3542-Pos Board B589

Dynamic Measurement Of The Membrane Potential Of Phagocytosing Neutrophils By Confocal Microscopy And SEER (Shifted Excitation And Emission Ratioing) Of di-8-ANEPPS

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Neutrophils engulf and kill invading microorganisms in a process called phagocytosis. Accompanying phagocytosis is the Respiratory Burst, a surge of activity by the enzyme NADPH oxidase. The enzyme transports electrons from the cytosol to the phagosome, where it creats ROS that help to kill bacteria. This electrogenic transport of electrons must be compensated for, so that the enzyme can function continuously. Here we report for the first time the membrane potential of individual phagocytosing neutrophils using a highly sensitive confocal techinque.

Isolated human neutrophils were incubated with 10 μM di-8-ANEPPS for 15 minutes and allowed to adhere to glass coverslips. The cells were then washed with Ringer's solution and treated with either 60 nM PMA or opsonized zymozan (OPZ). SEER consisted of ratioing two simultaneously acquired images of fluorescence, excited at 488 nm and 545 nm and collected at emission ranges 470-560 nm and 570-700 nm respectively. Using this approach we were able to detect a 26 % change in ratio from −50 to +50 mV.

Neutrophils stimulated with 60 nM PMA depolarized 80 ± 6 mV (n=5) after a delay of ~ 3 minutes. Addition of the NADPH oxidase inhibitor DPI repolarized the membrane, confirming that the depolarization was due to NADPH oxidase activity. Neutrophils challenged with 2 mg/ml OPZ showed a depolarization spike of 86 ± 11 mV (n=5) that coincided with the onset of phagocytosis. In both cases the depolarization lasted several minutes before subsiding spontaneously.

This study confirms that phagocytosis by human neutrophils is accompanied by a large depolarization of their plasma membrane, a depolarization that can now be monitored quantitatively and dynamically in individual cells.

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3543-Pos Board B590

Smart Packaging: A Novel Technique For Localized Drug Delivery

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In this project, we have developed a model drug delivery system consisting of non-ionic surfactant vesicles (niosomes) packaged within a biodegradable, temperature and pH sensitive hydrogel network. We have characterized the behavior of individual niosomes when exposed to environments that mimic body fluids. Diffusion and mass transfer characteristics of 5,6-Carboxyfluorescein, a dye with similar physical properties as therapeutic drugs for cancer, is used to determine release rate from the niosomes. The release rate can be controlled to last from 24 hours to more than 144 hours depending on the conditions to which individual niosomes are exposed. We used a cross-linked hydrogel (chitosan) network into which the niosomes were embedded. The hydrogel provides another layer of control, gives a stable environment for the niosomes, and enhances the release rate. The niosome-hydrogel system, which is a liquid at room temperature, starts gelling once inside the body since it undergoes a phase transition at 37°C. Surface characteristics, such as the interaction between the niosomes and chitosan, Van der Waals forces and chemical bonding are being measured by the Surface Force Apparatus (SFA) technique. One of the systems that we are targeting with this study is intraperitonial cavities after ovarian cancer is discovered and removed to increase the life span of patients. Single administration rates have been tested and compared to local delivery via an external catheter. We found that our system lasted longer than catheter administration leading to less frequent administration and also resulted in reduced toxicity. Our results will help in the development of a low cost and improved method for drug delivery with application to intracavitary ovarian cancer treatment and other cancer types.

3544-Pos Board B591

Localized Drug Delivery System For The Treatment Of Cancer

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Localized drug delivery can provide better treatment for residual cancer tumors. Our research proposes a ''package within a package'' drug delivery system which results in an effective controlled release of the drug to the targeted site and also reduces its toxic effects to other parts of the body. The first part of the system is a non-ionic surfactant vesicle or niosome and the second a biodegradable and temperature sensitive crosslinked hydrogel. The vesicle is prepared by hydration of amphiphilic films with a PBS buffer solution and a fluorescent marker, 5,6-carboxyfluorescein. We chose to encapsulate this particular marker to imitate and test the release rate of the chemotherapy drug from the vesicle interior. After hydration, the vesicles were extruded to constrict their size distribution. Unentrapped dye was removed using ultracentrifugation and gel exclusion chromatography. The dye encapsulated vesicles were placed in a semi-permeable cellulose membrane and immersed in a solution of either PBS or Milli-Q water. The environment in the PBS solution would closely mimic the vesicle's behavior in the hydrogel and the water environment would closely follow the behavior near a residual cancer site, where the pH tends to be lower compared to blood. Diffusion of the dye through the membrane was determined at different time intervals by using fluorescence spectroscopy and its release rate was determined. The maker encapsulated niosomes will then be embedded into the hydrogel network which will begin to degrade with time exposing the niosomes to a different pH that will cause them to rupture. The thermal sensitive hydrogel is a polymer, chosen to be Chitosan. Chitosan is a biomaterial obtained abundantly in nature. This system will then have antibodies to provide specificity. Using this methodology, we hope to provide a better treatment technique for cancer patients.