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MEMS technology for nanobio research

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Micro- and nanotechnology have gathered 20 years of increasing research efforts. This research activity began and developed with the design and the fabrication of micro- and nanomechanisms, sensors and actuators, which range from 10 nm to 100 μm. More recent trends focus on the transfer of this technology know-how towards nanobiological topics and very wide range applications can be addressed. Among them, this review proposes various examples that include MEMS tweezers for molecular direct handling and characterization, single molecular characterization in femto-L chambers and dynamic microarray for cell positioning. The micromachined devices are described with bio-oriented experiences that are relevant to foresee their future contribution to drug discovery.

Over 20 years, we have witnessed remarkable progress in the investigation of the design, fabrication and application of micro- and nanomechanisms and actuators that range from 10 nm to 100 μm in size [1]. Fabrication technology, called micromachining, is based on semiconductor processes [2]. The final goal is to build a smart micronanosystem through the integration of moving mechanisms, sensors and electronics in a chip-size system [3]. Applications include optics [4], biotechnology, nanotechnology and information technology. The microelectro mechanical systems (MEMS) technology permits the manufacturing of smart microdevices that have sensing, data processing, communication and actuation capability; thus, it will provide us with indispensable equipment capable of improving quality of life in future society. The combination of MEMS and nanobiotechnology is especially beneficial for future society through advances in medical equipment and the development of new medicine (Fig. 1). Therefore, this review deals with the latest achievements on the MEMS applications to nanobiotechnology.

Silicon micromachining technology has made remarkable progress in terms of structural complexity, miniaturization and heterogeneous integration of material variety. Thus, the MEMS devices, fabricated with these improved processing capabilities, are sufficiently advanced to be capable of performing scientific analyses of bio-molecules and cells. A pair of probes with 10–50 nm tip radius can be micromachined with integrated microactuators and displacement sensors. The device, called nanotweezers, is able to handle DNA molecules. For the bioassay of enzymes, microchips with fL-chambers and microheaters were fabricated in which the enzymatic product of individual molecule was separately evaluated. It is also possible to utilize MEMS fluidic devices to arrange beads or gel-encapsulated living cells in a reconfigurable array. Following bioassay on beads or cells screening, each individual bead can be retrieved selectively.

Micromachined tweezers for DNA handling

Single-molecule micromanipulation experiments [5] have produced fundamental information on the physical properties of biological systems that have been awaited a long time. Quantitative data, such as how long a molecule can extend when subject to stretching force, are invaluable to understand underlying mechanisms such as DNA conformation [6], chromatin organization [7] or biomolecule interaction dynamics [8,9].

Besides near field spectroscopy such as atomic force microscopy [10], a variety of techniques such as electric [11], magnetic [6] and optical traps [12] have been used to position and characterize nanoscale objects and molecules.

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1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2008.07.011

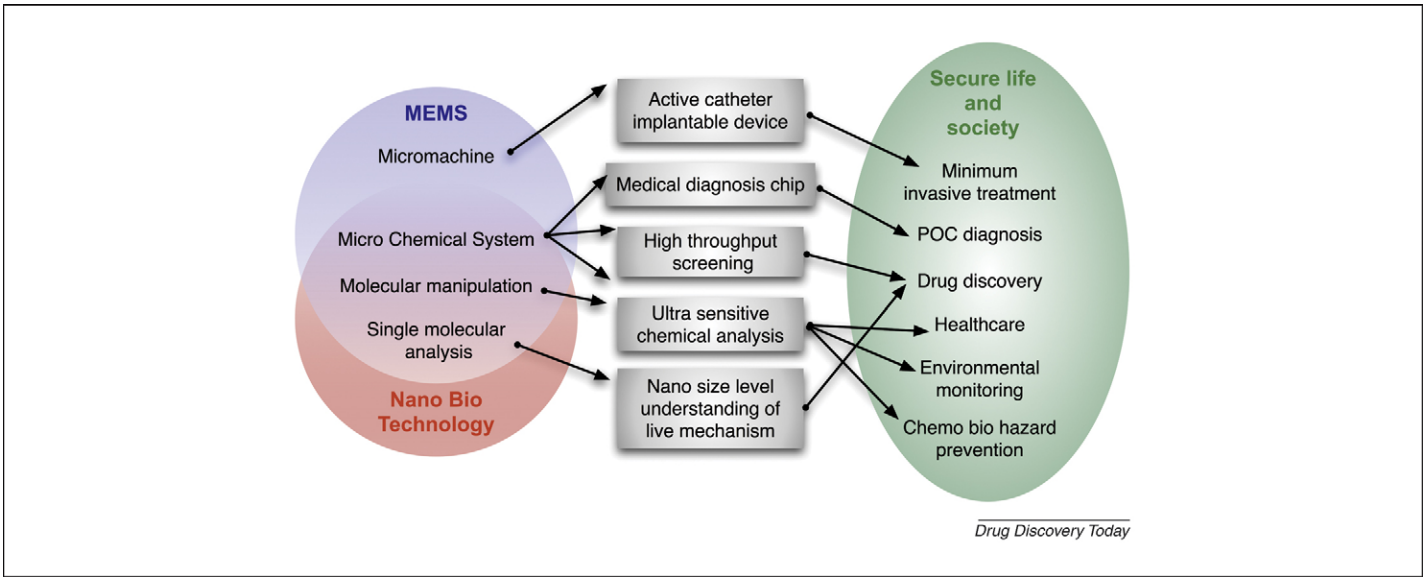

FIGURE 1

Contribution of MEMS and nanobiotechnology to the quality of life for society in the future through medical and pharmacological applications. From MEMS technology (blue) and nanobiotechnology (red) to secure life and society (green) through dedicated analysis (grey).

Beyond these fundamental biophysical investigations, a huge interest exists in pushing molecule manipulation techniques towards systematic analysis to anticipate future biological and medical applications [13]. This next step requires quick access experiments on low-cost equipment. However, the techniques that have been developed and used so far hardly fit these requirements, because they are only capable of low levels of data throughput. The setup and molecular preparation have to be performed one at a time and parallel operation is not possible.

To overcome such limitations and to achieve applicative goals, the MEMS concept has to be explored because it can integrate very accurate molecular level engineering tools [14] and can be cheaply produced by highly parallel fabrication processes. From microsystem know-how, numerous basic functions (electronic, optical, chemical and mechanical) and technologies (microtweezers and microfluidics) are available for this task [15].

It turns out that MEMS-based tweezers with sharp opposing tips are appropriate to trap and handle molecules (Fig. 2a). This technology enables the easy integration of actuation (force application) and displacement sensors (molecule extension sensing) in a monolithic device. To have compact tweezers, electrostatic comb drive actuation has been chosen to stretch the molecule, and the produced extension is measured by differential capacitive sensing [16]. These two integrated electromechanical components only need connections to electronics apparatus. The use of specific equipment or complex optical setting is avoided, providing the tweezers with simple and quick operations.

The two sharp tips can act as electrodes for dielectrophoresis. One electrode is fixed, while the other is actuated with the comb drive actuator. The gap between the electrodes can be monitored in real time thanks to the differential transverse capacitor that measures the relative displacement of the moving electrode. Compensation spring structure was also implemented to force a pure translation (no rotation) displacement of the moving arm. This design gives a mechanical stiffness of the arm with respect to the motion direction close to 35 N m^{-1}.

The device was fabricated from a silicon-on-insulator (SOI) wafer with a three-mask process including KOH etching for the tips formation and deep reactive ion etching (DRIE) for the structural patterning.

When the opposing tips are immersed in a DNA solution, a high AC electric field (1 MHz, 1 V μm^{-1}) is applied for few seconds between the two arms of the tweezers. Owing to the intense electric field, DNA molecules are stretched and attracted towards the tips [17] and finally form a bundle between them. Finally, the DNA bundle can be easily retrieved from the solution [18]. The molecular bundle was rigid enough to be bent or extended mechanically. This biomechanical testing of molecules can be performed in static mode, as for optical or magnetic tweezers, or by harmonic analysis. In the latter case, the increased resonant frequency of the tweezers because of the added rigidity of the molecule bundle can be easily detected (Fig. 2b). Furthermore, the bundle electrical ionic conductivity can even be measured during mechanical extension [19]. These experiments can also be performed in solution by keeping the tweezers' tips immersed. Additional research is intended to isolate single-molecule before trapping. Single-molecule isolation can be achieved by microchannel (2 $\mu m \times 3$ μm in cross-section) [20] and trapping of a single DNA molecule on fixed electrodes has been demonstrated in a microfluidic device [21].

Manipulation by tweezers has proven to be very useful and more functionality can be added. For example, microfluidic devices can select and introduce different kinds of biomolecules in the solution [20,22], before the molecular trapping. So specific molecular interaction can be detected and analyzed by the tweezers. Thus, it is not unreasonable to suggest that in a near future, MEMS tweezers are likely to be able to be used for real time biosensing.

Observation and utilization of biomotor molecules

Interrogating individual molecules has the potential to bring a drastic change in biotechnology. This approach aims to replace statistical methods such as the conventional test tube or even

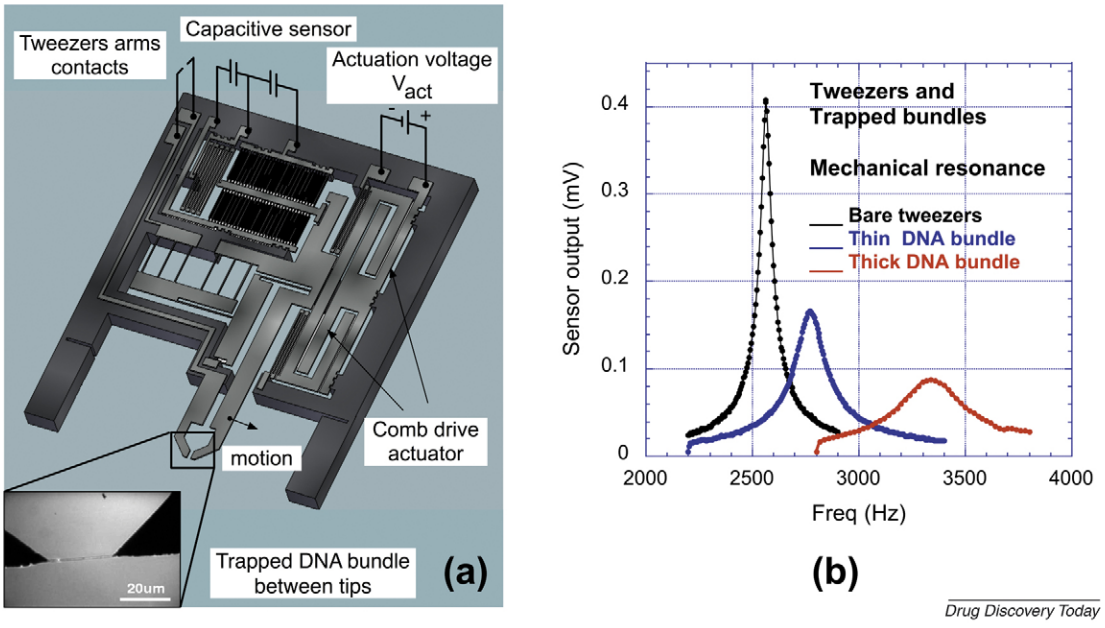

FIGURE 2

Molecule capture and characterization with MEMS tweezers. **(a)** 3D schematic view of the silicon nanotweezers (external dimensions: 4.5 mm × 5.5 mm). The mobile electrode is electrostatically actuated (actuation voltage V_{act}) and its motion is sensed by the capacitive sensor. Dielectrophoresis is applied between the opposing tips. Insert: SEM view of a trapped DNA bundle. **(b)** Frequency analysis of the mechanical response of the tweezers with different trapped DNA bundles. The resonance frequency increases with bundle thickness.

microfluidics approaches by direct assay at the single-molecule level. Nevertheless, single-molecule observation is hardly achievable, because individual behavior and characteristics of molecules are usually hidden in the ensemble- and time-averaging of bulk experiments.

Biomolecular motors are appropriate targets for single-molecule observation, because their motion corresponds to their activity and can be recorded and analyzed with high resolution [23–25].

Of those motor proteins that have been studied, enzymes such as kinesin [26], dynein [27], myosin [28], DNA helicase [29] and RNA polymerase [30] act as linear motors that move either proteins or DNA. Bacterial flagella protein complex [31] and a subunit of ATP synthase F_1 act as rotary motors [23].

The ATP synthase F_1 (Fig. 3c bottom) has received a great deal of attention, as it is the smallest known rotary motor, with height and diameter in the 10–14 nm range [32]. It rotates in an anticlockwise direction as it hydrolyses ATP [33] (Fig. 3e, left-oriented arrow). Micro- and nanotechnology were implemented to analyze its behavior in real time resulting from temperature [34] and chemical changes [25].

From bulk experiments, the activity of proteins is known to be temperature dependent [35]. The change in ATP synthase F_1 activity can be measured by analyzing its rotation speed.

To visualize F_1 activity over a wide temperature range, a device that rapidly controls the temperature under continuous microscope observation is required. Conventional systems, such as platforms based upon the Pelletier effect present two drawbacks: (i) they suffer from low response time and (ii) the generated heat diffuses and causes thermal expansion of the microscope stage, making observation impossible owing to continuous defocusing. Micromachining provides an elegant solution by permitting the integration of the microheater-sensor on the glass plate on which the ATP synthase has been tethered.

A local heater and a sensor were fabricated by patterning Ni on a glass plate (Fig. 3a). The circular shape of the heater ensures a uniform temperature inside the internal area [36] (variation less than 5°C) where the F_1-ATP synthase is localized. The device is completed by closing the flow cell with a cover glass and parafilm spacers and by performing the wiring.

The motor protein is attached to the glass surface by infusing F_1-containing buffer into the flow cell. Subsequently, 0.5-μm diameter microbeads were attached to the rotary axis of the F_1 motor protein (Fig. 3c, bottom) [34]. Microbeads are required as the dimensions of the proteins are too small to be observed by optical microscopes. Protocols for the rotation assay have been described in a previous report [23].

The heater was connected to a current source and the rotational speed of the F_1 was recorded while measuring local temperature by the sensor. The rotational speed is proportional to temperature increasing from 1.2 rps at 18°C to 4.4 rps at 50°C and back to 1.7 rps at 20°C (Fig. 3b). The short response time of the integrated heater/sensor permits the analysis of the transient behavior of the F_1-ATPase at a peak temperature of 50°C. Conventional devices would be incapable of performing this experiment because they would alter the molecule by keeping it at high temperature for too long time as a result of their long response time.

By further reducing the volume of the chamber in which the molecule is confined, more detailed information of the mechanochemical transduction of the protein motor can be accessed [25]. A single molecule of F_1-ATPase has been isolated in a chamber of a

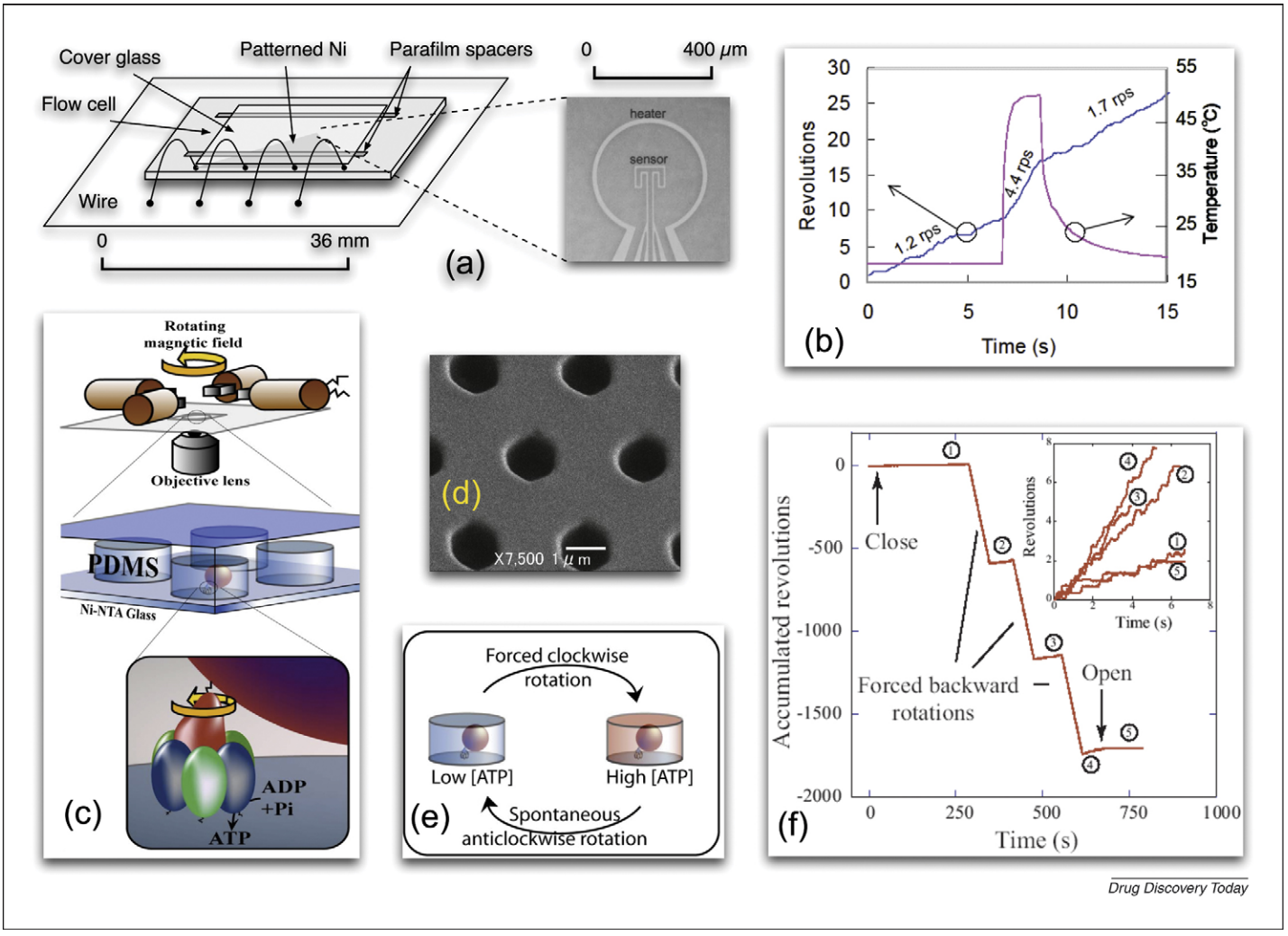

FIGURE 3

Analysis of F_1-ATPase motor rotation in micromachined devices. **(a)** Device for the analysis of the F_1 rotation with temperature change. The flow cell integrates the heater and the thermal sensor. A schematic view of the wiring is given. Right: close-up view on the heater surrounding the sensor. The analyzed F_1-ATPase is located inside the heater ring. **(b)** Time course of the rotation of F_1 while switching the temperature. Angular velocity changes from 1.2 to 4.4 rps and back to 1.7 rps. The ATP hydrolysis rate also varies from 3.6 molecules s^{-1} to 13.2 s^{-1} and to 5.1 s^{-1}. **(c)** Schematic of the F_1-ATPase to which a magnetic bead is attached. The F_1 is encapsulated in a fL chamber. The upper plot shows the magnetic tweezers and the visualization set-up. **(d)** Scanning electron microscopy image shows the PDMS sheet with cylindrical cavities making the chamber. **(e)** ATP synthesis is detectable though the enzyme itself: after the magnetic field is released the F_1 resumes its ATP hydrolyzing anticlockwise rotation. **(f)** Before and after a forced clockwise rotation by the magnetic tweezers, spontaneous anticlockwise is recorded as a mean of detection for the ATP synthesis through the increased speed (see insert).

few micrometers in size (Fig. 3c middle); the chamber, having a volume in the fL range, was a replica in polydimethylsiloxane (PDMS) of a microstructured silicon mold [24]. After being removed from the mold, the PDMS sheet (Fig. 3d) was gently pressed onto the glass plate on which a low density of F_1 had been incubated. Individual F_1 molecules were encapsulated in a chamber with its buffer containing a predefined ATP concentration.

Initial experiments focused on the enzyme's motor action, that is its ability to rotate in hydrolyzing ATP [37] (Fig. 3e, left-oriented arrow). Just after encapsulation, the rotation of F_1-ATPase was recorded. Because this rotational speed is directly proportional to the concentration of ATP, a temporal decrease in speed of a F1 molecule was observed owing to the consumption of ATP in the closed chamber. By summing up the total number of turns and the total number of ATP molecules in the chamber at the start, a coupling ratio of 3 (3.15 ± 0.5) ATPs per turn was calculated, confirming previous estimations [38,39].

In another set of experiments, the motor was forced to rotate in the opposite direction to address the more challenging question of ATP synthesis. As before, single enzymes, upon which a magnetic bead had been attached, were encapsulated in a chamber. The rotation was forced in the reverse direction (clockwise, right-oriented arrow in Fig. 3e) using a rotating magnetic field and the magnetic bead as a handle (Fig. 3c top). An increase in ATP concentration produced by synthesis was confirmed by observing spontaneous rotation after forced clockwise motion (Fig. 3f). More detailed analysis confirmed that 3 (2.3 ± 1.6) ATPs were synthesized for every forced revolution [25].

The use of micro- and nanotechnology to access detailed molecule behavior can be extended to other molecular proteins, like kinesin, that experience linear displacement on microtubule [40].

These molecular motors can even be hybridized to microdevices to transport them in a confined environment [41,42].

Dynamic microarray chips

The development of cell-based microsystems has been a subject of a great deal of research, because microsystems can more closely mimic the extracellular environment than standard tissue culture-based approach. These cell-based microsystems are useful in the studies of pathological and physiological [43,44] phenomena in cells, which will have enormous potential for cell-based diagnostic applications including drug testing [45,46] and toxicology studies [47]. However, to realize this potential fully, a reliable multifunctional platform is required to transport and immobilize particles, infuse reagents, observe the reaction and retrieve selected cells. Micromachined devices provide a solution to these requirements, thanks to the realization of dynamic microarrays [48]. This technology allows the integration of all the required functions mentioned previously in a single device, through the combination of hydrodynamic and optical approaches. The micromachined platform first performs (i) the cell encapsulation in alginate hydrogel beads. Encapsulation allows the use of both adherent and non-adherent cell types, protects cells from direct mechanical stresses and the size uniformity of beads facilitates trapping in the micro-array system. In a second phase, (ii) microfluidic traps are formed through hydrodynamic confinement to position the particles in an array. Hydrodynamic forces allow simultaneous transportation and immobilization of a large number of particles without the need for elaborate control. The last operation (iii) consists in the retrieval of particles with optical-based microbubbles. The bubbles push the particle out from the trap, and this technique provides a dexterous handling of individual particles without sophisticated circuitry.

In the device, an internal gelation method is combined with T-junction droplet formation to produce monodisperse alginate hydrogel beads [49]. Droplets are formed when sodium alginate solution containing insoluble calcium carbonate particles is introduced into the stream of oil at the T-junction (Fig. 4a). A reduction in pH downstream releases Ca^{2+} from the insoluble complex, causing gelation. These alginate hydrogel beads exhibited a narrow size distribution, with a coefficient of variation <2.9%. The viability of the cells after the encapsulation was studied. Jurkat cells viability using trypan blue (Fig. 4b) revealed that the ratio of live cells in the alginate hydrogel beads could be as high as 75%.

The accurate arrayed localization of the beads was performed by a microfluidic device. The microfluidic trap is made up of a square

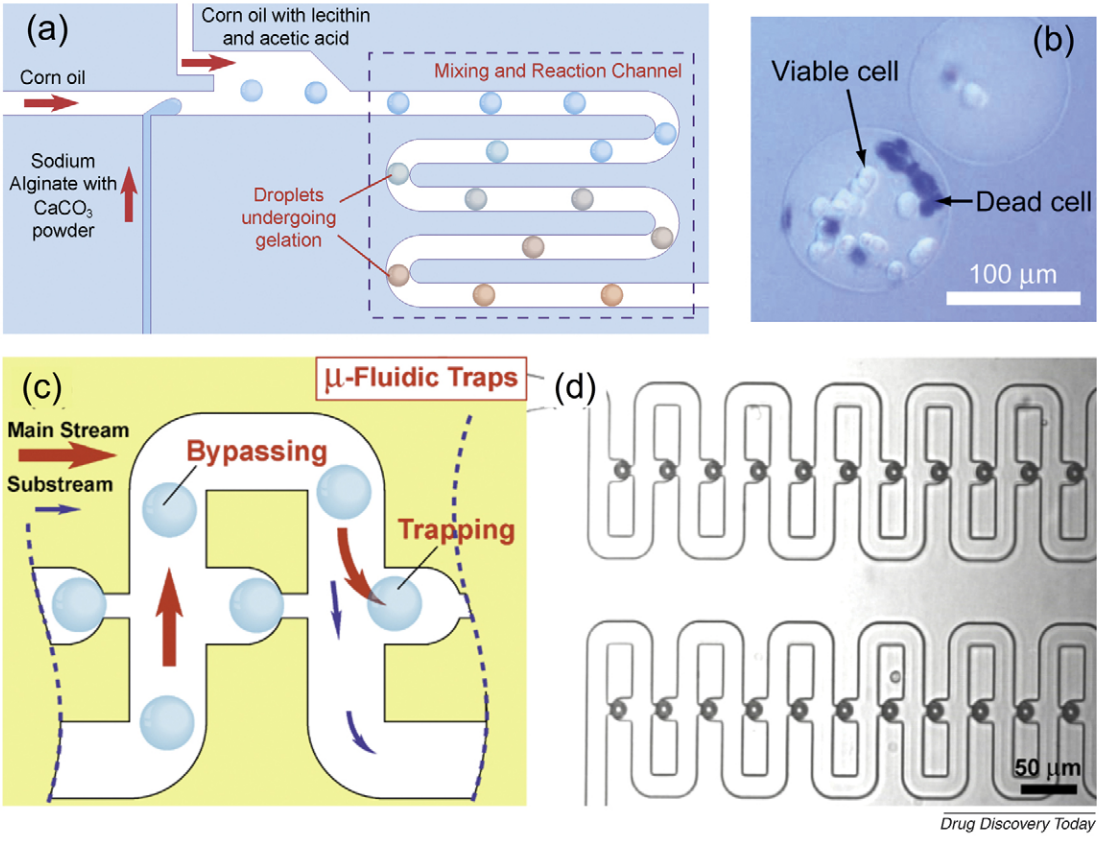

FIGURE 4

Device for positioning cells in array. **(a)** Schematic view of the device to produce monodisperse alginate hydrogel beads. Droplets of sodium alginate containing $CaCO_3$ powder are formed at T-junction. Gelation occurs when the pH reduces and Ca^{2+} combines to form calcium alginate gel downstream. **(b)** Viability test with trypan blue. **(c)** Principle of the cell trapping. **(d)** Microscope image of microfluidic trap device (20 × 5 traps in series).

wave shaped channel superimposed onto a straight channel (Fig. 4c). The traps are narrowed regions within the straight channel. When the trap is empty, flow resistance along the straight channel is lower than that of the loop channel, and the main stream flows along the straight channel. A bead in the flow will be carried by the main stream into the trap (Trapping mode). Once the trap is filled, flow resistance is increased drastically along the straight channel and the main flow is redirected along the loop channel. Subsequent beads will be carried along the loop channel, bypassing the filled trap (Bypassing mode). Owing to the unique design of this microfluidic trap, beads will not be able to enter already-occupied traps. The operation of this trapping and positioning techniques has been validated with microbeads of 15 μm in diameter (Fig. 4d). As the efficiency of this trap is near 100%, it is suitable for use with small sample volumes. Theoretically, this trap should work for scaled-down versions, enabling single cells to be directly trapped and arrayed for cell assays.

The device also includes the functionality to release beads, locally, after trapping. This operation is possible because of aluminum patterns that act as heaters close to the trapping site [48]. When illuminated by laser, bubbles form and push the trapped bead into the main flow that carries it towards the outlet.

The device is highly amenable to automatic processing, and can be easily scaled up to cater for fast, high-throughput, and highly parallel screening.

Perspectives and conclusion

Advanced research related to MEMS for nanobio applications can obviously bring new functionalities in the manipulation, the analysis and the testing of biological samples. These MEMS/NEMS devices are sufficiently versatile to fulfil real needs in medical and pharmacological applications.

These new capabilities are illustrated by dedicated tools targeting the direct manipulation of filamentary molecule, in the observation and analysis of motor proteins and, finally, the capability of cell positioning on dense arrays.

A pair of probes with 10–50 nm tip radius can be micromachined with integrated microactuators and mechanical sensors. The device acts as nanotweezers that catch and handle DNA molecules and are able, to stimulate mechanically and sense them. The results that have been demonstrated for DNA molecules can be achieved for other filamentary molecules both in air, but more interestingly, and in solution. Effects of buffer content on the trapped bundle and interaction with molecules can be sensed with these tweezers.

To increase the throughput of molecular interaction analyses, molecules could be prepared in many small chambers [50] integrated in a microfluidic device. Then, multiple tweezers (or device with multiple arms) could trap the molecules in these different chambers, and simultaneously perform the bioassays. Highly parallel analyses can be foreseen; the tiny width of the tweezers arm and the integration of the microfluidic function in a single device [51] can reduce the pitch distance between wells down to several millimeter. This approach can be cost effective, too; the tweezers are fabricated by collective MEMS processes, and the microfluidic parts are produced by molding. In a long-term view, this system could be considered as a new generation of multi-well pipettors capable of performing analyses at the molecular level, so with noise drastically reduced compared to bulk experiments, with even more chambers than currently available.

In a second type of devices, flow cells and microfluidic devices with tiny fL reservoir volumes allow the directly visualization of the activity of rotational biomolecular motors and the ability to derive quantitative data on their chemo-mechanical transduction. F_1-ATPase can be immobilized on a chip equipped with a microheater. The enhanced activity of this enzyme with temperature can directly be quantified through the recording of its accelerated rotational speed. By encapsulating a single molecule in an extremely small chamber, its chemical activity that consists in the consumption and synthesis of ATP molecules, was correlated with its rotational motion. The change of the proteins activities induced by interaction with other molecules or chemical compounds can be analyzed or measured in the same way.

The fL-chambers system provides spatial localization that is relevant for the parallel observation of numerous single enzyme activities. Microfluidic network can address the chambers for dispensing different drugs to the encapsulated target molecules. With this parallel observation capabilities and fast response that can be obtained when the proteins are localized in a tiny volume, high-throughput analyses can be expected. The devices are also fabricated by cost-effective molding of PDMS from a nanostructured silicon master. Thus, devices combining microchambers array and microfluidic feedthrough could be considered as a future possible solution for high-throughout screening of drug candidates when the chemo-mechanical activities of enzymes, motor proteins and even in cell transporters are targeted.

Finally, the third example addresses the microarrays technology whose purpose is to have extensive application, not only for basic studies, but also for drug-discovery [46,45] and diagnostic purpose [47]. Compared to static microarrays, where biomolecules and chemicals are spotted on a static solid support, the dynamic arrays, as the one presented, transport the molecules via mobile substrates, like beads or cells. Compared to their static counterparts, dynamic microarrays present several advantages such as the ability to mix and match the beads or the cells to cater for the type of screening to be performed, and introduce them into the microarray on demand; the beads or cells can be replaced, thus resulting in a reusable format that greatly reduces the cost of operation; and finally, the reaction on beads tends to be faster compared to conventional planar surfaces, as microbeads have increased surface area, thus higher binding capacity.

The presented device shows that dynamics microarrays provide reliable multifunctional platforms to transport and trap particles. After the immobilization of particles or cells in predefined location, the device allows to infuse reagents, observe the reaction, and retrieve selected particles. Such device has the real potential to become an adequate tool for screening methods such as the 'one-bead-one-compound' (OBOC) combinatorial library method [48].

Combining this highly parallel cell-based microfluidic devices together with automation will allow high-throughput screening. Moreover, it is possible to construct complex devices that more closely mimic the cell–cell interactions found in animals. Instead of using a single cell type for screening, different cell types connected by channels can be cultivated in a single microfluidic device to study the effects of one cell type's metabolic activity on another in the presence of the drug [52–55]. This kind of device

more accurately reflects actual physiological conditions in animals and may become an important tool for drug screening.

State-of-the-art MEMS technology for nanobio research provides appropriate manipulation and analysis means to sense the characteristic or the activities of bio elements, such as filamentary biomolecules, motor proteins and cells. These tools can integrate fluid handling, and the interaction of the bio samples with other molecules and chemical agents can be analyzed in a time-controlled way. All these new capabilities are believed to become productive in drug screening method and hopefully for new drug discovery.

Acknowledgements

Part of this work was supported by the Japan Society for the Promotion of Science (P06373), and a JST Grant for Advanced Instrumentation (Development of Handling and Characterization Tools for Nano Objects). Support from the Centre National de la Recherche Scientifique (CNRS, France) and by the Swiss National Science Foundation (grant PBEL2-107898) is acknowledged.

The photolithography masks were fabricated with the 8-in. EB writer F5112+VD01 donated by Advantest Corporation to the VLSI Design and Education Center (VDEC, the University of Tokyo).

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