THE ART AND SCIENCE OF ENGINEERING HYBRID LIVING/NON-LIVING MECHANICAL DEVICES

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Abstract

We have demonstrated the construction of hybrid systems comprised of micro- and nanofabricated parts and biological units. This integration has been accomplished at both nanoscale and microscale levels. The integration of biological parts with nano- and microfabricated structures opens up the possibility of incorporating biological complexity to the resulting systems, ultimately leading to the realization of systems that were not previously feasible. Biological motility structures, ranging from their basic constituents – such as the actinomyosin complex – to myotubules and complete muscle tissue have very high efficiency not currently matched by other commonly used methods of MEMS actuation. Potential applications range widely from medical implants to autonomous reconnaissance systems. Specifics of our fabrication processes and integration protocols are discussed, as well as integration issues that are likely to impact on future work.

Introduction

The indisputable progress and fundamental breakthroughs accomplished in the fields of microelectromechanical systems, nanotechnology, and molecular biology in recent years have naturally led to the interest in bringing such technologies together to build yet more complex systems with increased functionality. Additionally, fundamental progress has been made in essential supporting fields of knowledge such as the work done in self-assembling technology. As MEMS technology progressed, medical applications became one its most promising objectives, which prompted some key issues: autonomy, compatibility, selectivity, and durability. Autonomous operation is of paramount importance particularly for *in vivo* applications; not only should one be able to independently power an implanted system for a long period of time, but it is equally important to be able to control such system without external supervision or with remote supervision. In many situations the use of control lines such as merely switching a system on and off is not acceptable. Systems have to be made compatible with the biological media; the materials used have to be amenable to biological use and able to withstand the rigors of the biological environment. Systems also have to be able to identify and select their targets. For example, biological filtration systems typically have a selectivity of 1:10,000 while man-made filters at best reach a selectivity of 1:100 [1]. This shortcoming becomes even more severe when we consider that due to their intrinsically reduced size MEMS cannot handle large volumes of fluids. Lastly, systems have to be made durable, which has to do not only with autonomy and compatibility but also with reliability.

Having all these considerations in mind, the choice of incorporating biological elements into our micro- and nanosystems becomes increasingly appealing. Because such elements are powered by the same source that feeds the hosting organism, the system can be made autonomous. Furthermore, it can be tailored to respond to chemical or physical signals coming from the hosting organism itself. In the process of achieving hybrid integration, compatibility is addressed at the earliest building stages and becomes an intrinsic feature. Selectivity is achieved by making direct use of biological entities that possess this attribute – rather than attempting to mimic it through man-made structures. Finally, reliability is attained thanks to the fact that the system is immersed in an environment that is naturally favorable to its survival.

We have worked on at least two fronts to achieve hybrid integration. The first has been done at a nanometer scale, making use of naturally occurring biological motors and nanofabricated parts. The second involved larger, micrometer scale units consisting of both dissected and lab-grown muscle fibers, and microfabricated structures. Specific issues have arisen in terms of tailoring biological and fabricated parts alike, as well as creating new protocols for the integration process.

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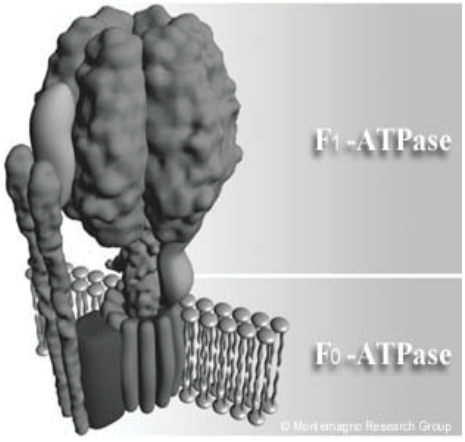
Material and Methods

I – Nanoscale Hybrid Integration

There are a number of naturally occurring biomolecular motors that can be explored for hybrid integration. Of particular importance – partly due to their high efficiency and stability regarding biological manipulations – are ATP synthase and the actinomyosin complex. ATP synthase is responsible for the hydrolysis and synthesis of adenosine triphosphate (ATP), the common source of chemical energy in all living organisms. The F_1 portion of the ATP synthase molecule can act independently as a motor, with ATP as the energy source, a theoretical efficiency above 80% and torque in excess of 80 pN.nm [2]. ATP synthase is in essence an electrostatic stepper motor with three steps per revolution. The stator consists of three pairs of alpha and beta subunits and the rotor of a gamma subunit (Figure 1.a).

We have engineered the F_1 ATP synthase molecule [3]. This included engineering a 6x histidine tag on each of the beta subunits and adding a cysteine residue at the tip of the gamma subunit. The molecule was subsequently biotinylated through disulfide linkage between the cysteine residue and biotin malemide in N'N'-dimethylformamide. Such dissimilar tags allowed self-assembling onto nickel dots with the desired orientation (no upside-down assembling). Initial calculations showed that drag forces near the substrate surface would impede device rotation, therefore we built posts so as to elevate the motors above the substrate. Finally, nickel rods coated with biotinilated histidine-rich peptides self-assembled onto the gamma subunit through a biotin-streptavidin link. Figure 1.b shows a sketch of the assembled system. The fabrication of the substrate consisted of electron-beam patterning of 50 to 120 nm dots on a thermally oxidized silicon substrate. This was followed by the evaporation of a nickel layer and lift-off. The remaining nickel dots on the SiO_2 surface were then used as an etch mask to form the posts. The nickel rods for the rotors were fabricated using e-beam lithography on a silicon substrate, followed by nickel evaporation. The rods were then released in KOH, suspended, dialyzed and incubated in biotin-histidine for the self-assembling process described above.

The assembled devices observed were made with a light microscope, and the propeller rotation was captured using a CCD video camera. Buffer A was used to fill the custom flow cell containing the device until the patterned array (i.e., alignment marks) was located, at which time the buffer was replaced with Buffer A + 2 mM Na_2ATP. To demonstrate the rotation dependency of the propeller, 10 mM sodium azide (NaN_3) was added to the flow cell while observing a functional device to inhibit the activity of the F_1-ATPase motor. Rotation of propellers in the absence of ATP (i.e., Buffer A alone) also was examined to demonstrate the functional dependency of the device on the F_1-ATPase biomolecular motor.



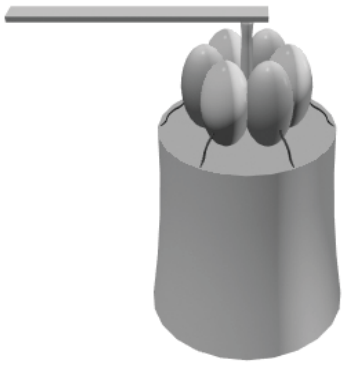


Figure 1: (a) Sketch of ATP synthase molecule inserted into a bilipid membrane. The top portion is the F1 motor, which can act independently. (b) Sketch of the hybrid device. The motor self-assembles onto a nickel dot atop a post, which reduces drag; a nickel rod then self-assembles to the gamma subunit (rotor).

II – Microscale Hybrid Integration

Our microscale hybrid integration work was geared towards exploring muscle-driven actuation, which is known to be considerably more efficient than any other MEMS actuation method currently in use. In particular, when compared to electrostatic actuation, much larger displacements are attainable with an overall power density approximately ten times greater [4]. In particular, for biological applications, the use of high actuation voltages or currents – which is needed for electrostatic and thermal actors, among other types – is prohibitive; furthermore, power and control requirements may render the resulting packaging solutions impractical. Muscles offer the possibility of direct electric control (i.e. triggering by an electrical impulse), chemical control (as it commonly occurs in living systems, where nerves actuate muscle fibers through Ca^{++} release), and even spontaneous contraction such as that found in cardiac muscles. Whether system power comes from glucose (in the case of cell- and tissue-level systems) or directly from ATP, the source of energy is the same as the living organism hosting the system, which not only facilitates packaging but also ensures an increased reliability and lifetime.

Muscle-MEMS integration relies heavily on self-assembly. We have been investigating the preferential growth of myoblasts – a type of stem cell that functions as a precursor for muscle growth – on different types of materials while keeping in mind the need for material compatibility as far as the integration with more standard fabrication techniques. Figure 2 shows an initial group of myoblasts and the resulting muscle fibers selectively grown on polymer.

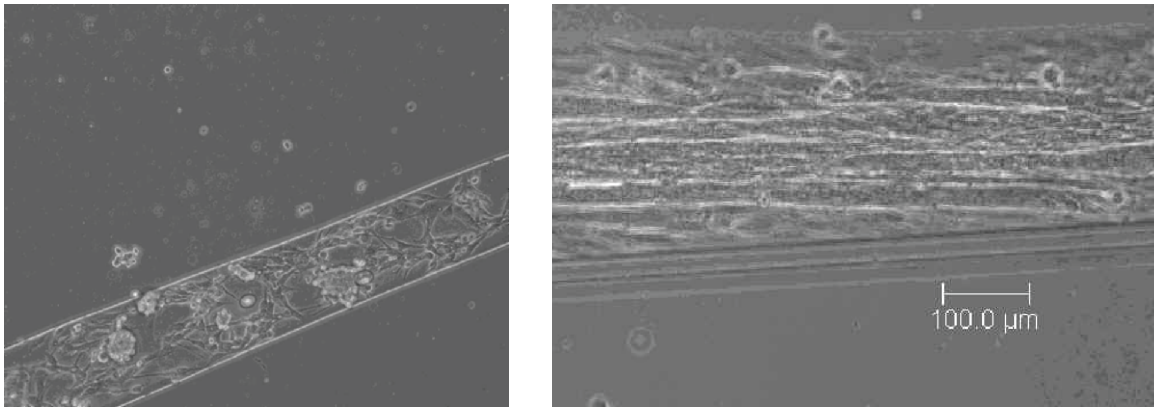


Figure 2: Optical micrograph showing the selective growth of myoblasts (left) into myotubules (right) over a period of eight days.

For the fabrication of our MEMS structures we have chosen SCREAM (Single-Crystal Reactive Etching and Metallization) [5]. SCREAM can lead to very high aspect ratios (in excess of 100:1), which facilitates sidewall attachment, as well as large distances between the fabricated structures and the substrate, which is important for post-processing for muscle growth. Figure 3 shows details of a MEMS structure for muscle tissue evaluation using a capacitive sensor.

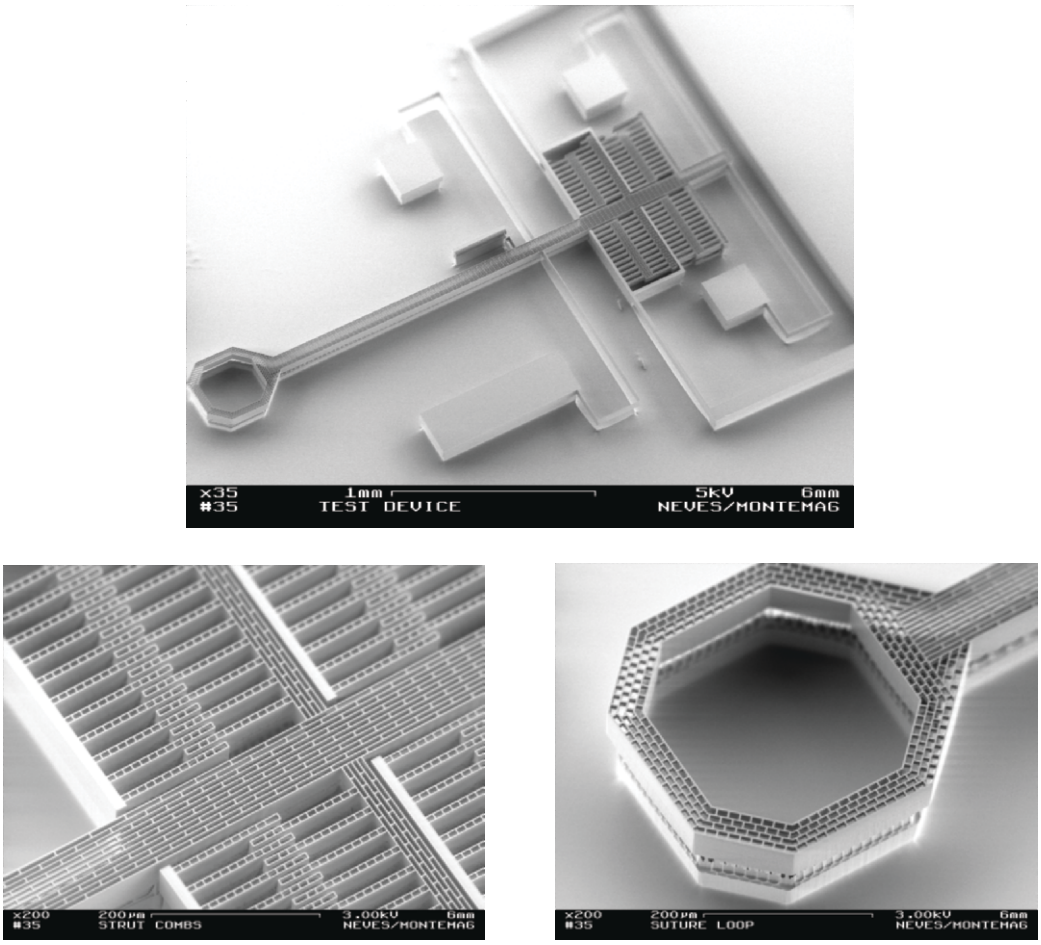


Figure 3: SEM micrographs of the MEMS structure for muscle tissue evaluation. Tissues are attached to the circular structure at the end of the trussed arm (bottom right micrograph) and the strain is measured capacitively. The device is capable of large displacements (in excess of 100 μm).

Results and Discussion

I – Nanoscale Hybrid Integration

Approximately one out of every 75 propellers rotated continuously in an anticlockwise direction. Rotating propellers were generally attached toward the end of the propeller (1/4 to 1/3) as opposed to the center. The majority (~80 %) of the non-rotating propellers did not display any Brownian fluctuation suggesting that the propellers were either (1) attached to more than one F_1-ATPase motor or (2) bound to the motor as well as the substrate. However, it is highly unlikely that the propellers (750 nm long) were attached to multiple motors due to the spacing (2.5 μm) of the Ni posts.

The rotational velocity of the device varied considerably from a minimum of 0.74 r.p.s., to a maximum of 8.3 r.p.s., with a mean velocity of 4.8 ± 0.8 r.p.s. Variation is likely due to the varying lengths of the nano-propellers used in different experiments. Detailed analysis of two propellers of differing lengths demonstrated two distinct rotation velocities, proportional to the length of the propeller. The mean rotation velocity of rods that were 750 nm and 1400 nm long was 8.0 ± 0.4 and 1.1 ± 0.1 r.p.s respectively.

The results of these experiments clearly establish the feasibility of creating functional hybrid nanoscale mechanical devices

II – Microscale Hybrid Integration

We have successful cultured function myoblasts that are selectively attached to surfaces by locally manipulating the nanoscale surface topography and chemistry. Controllable contraction is achieved after the myoblasts grow to a length of approximately 200 μm. Typically the when stimulated with a 3 V pulse the myoblast will contract 10% of its length with a force of approximately 1 nN.

Using surgically excised individual frog muscle fibers have been attached to MEMS cantilevers to assess the structural compatibility of the design. Both the voltages required for contraction and the forces generated during contraction were consistent with the cultured muscle myoblasts. After modification we have establish an actuator design which can accommodate the force loads and displacements generated by muscle fibers. Efforts are now focused upon local surface modification of MEMS actuators to facilitate a self-assembled Muscle MEMS system.

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