Invited Paper

Optical manipulation of hot nanoparticles can mediate selected cell fusion

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Abstract

Metallic nanoparticles with diameters from 10 nm to 250 nm can be optically trapped and manipulated in 3D using a single tightly focused near infrared laser beam. This will result in a significant heating of the particle and its vicinity, with temperature increases easily reaching hundreds degrees Celsius. If such a hot metallic nanoparticle is brought into the contact zone between two cells or vesicles, this local temperature increase can cause a total fusion of the selected cells or vesicles. Upon fusion, both the membrane and the cargos become completely mixed and we also show that the cells remain viable after fusion. The presented method has potential for single-cell targeted drug delivery and for the creation of hybrid cells.

Keywords: plasmonics, optical tweezers, gold, nanoparticles, cell, fusion, vesicle

Introduction

Metallic nanoparticles have unique optical properties. Individual gold and silver nanoparticles can readily be manipulated by a singly tightly focused laser beam, an optical trap[1,2] operating in the near infrared (NIR) region. A choice of wavelength in the NIR region is particularly useful for studying biological specimen because the absorption of NIR light in biological tissue is minimal. Upon NIR irradiation a metallic nanoparticle will absorb part of the incident light, with its absorption and scattering cross sections being highly dependent on the particle's size, shape, composition and orientation with respect to the polarization[3,4].

The energy absorbed by the particle will be dissipated as heat in the immediate surroundings with temperature increases easily exceeding hundreds of degrees Celsius[3,4,5]. This effect can be advantageously used, e.g., for plasmonic nanoparticle based cancer therapy[6].

If an optically trapped nanoparticle is placed in the contact zone between two selected cells, between two vesicles, or between a cell and a vesicle, these two separate entities can be fused to a single entity simply be laser irradiating the nanoparticle in the contact zone. The fusion is complete, the membranes of the two cells or vesicles completely mix and the two cargoes completely mix too. The cells even remain viable after fusion. This technique can be advantageously used for drug delivery on a single cell level, to design cells with the capacities of two cells types, or to perform controlled femtoliter chemical reactions, as demonstrated in this presentation.

Vesicle-vesicle fusion

In order to facility optical manipulation of giant unilamellar vesicles (GUVs) they were loaded with sucrose which has a significantly higher index of refraction than the surrounding aqueous medium[7]. The membranes or cargos of the vesicles were labeled with appropriate fluorophores, details of the labeling

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procedures are given in Ref.[8]. The GUVs were flushed into a chamber which was mounted on a Leica SP5 confocal microscope. An optical trap, based on a NIR laser (1064 nm) was implemented in the confocal microscope, thus allowing for simultaneous optical manipulation and confocal visualization[9]. To prepare for the fusion event, two vesicles were selected in the sample, trapped, and brought into contact. Figure 1 shows data from a typical experiment, where a gold nanoparticle with diameter 80 nm was optically trapped in the contact zone between the two selected vesicles using a laser power of ~200 mW at the sample. The heat liberated from the gold nanoparticle caused the two adjacent membranes to rapidly form a fusion pore which gradually expanded as shown in Figure 1. This caused a total mixing of the cargos of the two fusing vesicles as well as a total mixing of the two membrane systems[8].

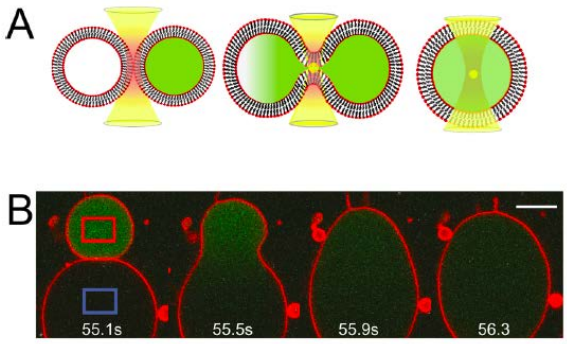

Figure 1. Fusion of two vesicles mediated by optical trapping of a gold nanoparticle in the contact zone. A) Sketch of the fusion process where two vesicles are brought into close vicinity and fusion is mediated by optical trapping and heating of a gold nanoparticle in the contact zone. B) Confocal images showing the fusion process between two fluid phase GUVs. The membranes of the GUVs are labeled with Texas Red fluorophores. The lumen of the upper GUV contains calcein (a green fluorophore). The scalebar is 10 μm. Figure is reproduced with permission from Ref. [8].

Fusion of two vesicles with femtoliter cargos allows for performing controlled nanoscale chemical reactions. As an example, we demonstrate fusion of acidic vesicles with neutrally charged GUVs containing I-BAR protein. Fusion between these two types of vesicles causes membrane tabulation[8]. (2)

Fusion of living cells

Following a similar methodology two individual selected cells can be fuse. In the experiment depicted in Figure 2, two HEK cells labeled with two different lipophilic fluorophores, vibrant DiD (red) and vibrant DiO (green) were optically manipulated and brought into contact. Then the optical trap was placed in the contact zone between the cells and at least one 150 nm gold nanoparticle was allowed to diffuse into the trap. Within very few seconds the membranes of the two cells completely fused. Also the cytoplasm of the two cells fused and a syncytium was formed with two nuclei. A sketch of this process is shown in Figure 2A and the corresponding confocal images are shown in Figure 2B.

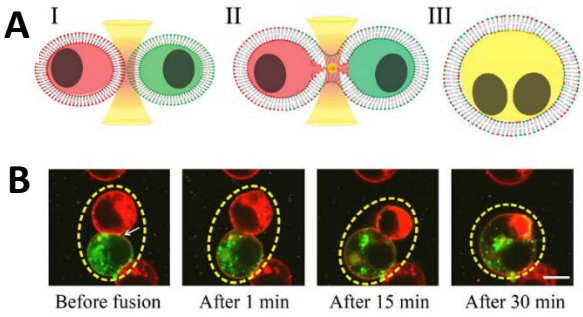

Figure 2. Hot nanoparticle mediated fusion of two selected cells. A) Sketch of the fusion process. An optically trapped hot nanoparticle in the contact zone mediates total fusion. B) Confocal images showing the fusion process between two HEK cells. The white arrow points at the trapped 150 nm gold nanoparticle. Reproduced with permission from Ref. [10].

After fusion the viability of the syncytium was investigated using a Calcein AM assay and using an MTT assay, details of this are given in Ref. [10]. Both independent viability tests showed that after fusion of two cells the newly formed syncytium with two nuclei was metabolically active and viable, however, on average with viability slightly comprised in comparison to un-fused control cells.

The hot-nanoparticle fusion method has also been demonstrated useful for fusing a living cell with a GUV. In this case the membranes fully mixed and the cargo of the GUV was delivered to the cell's cytoplasm. After fusion the cell remained viable, although with viability slightly comprised compared to control cells[10].

This novel hot-nanoparticle fusion method offers a way to create novel hybrid cells that have characteristics from two different cell types. For instance, it could be highly interesting to fuse stem cells with fully developed tissue cells, whereby the regenerative properties of the stem cell might assist tissue repair and regeneration. Also, fusion of a GUV to a living cell is a realization of single-cell drug delivery and will be highly useful for manipulating, e.g., cellular regulation.

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