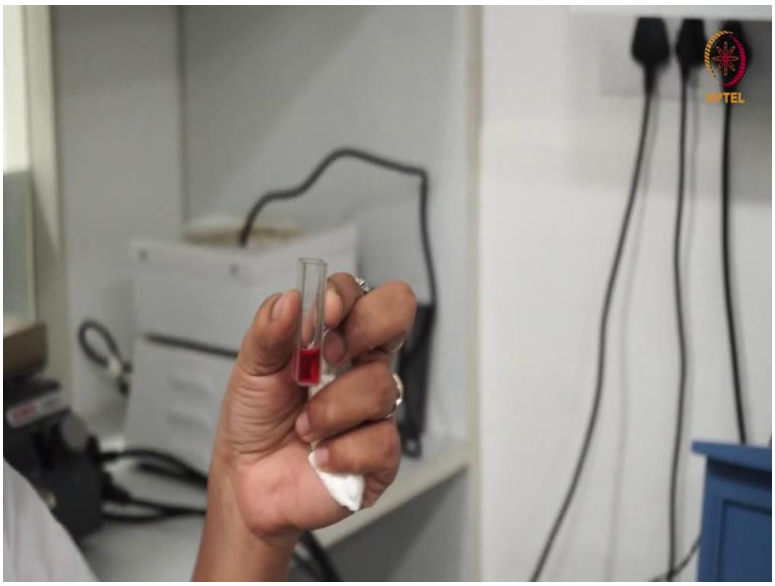

So now, I have taken my test solution in the QWET and going to analyze the absorbance of the solution with the help of UV visible spectrophotometer, then we quantify the solution absorptions in this display.

Then with this absorbance value we can plot a graph in your y axis, you will get percentage of hemolysis in your x axis you may get sample concentrations or variables in the test specimen. With this assessment we have analyzed the compatible properties of bioactive materials specifically, bioactive glasses compatibility in erythrocytes.

After all these steps, now we come to the end. With this assessment we can analyze the biocompatible properties of our material specifically quantified erythrocyte rupture rate with the presence of copper bioactive glasses.

Usually, we follow ASTM and ISO standards; in case of ASTM standard F2888 standard we follow. Similarly, in case of ISO 10993 standards we usually follow to compare our results generally. As per ASTM standard 5 percent lysis is compatible, below 2 percent is slightly hemolytic, 5 percent is hemolytic, beyond 5 percent is highly hemolytic.

So, a material should express compatibility below 5 percent rate then only it is acceptable for biomedical applications.

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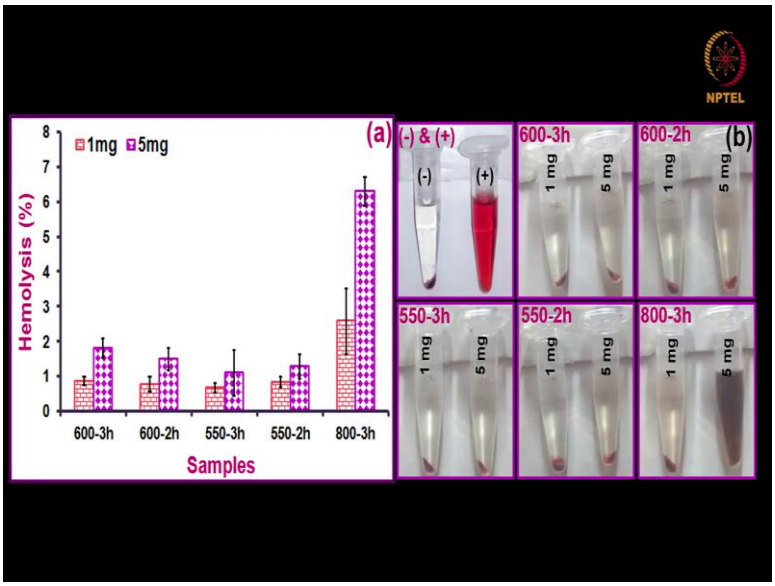

With the study I have analyzed the erythrocyte compatibility rate of bioactive glasses.

Thank you.