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Review

Biofunctionalized nanoneedles for the direct and site-selective delivery of probes into living cells ☆

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ABSTRACT

Background: Accessing the interior of live cells with minimal intrusiveness for visualizing, probing, and interrogating biological processes has been the ultimate goal of much of the biological experimental development.
Scope of review: The recent development and use of the biofunctionalized nanoneedles for local and spatially controlled intracellular delivery brings in exciting new opportunities in accessing the interior of living cells. Here we review the technical aspect of this relatively new intracellular delivery method and the related demonstrations and studies and provide our perspectives on the potential wide applications of this new nanotechnology-based tool in the biological field, especially on its use for high-resolution studies of biological processes in living cells.
Major conclusions: Different from the traditional micropipette-based needles for intracellular injection, a nanoneedle deploys a sub-100-nm-diameter solid nanowire as a needle to penetrate a cell membrane and to transfer and deliver the biological cargo conjugated onto its surface to the target regions inside a cell. Although the traditional micropipette-based needles can be more efficient in delivery biological cargoes, a nanoneedle-based delivery system offers an efficient introduction of biomolecules into living cells with high spatiotemporal resolution but minimal intrusion and damage. It offers a potential solution to quantitatively address biological processes at the nanoscale.
General significance: The nanoneedle-based cell delivery system provides new possibilities for efficient, specific, and precise introduction of biomolecules into living cells for high-resolution studies of biological processes, and it has potential application in addressing broad biological questions.
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1. Introduction

Nanotechnology has recently found increasing applications in biology by providing new nanotechnology-based tools and materials to probe and manipulate biological processes at the nanoscale (~1 to 100 nm) [1], which is the length scale where many fundamental biological processes occur. For instance, fluorescent semiconductor nanoparticles, or quantum dots [2,3], have been used as probes to visualize dynamic processes in living cells, including the dynamic movement of singe membrane receptors [4–8], motor proteins [9], nerve growth factors [10], and synaptic vesicles [11,12]; and magnetic nanoparticles have been used to manipulate individual membrane receptors to control signal transduction in living cells [13].

One-dimensional nanomaterials, such as nanotubes and nanowires, have also been used as intracellular biosensors, delivery carriers, and imaging agents [14–21]. In particular, with their unique physical and chemical properties distinct from both individual molecules and bulk materials, chemically synthesized nanomaterials have presented new opportunities and applications in biology and medicine, from basic biophysical studies at the single-molecule level to the diagnosis and treatment of diseases [22–24]. In addition, with their needle-like nanoscale geometry and excellent mechanical and electrical properties, these high-aspect ratio nanostructures have been explored as membrane-penetrating nanoneedles that can manipulate and sense biological processes inside cell with minimal intrusiveness and toxicity [24–31]. For example, surface-functionalized nanotubes have been used to deliver biomolecular species into living cells with high spatial and temporal precision [27,30,31]. Conductive nanotubes have also been envisioned as an electrochemical nanoprobe to measures electrochemical events, redox environments, and signaling processes occurring inside cells or between neighboring cells [31,32].

The transfer of biomolecules into living cells is a general practice used to monitor or modify molecule-specific intracellular processes. It

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provides an efficient way to study the temporal and spatial regulation of protein systems that underlie basic cellular functions [33]. Many methods have been developed for this purpose [33–41]. Each of them has its characteristic advantages and disadvantages with respect to cell viability, transfer efficiency, general applicability, and technical requirements [33]. In this review, we discuss a new type of nanotechnology-based methodology for the introduction of biomolecules into living cells and its potential implications in addressing biological questions.

2. General description of the nanoneedle-based intracellular delivery

Similar to a micropipette-based injection system, a nanoneedle-based intracellular delivery system comprises a nanoneedle (a nanotube or a nanowire) on a macroscopic handle (an etched metallic wire or simply a pulled glass micropipette) and a manipulator (a standard piezoelectric micromanipulator) integrated with an inverted optical microscope [26,27,30,31]. Similar also in practice to the use of a standard micropipette-based injection system, the nanoneedle is manipulated with the micromanipulator to penetrate into a target cell under the observation of an optical microscope. The major difference between the nanoneedle-based system and the micropipette-based injection system is that in the nanoneedle-based system a sub-100-nm-diameter nanowire is used (compared to the micrometer-sized micropipette used in the injection system) to penetrate the cell membrane, which introduces minimal damage to the cell membrane and minimal disruption to the interior environment of a cell, and the materials to be delivered into the cell are carried by the nanoneedle surface and released through a predesigned surface chemistry [27,30,42–44] and not through a pressure-driven injection flow.

Such a configuration also allows the direct visual monitoring of the whole nanoneedle-based delivery process (Fig 1A) and requires no additional setup beyond what a biological science laboratory typical has. The drawback is that its operation is limited by the resolution of the optical microscope; thus, only nanoneedles with relatively large diameter and length (diameter larger than ~30 nm and length larger than ~3 μm) can be visually monitored and thus used. Other configurations have used a nanoneedle mounted on an atomic force microscope (AFM) probe and manipulated by an AFM. The advantage of such an AFM-based nanoneedle system is that the force–displacement dependence behavior when the nanoneedle approaches towards and breaks through the cell membrane, and advances into the cell interior can be quantitatively monitored with very high resolution (Figs. 1B and C) [26–28]. However, the limitation is that the direct visualization of the nanoneedle is difficult as the AFM cantilever blocks the direct view of the nanoneedle, and the nanoneedle motion is restricted along the vertical direction. Some AFM-based systems have also been integrated with a confocal microscope; this allows the three-dimensional imaging of the nanoneedle and the target cell (Figs. 1B and C) [26,43]. However, because of the long acquisition time needed in confocal imaging, real-time monitoring of the nanoneedle operation and the dynamic cellular processes is difficult [45].

Overall, an ideal nanoneedle-based delivery system that can control the nanoneedle at the nanoscale resolution, directly visualize the nanoneedle and the target cell, and monitor the force exerted on the nanoneedle would be desirable for the wider use of nanoneedles for biological studies in living cells [45,46].

In the following, we discuss the fabrication and functionalization of a typical nanoneedle for the intracellular delivery application.

3. Fabrication of nanoneedles for intracellular delivery

The most critical component in the nanoneedle-based system is the nanoneedle, which is often attached to a macroscopic structure for the ease of manual handling. For the intracellular delivery purpose, the nanoneedle, in general, needs to have a needle-like structure with nanoscale diameter (less than ~100 nm) and microscale length (~1 to 20 μm, long enough to reach the target area inside a cell), be mechanically rigid to survive the operation in an aqueous media and to penetrate through the cell membrane [25,28], and have a surface that can be chemically functionalized to attach the cargo on its surface or to convey other functionalities to the nanoneedle [28].

Such a nanoneedle can be typically made by the following two methods. First, chemically synthesized one-dimensional nanostructures, such as nanotubes and nanowires, can be directly used as nanoneedles [27,28,30]. For example, chemically synthesized nanotubes (carbon nanotubes or boron nitride nanotubes) have ideal properties as nanoneedles for intracellular delivery: they have the needle-like structure with nanoscale diameter (~1 to 100 nm) and microscale length (~1 to 100 μm) [47], large Young's modulus (~1 TPa) and high tensile strength [48–51], while in the meantime, are resilient [48,49,52,53]. There are well-developed methods to synthesize such one-dimensional nanostructures with controlled sizes and shapes and they are mostly commercially available. However, the precise alignment and stable assembly of such nanostructures into useful individual nanoneedles are still challenging [25]. Reported methods for the assembly of the nanostructures into needle-like structures include direct attachment of nanotubes by using a manipulator [27,28,30,52–55], catalyst patterning and direct growth of nanotubes by chemical vapor deposition [56–58], alignment and assembly using dielectrophoresis or magnetic fields [25,59–66], and transplanting of single nanotubes encapsulated in polymer blocks [67,68]. However, these methods do not reproducibly produce nanoneedles in large quantity or produce high-aspect ratio "water-stable" nanoneedles (that can survive the meniscus forces involved in the intracellular delivery operation) [25,28,54]. Second, a nanoneedle can be fabricated by nanofabrication, such as focused ion beam (FIB) machining [26] and direct-write nanofabrication techniques [69–71]. For example, Nakamura et al. have fabricated Si nanoneedles by sharpening Si AFM tips with FIB [26,42–44,72–76]. They fabricated Si nanoneedles with diameters of ~200 to 800 nm and lengths of ~5 to 10 μm and demonstrated the capability of these Si nanoneedles to penetrate through both the cellular and nuclear membranes of living cells. However, in general, nanofabrication methods produce nanoneedles with relatively large diameters (in most cases, larger than 100 nm) and short lengths.

4. Functionalization of nanoneedles for intracellular delivery

Several research groups have developed the nanoneedle-based delivery system that uses the outer surface of the nanoneedle for carrying the cargo for intracellular delivery (Fig. 2) [27,30,42–44]. This requires that the cargo is conjugated on the surface of the nanoneedle and is able to be released from the surface of the nanoneedle once transferred inside cells. There are various surface chemistry and bioconjugation methods to functionalize the surface of the nanoneedle and conjugate the cargo on it. For example, the surface of the nanoneedle can be directly functionalized (Fig. 2A) [27,43,66] or can be first coated with other materials (e.g., gold) and then functionalized (Fig. 2B) [28,30].

In the case of a carbon nanotube-based nanoneedle, it can be functionalized by either covalent methods or noncovalent methods. The covalent methods use chemical reactions to chemically bond functional groups directly on the surface of the CNT (e.g., carboxyl groups by oxidation) [20,21,66,77]. The noncovalent methods use hydrophobic and π–π interactions [20,21,27,77] to attach functional groups on the CNT surface. For example, Chen et al. [27] used a linker molecule that contains a pyrene moiety and a biotin moiety to functionalize the CNT nanoneedle with nanoparticles: the pyrene moiety binds to the CNT surface through π–π stacking and the streptavidin-coated nanoparticles are attached to the biotin moiety (Fig. 2A).

A Si nanowire-based nanoneedle can be functionalized by forming self-assembly monolayers (SAMs) of alkylsilanes on the Si surface through the silane coupling reaction. For instance, Nakamura et al.

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