Cell Surgery: A Novel Living Cell Manipulation Technology Using Nanoneedle and AFM

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Recently, applications of atomic force microscopy (AFM) have been extended to the field of cell biology. Direct imaging of living cell is powerful tools to analyze the architecture of living cell surface without staining or chemical pretreatements. We are utilizing an AFM system to manipulate a living cell. We expect that substances on a surface of the AFM tip can be forcibly and precisely transferred into a living cell, like a kind of surgical operations. Here we show a new low invasive single cell manipulation and gene delivery technology using an ultra thin needle (nanoneedle) and AFM. An AFM tip is etched and sharpened using focused ion beam to form a needle shape of 200 nm in diameter and 10 μm in length. The insertion process of nanoneedle into a cell can be monitored with a force exerted to the AFM cantilever. A needle penetration event into a cell is represented as a sudden repulsive force relaxation appearing in the force curve (Fig. 1). The invasiveness of the nanoneedle of 200 nm is so low that even two hours continuous insertion of the nanoneedle does not cause any cell death. Therefore we can manipulate a single cell sequentially.

The nanoneedle insertion could be applied for high-efficiency DNA transfer to living cell. The needle surface was modified with a positively charged peptide, poly-lysine. The DNAs adsorbed on the surface of nanoneedle electro-statically at pH 7.4 same as a culture medium. When the DNA immobilized needle was inserted to a living cell and was kept for several minutes, DNA molecules were efficiently released from the needle surface in cytosol autonomously by decrease of pH. We achieved high-efficiency gene transfer into the human primary cultured mesenchymal stem cell, with over 70% efficiency.

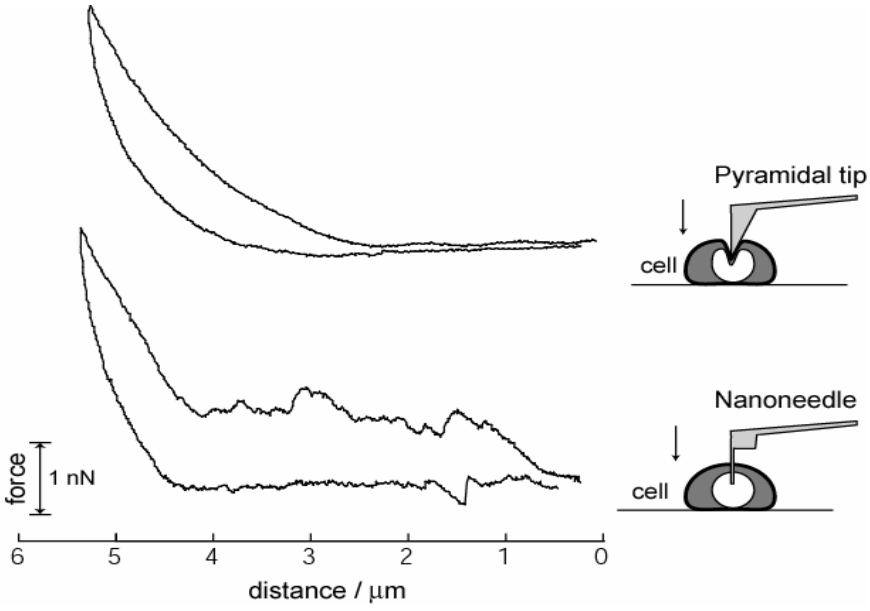

Fig. 1 Force-distance curves when a normal AFM tip (upper) and nanoneedle (lower) were approached to a living cell.