

The Final Report:

Phase I Advanced Aeronautical /Space Concept Study

NIAC CP 00-02

**DIRECTED APPLICATION OF NANOBIO TECHNOLOGY FOR THE DEVELOPMENT OF
AUTONOMOUS BIOBOTS**

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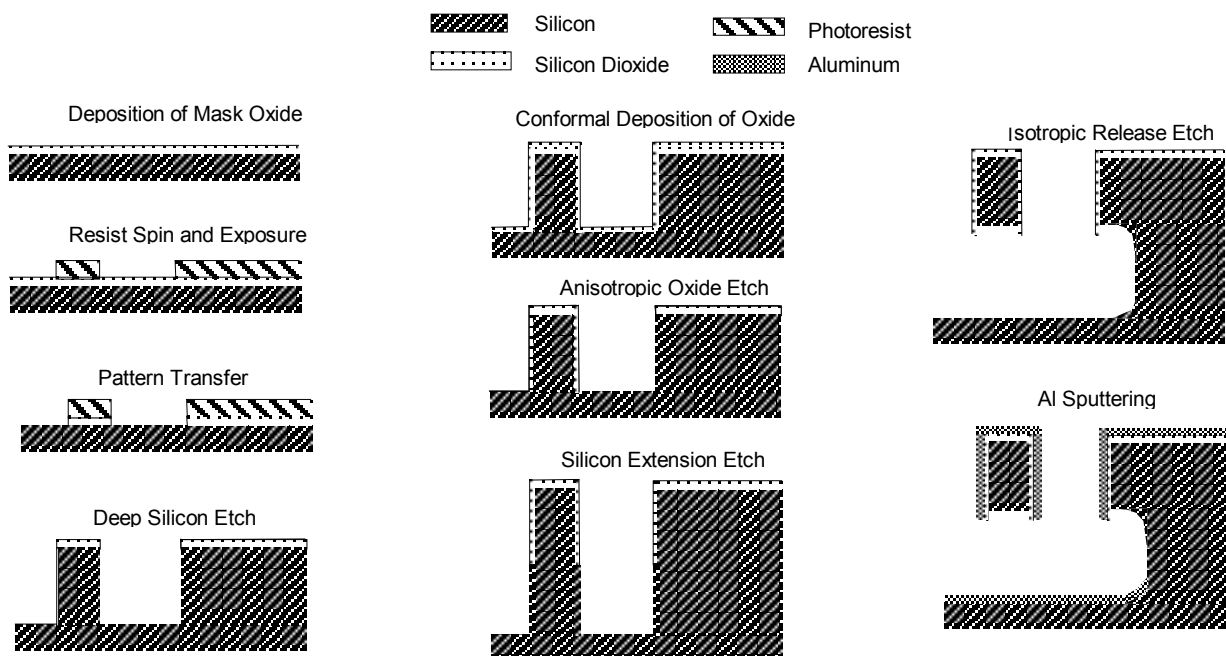
We have proposed two major goals in the Phase I study period:

- 1) **Characterize the engineering performance of single muscle fibers to assess their potential as a motive force for Biobots.**
- 2) **Evaluate the potential of nanoscale chemical and surface topology modification of surfaces to enable the directed self-assembly of Biobots.**

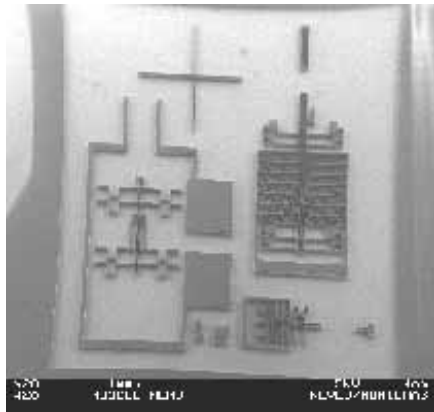
Indeed, the two goals are closely related to each other with respect to muscle fiber research, this report is organized to deal with different headings.

Fabrication of MEMS

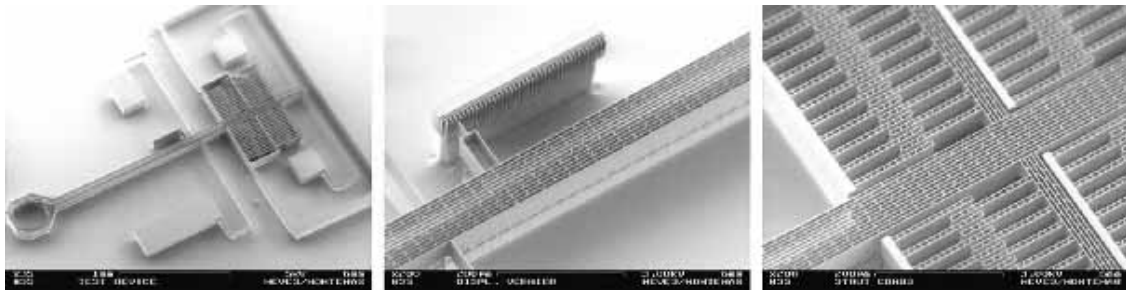
We have begun designing and fabricating a MEMS, on which a muscle fiber would be attached. The process of choice is SCREAM (Single Crystal Reactive Etching and Metallization), developed at Cornell University. It is a simple, fast turnaround process based on a single lithography step.



Overview of the SCREAM process for fabrication of the integrated MEMS device.



This design, however, although more complexed, was not suitable for constructing muscle-MEMS, especially for beginning of the study. After several revisions, we have constructing the version, shown below. We are currently using this device for constructing muscle-MEMS.



SEM images of the most recent MEM fabrication. Left image, low magnification view of the entire MEM. Middle; a part where muscle fiber attachment takes place. Right; alternated calms to be used for capacitance change measurements (-i.e. monitoring system),

Use of Muscle Fibers.

Although we have achieved to produce differentiated, multinuclear myotubes from undifferentiated myoblast cells (see section 2. below), the myotubes are not myofibers found in a muscle morphologically nor physiologically. There is no research report which demonstrate production of fully differentiated myofiber from myoblasts that is indistinguishable to that from a muscle.

Consequently, we have decided to isolate the muscle fibers, and use them to construct a muscle MEMS. E chose a frog *Xenopus laevis* as a source of muscles. Leg muscles were excised, and muscle fibers are dissected out using microsurgery apparatus.



Isolated bundle of myofibers from *Xenopus* leg muscle. Striations of actomyosin fibers are seen, indicating that the thin slice has only one layer of the muscle fibers. The fiber is capable of undergoing contractions with external electrical stimuli (below).



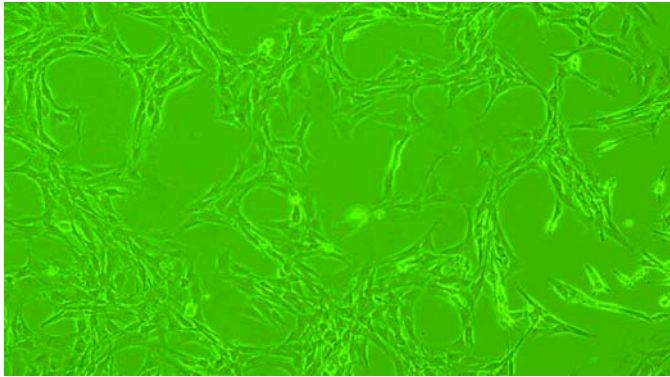
The isolated bundles were further dissected to obtain individual fibers.

We have initially followed many frog physiology protocols to isolate muscles and fibers, and found that they do not maintain their physiological activities (-ie. contractions) for long; only for one to two hours. Then, we have found when the muscles and fibers were kept in the commercial 'lactated Ringer IV solution', they maintained the activities much longer - even a day (or possibly more). This is a very practically beneficial finding for the current stage of the muscle-MEMS development, since we do not have to keep dissecting frogs for every few hours. It also helps in future in designing total Biobot system.

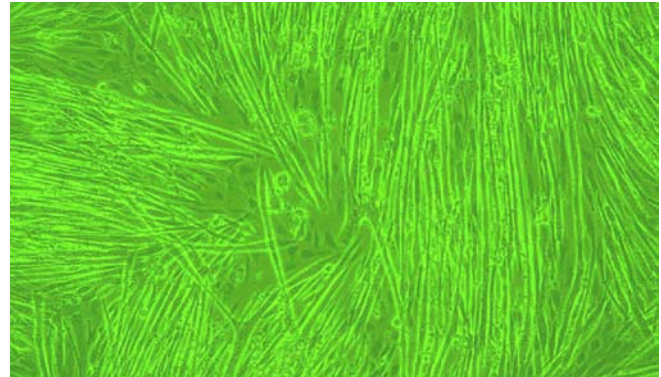
Now we have obtained 'workable' MEMS and isolated muscle fibers, we are constructing muscle-MEMS finally. At moment, however, we have encountered a problem; how to attach the muscle tissue to the MEMS. We have tried cyanoacrylate (Krazy Glue), aluminum wire, and surgery suture; without a precision 3D micromanipulator that we currently do not own, it appears extremely difficult to make this connection successfully. Many times, the biological tissue died during or right after the connection has been made, presumably due to physical injury, or effect from the chemical glue. Other major consequences were that the MEMS were fractured during the attachment process. Use of a micromanipulator (requested as a equipment in the Phase II proposal) would greatly help this process.

Production of differentiated muscle cells from stem cells on a selected material surface.

We have started to culture myoblast cell line C2C12. Myoblast cells are cultured in DMEM containing 10% fetal calf serum (FCS) depleted of the thyroid hormones at 37°C. Under the conditions, each cell was undifferentiated, and was not fused to form myotubules, as expected. 4 days after the tissue culture medium was switched to 2% horse serum in Dulbecco's Modified Eagle's Medium (DMEM), the cells became aggregated and fused to form myotubes (B, below).



A) Undifferentiated myoblast cells



B) Myotubes

In the Phase I proposal, we have proposed to make a special cell-growing chamber for future de novo muscle fiber formations within the Biobots. While we were culturing the myoblast cells in regular polystyrene tissue-culture dishes, we have tried several compounds that were available to us in our lab. We have painted those compounds on the bottom surface of the dishes like striped-patterns, then placed the myoblast cells to let them grow.

Among those compounds, PDMS (polydimethylsiloxane) had a significant effect to the cell attachment and/or growth. Even one day after one day of the incubation, the cells were mostly absent on the PDMS surface, whereas cells remained attached and even grew on the neighboring surface where the other compound, either Polysulfate, or Polycaprolatone (see the figure below) was on the surface. The cells even underwent differentiation to form long myotubes later. This discovery will benefit us for the construction of the Biobots: Larger amount of myoblast cultures will be grown on narrow-strips of a material on which the cell-friendly compound, such as Polysulfate or Polycaprolatone, for instance, will be coated, meanwhile the rest of the surface will be coated with a cell-unfriendly compound such as PDMS. Because the individual myotube varies one to the another, only the one which fits to the muscle-MEMS will be selected and then placed onto the device. It is also possible that with a modification, we may be able to replace damaged myotube with the new one with this 'strip growth and transfer' methods in future.

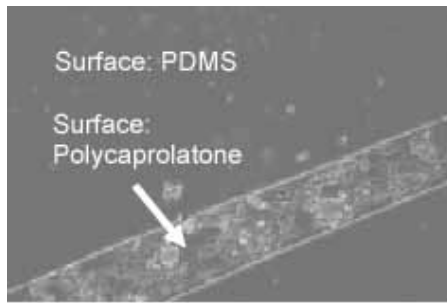


Fig. 6A: Day 1

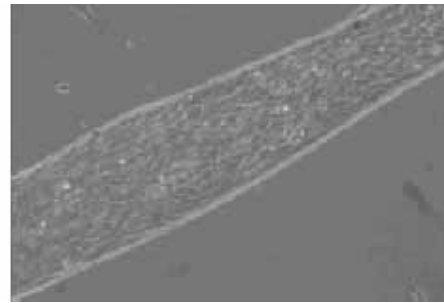
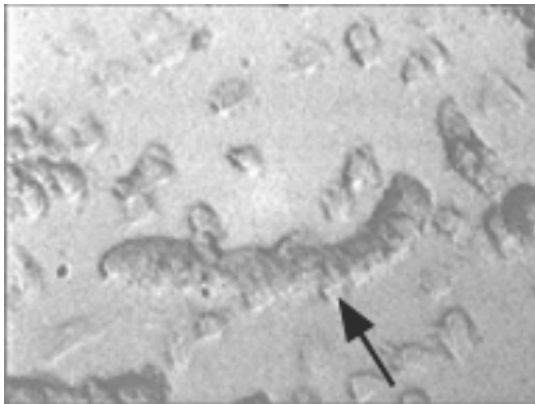


Fig. 6 B: Day 2

Myoblast cell growth on a selected surface. Myoblast cells were cultured on a surface that has been coated with two different substrate: PDMS (polydimethylsiloxane) and Polycaprolatone for two days. The cells initially on the PDMS surface were either quickly moved into the Polycaprolatone-coated region, or died. Those on the PDMS, however, continued to grow and started differentiations to form myotubes (below).



The myotubes, however, does not have characteristics of a typical myofiber isolated from a muscle, such as cell-end differentiations, which will be important for transmitting the motion of



The isolated myotube (arrow) which exhibited spontaneous shortening.
(the image is taken from a movie file:
the movie is available upon request.)

the shortening muscle fiber to the MEMS, or rapid response to a electric stimulus. Though, no other biological/medical research has achieved beyond what we have achieved here for producing the myotubes in a particular manner, we will continue the tissue culture research to produce better myotubes, or completely differentiated and functional myofibers.