Tu-Pos596

POLYMETHACRYLIC NANOPARTICULES USED AS BIOPHYSICAL VECTOR. **A. Allard and E. Rousseau* (Intro by J. Sygush). Dept. of Physiol./Biophys., Univ. of Sherbrooke, Sherbrooke, Que., Canada.**

The polymethacrylic nanoparticules (nP=0.3 μm ϕ) were developed as a new drug delivery system (Rolland et al. Int. J. Pharmac. 1989, 53). Doxorubicine (DXR) might interact with a 96% absorption yield on nP. DXR is also known to bind intracellular membrane proteins as previously reported (Zorzato et al., J. Biol. Chem. 1986, 261). Suspensions of nP-DXR complex were mixed with either i) SR membranes or ii) solubilized SR membrane proteins in 1.5% CHAPS + 0.5 M NaCl. Following incubation (1/2 h at 22°C), DXR was covalently bound to the proteins by UV-photolysis. SR membranes were solubilized as in ii. The nP-DXR-protein complex was sedimented. Non specific binding was assessed in presence of an excess of free DXR. Bound proteins were freed and revealed by SDS-PAGE. Both approaches yield similar results: a single high molecular weight protein band, ≈ 400 KDa, was consistently observed within the complex. Methodological improvements will be discussed and proposed for this promising system.

*A. A. was a fellow of ACIM; E. Rousseau is a Scholar of CHF.

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MICROSCOPIC MAPPING OF SUBNANOMETRIC MOTION. H. Lin and M. Sharnoff, Univ. of Delaware.

For several years we have had at our disposal a microscope-based holographic system capable, theoretically, of detecting motion of 10 Angstroms or less by objects well-resolved in the microscope. We have recently calibrated the system's sensitivity to motions of this size by observing the drifting of air bubbles introduced into a glycerine-filled glass capillary. The capillary was placed on the stage of our microscope with its axis nearly horizontal. A millimetric scale was set beside it, and observations made over several hours confirmed that the bubble trajectories were smooth and rectilinear. Drift velocities, ca 1 mm/hour, were different for bubbles of different diameter. The capillary and scale were illuminated by a laser beam at ca 30° to the horizontal, and the scattered light was collected through a 2.5X/0.08 objective and sent to a holographic plate. Difference holograms with interflash intervals as short as 2 msec were made. Images made from these holograms distinguish bubbles according to their velocities. A stationary bubble included in each scene gave a fixed reference point. Photos of the images, unenhanced by any electronic means, reveal absolute displacements as small as 11 Angstroms. Yet smaller relative displacements were also discernible.

Tu-Pos597

MEASUREMENT OF INTRACELLULAR FREE Ca^{2+} CONCENTRATION IN FRACTIONS OF ISOLATED GROWTH PLATE CHONDROCYTES USING FURA-2. M. Zuscik, R. Rosier, K. Gunter, E. Puzas and T. Gunter, Depts. of Biophysics & Orthopaedics, U. of Rochester, Roch., NY

Isolated avian growth plate chondrocytes convert the AM form of fura-2 quickly and completely to the FA form. This conversion is 65% complete in 40 min at 37°C. Control experiments showed that almost all of the converted indicator could be identified as intracellular fura-2FA and that the K_d of intracellular fura-2FA was very close to that at a similar ionic strength and pH outside the cell. A spectroscopic procedure was used to determine free cytosolic $[Ca^{2+}]$ that corrects for errors which could be caused by Ca^{2+}-insensitive partial conversion products (found under some conditions of incubation) and fura-2 signals from the medium. Free cytosolic $[Ca^{2+}]$ determined in freshly prepared chondrocyte suspensions containing all maturational stages varied over the range 75-95 nM. When cells from these various stages were separated by countercurrent centrifugal elutriation, the free cytosolic $[Ca^{2+}]$ in the hypertrophic cells was four times higher than that in the resting cells.

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MONTE-CARLO (MC) MODELING OF PHOTON TRANSPORT IN TISSUE (PTT) I. SIGNIFICANCE OF SOURCE-DETECTOR CONFIGURATION. Barbour, R.L., Graber, H., Lubowsky, J. (Intro. by Rushbrook, J.) Depts. of Path. and Biophysics, SUNY Health Sci. Center at Brooklyn, Brooklyn, NY, 11203.

Advances in the analysis of optical measurements of tissue will require the ability to infer the paths of scattered photons (SP) from directly measurable parameters. To examine these relationships we have modeled PTT by a MC simulation in a nonabsorbing, homogeneous, isotropic scattering medium. By examining the histories of SP we have determined the relationship between the intensity, I, of backscattered light (BL) and the average maximum penetration depth, <Z>, average number of collisions, <n>, and other non-observable parameters for photons emerging into selective detectors (D) for various source configurations. Results obtained showed that <Z> and <n> vary in a linear and quadratic manner, respectively, with distance, R, of D from a collimated point source (CPS). <Z> and <n> for SP emerging from media illuminated by a broad collimated or broad diffuse source had an angle dependence qualitatively similar to, but of much smaller magnitude than, that for a CPS. Unexpectedly, it was also determined that each D orientation has at least one "enantiomorph"; a second orientation at a different R with the same elevation angle but opposite azimuth, such that light received by both D has the same value of I, <Z> and <n>. These findings demonstrate that the subsurface properties of a random medium can be selectively interrogated by a position and angle scan of BL.