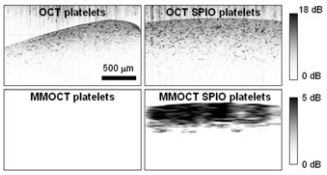
Rehydrated lyophilized human platelets (“RL platelets”), are chemically stabilized infusion hemostatic agents retaining viability. We investigate similar dried platelets with intracellular SPIOs (“SPIO platelets”) as imaging therapeutics. SPIOs are uptaken into the surface connected open canalicular system of platelets where they form clusters. Platelets are then stabilized and dried with the same methods as RL platelets. These SPIO platelets retain the primary hemostatic functions of RL and fresh platelets.

We found that MMOCT provides highly specific contrast to SPIO platelets at 1.5e6/μL in 1% agarose scaffolding (see figure). Furthermore, by sweeping the modulation frequency, a mechanical frequency spectrum is obtained, and resonance peaks are associated with the sample elasticity. This has potential for imaging sites of vascular damage and monitoring the local mechanical microenvironment to provide more detailed information about vascular pathologies.



3874-Pos

Imaging on Nano-Resolution Scale of Carrier Modifications Caused by Therapeutics & Diagnostics by Freeze-Fracture TEM

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The potency of nano- and micro-particles, loaded with therapeutic and/or diagnostics is frequently depending upon their morphology adopted in biological relevant environments. Freeze-fracture transmission electron microscopy (ff-TEM) as a cryo-fixation, replica TEM method is a powerful technique to monitor self-assembling of lipid-, polymer-, as well as protein/peptide-based carriers encapsulating drug-, gene-, vaccine, antimicrobial- and imaging molecules[1]. At a 2 nm resolution limit we are able to study structural modifications of such carriers related to their pay load, application milieu, and during cell interaction.

Using ff-TEM we studied the morphology of a wide variety of nano- and micro particles suitable as carriers for diagnostics as well as therapeutics including quantum dots (coupled to drug-loaded immunoliposomes)[2], gold nanoparticles, superparamagnetic iron oxide nano-particles loaded in polymeric immunomicelles[3], micelles (spherical-, disc-, and worm-type micelles)[4,5], small unilamellar liposome[6], multilamellar liposome, niosomes, cationic liposome/DNA complexes, integrin-targeted lipopolyplexes[7], polymer- or lipid-stabilized gas bubbles[8], cochleate cylinder, depofoam particles, and drug crystals. Recently we explored liposome-, virosome-, and virus-based vaccines, including measles vaccine powders, by ff-TEM. Furthermore, we explored structural modifications within bilayers such as domain-formation[1] but also transformations to non-bilayer structures such as hexagonal and cubic phases.

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3875-Pos

Synchrotron X-Ray Fluorescent Imaging and Spectroscopy Studies of the Role of Copper in the Stem Cell Niche Architecture of Adult Neural Stem Cells

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Improvements in sensitivity and spatial resolution of X-ray fluorescent (XRF) imaging and spectroscopy allowed us to study the distribution of metals in brain tissues. We discovered the specific Cu enrichment in cells in the subventricular zone (SVZ) of the lateral ventricle. This area in the brain contains adult neural stem cells (NSCs). NSCs niche architecture enables to continuously generate functional neurons in specific brain regions throughout life. Knowledge about the mechanisms controlling NSCs self-renewal, proliferation and differentiation is of critical importance for future therapeutic interventions of major brain disorders.

XRF-imaging with sub-cellular (200 nm) resolution on rat brains demonstrated that sub-population of cells in the SVZ builds up sub-cellular Cu accumulations. The Cu concentration inside these structures is as high as 50 mM. It is well established that SVZ has four types of cells: ependymal cells, type B progenitors, type C transit amplifying cells and type A migrating neuroblasts. Imaging the Br signal in brains of BrdU treated rats, we found that actively dividing cells are largely depleted of Cu accumulations. However, cells surrounding actively dividing cells, which are assigned to type B progenitors, demonstrate significant Cu accumulations. Cu K-edge micro-XANES demonstrated that Cu is in Cu(I) form with and spectrum has shape characteristic of a Cu(I)-thiolate multimetallic cluster. The Cu co-localizes with increased sulfur signal which allows quantitation of the Cu/S content.Copper is known to play an important role in the brain’s development and function. However, the role of Cu in the viability and control of the NSCs is presently unknown. Our study is a first attempt to look at the role of Cu in mechanisms controlling the NSCs.

3876-Pos

MRI of Thrombus Propogation after Plaque Rupture

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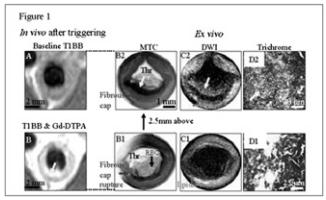
Atherosclerotic plaque disruption and subsequent thrombosis is the leading cause of acute cardiovascular events. We have used a rabbit model of controlled atherothrombosis and combined *in vivo* and *ex vivo* MRI and histology to study whether thrombus organization and composition could be detected based on its biophysical properties. In vivo MRI at the site of plaque rupture without (Figure 1A) and with gadolinium (Figure 1B) showed the luminal thrombus. Ex vivo MRI of disrupted aortic plaques revealed that thrombi propagated both anti-parallel **(Figure 1)** and parallel to blood flow. Examination of disrupted aortic plaques revealed that the % magnetization transfer was much lower and the apparent diffusion coefficient much higher in platelet-rich thrombi compared to organized, fibrin-rich thrombi. This permitted distinction of the thrombus at the site of plaque rupture (Figure 1B1 and C1) from the subsequently propagated thrombus (Figures 1B2 and C2).The conclusions drawn from MRI were validated by histology (Figures 1D1-D2).



3877-Pos

Development of an arganotypic System to Image Metastasis of Carcinoid Tumors in Situ

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Patients with neuroendocrine tumors of the small or large bowel commonly called carcinoid often develop liver metastases. Hepatic portal circulation is a predicted route for establishing liver metastases of carcinoid tumors or following intra-splenic injection of cancer cells. However, the cellular and molecular events that mediate this site-specific metastasis are not well understood. We developed a system to study extravasation, migration, invasion and proliferation of a human carcinoid cell line using mouse liver organotypic slice culture as a tractable preparation that more closely resembles the three-dimensional, multi-cellular tumor microenvironment than does a dispersed cell culture system. BON cells stably transfected with GFP were introduced to liver by portal vein injection. Organotypic slices obtained from the liver were monitored using fluorescence macroscopy and confocal/multi-photon microscopy. The seeded cancer cells adopted an elongated morphology and arrested in the lumens of red fluorescently-labeled venules. Seeded cells in the liver slices were monitored out to 14 days in culture. Although liver slices generally remained viable over the experiment, there was a general reduction in the number of seeded cells with the greatest reduction occurring by the 24 hr or 48 hr time points. After 72 hr to 96 hr, there was a detectable increase in GFP-labeled cells indicating that subsets of the remaining seeded cells were proliferating. These cells formed small “tumorlet” or spheroid structures that extended beyond the vasculature and into or on the surface of the parenchymal tissue. These tumorlets continued to increase in size to a maximum diameter of about 300 μm. We provide evidence that this organotypic slice/xenograft model is a promising tool with considerable potential as a means to probe the early events mediating metastasic tumor growth in the liver.