L.11: Mechanical and transport characteristics of tunneling nanotubes in tumor spheroids investigated using optical trap and microbeam

Tunneling nanotubes (TnTs) are long, non-adherent, actin-based intercellular connections which are believed to play important role in cell-to-cell communication through the transfer of several cellular components such as vesicles, cell organelles (mitochondria, lysosome), calcium signals etc. The studies reported so far have been made in monolayer of cancer cells in culture. It is only recently that the presence of TnTs between cells have been confirmed in tumor tissue also. However, since the cellular architecture and environment of cancer cells in tumor is much more complex as compared to cells in monolayer, the role of TnTs in cell to cell communication under *in vivo* conditions in cancer is not yet established.

Compared to the cancer cells grown in monolayers, tumor spheroid, a three dimensional (3D) culture of cancer cells grown *in vitro*, is considered to be a more representative tumor model. Unlike cells in monolayer where the TnTs are naturally stretched between the cells and can be easily viewed under a bright field microscope (Fig.L.11.1(a)), the major challenge in studying TnTs in 3D spheroids is that these are often buried under the close packing of the constituent cells, (Fig.L.11.1(b)). We made use of optical tweezers to visualize and investigate the functional and biomechanical aspects of TnTs between cancer cells in tumor spheroids of human oral cancer cells. For optical micromanipulation, collimated beam from a 1064 nm single-mode CW fibre laser was coupled to an inverted microscope to generate optical trap at the object plane using 60× (NA 1.42) oil immersion objective.

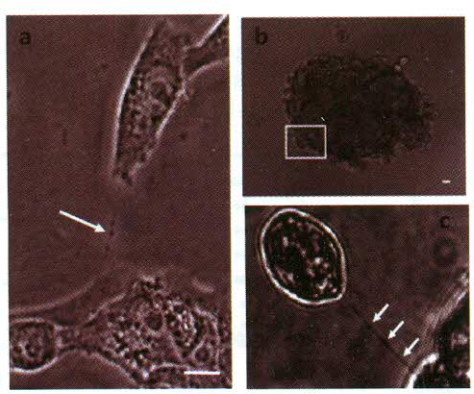

Fig. L.11.1: Cancer cells in monolayer (a), tumor spheroid (b) and optically trapped cells pulled away from the periphery of spheroid (c) to reveal the TnT and vesicles (white arrows). All scale bars, 10 μm.

A cell at the surface of the spheroid was trapped and pulled some distance away from the other cells using optical tweezers. This could make it possible to view TnTs present between the trapped cell and the cells connected to it (Fig.L.11.1(c)). The transfer of cytoplasmic vesicles observed between the cells (Fig.L.11.1(c)) suggested that the TnTs are functionally active. We also combined an optical microbeam, made of a pulsed femtosecond laser (810 nm single mode 200 fs, 76 MHz), with optical tweezers to selectively optoporate the trapped cell and inject a fluorescent, cell impermeable dye into the trapped cell. Because no dye got injected in the large tumor spheroid mass, by monitoring the fluorescence of the injected dye we could monitor its transport from the trapped cell to the connected cells via TnT (Fig.L.11.2(a) & (b)).

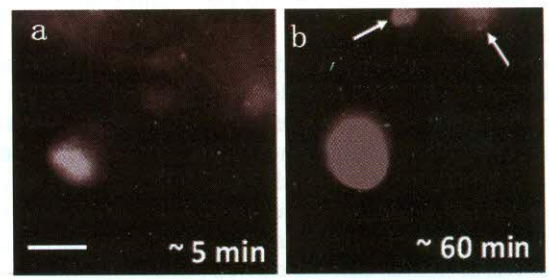

Fig L.11.2: Fluorescence image showing transfer of PI between two cells via TnT in tumor spheroid. (a) uptake of PI in cell after optoporation, (b) with lapse of time, PI fluorescence spread in entire cell and the two other cells at the spheroid surface (arrow) also started to pick-up PI. Scale bar, 20 μm.

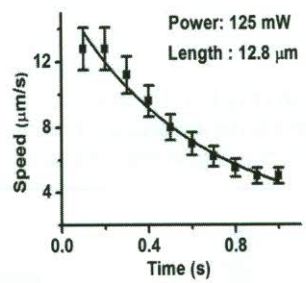

Fig. L.11.3: Velocity profiles of the cell after being drawn out of the optical trap at 125 mW trap beam power.

The ability of the optical tweezers to apply controlled force was also used to measure the elastic constant of the TnTs. For this, the trapped cell is pulled away from the spheroid by keeping the trap beam fixed and moving the stage so that the spheroid moves away from the trapped cell. As the cell is pulled beyond a certain distance, it escapes the trap and returns back to its original position on the surface of spheroid. This happens because the restoring force in the TnT holding the trapped cell with the spheroid becomes greater than the optical force. From the velocity profiles of the cells after being drawn out of trap the stiffness of the TnT was estimated to be ~ 0.61 ± 0.22 pN/μm which is in the same range as that for pure membrane tethers. For more details, please see Patheja et al., J. Biophotonics, Oct. 29, 2014.

Reported by:
A. Dube (okdube@rrcat.gov.in) and P. Patheja