Designing a Fluid Cell for an Atomic Force Microscope using Rapid Prototyping

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Abstract

The purpose of this research is to create a fluid cell that allows studying morphological changes in cancerous fibroblasts with an atomic force microscope. A thorough examination of a Veeco[12] contact mode fluid cell lead to creating a more convenient and cheaper version of a fluid cell. Using rapid prototyping a fluid cell was designed. Cells are cultured in sterile conditions and in a humidified environment. An ultraviolet light chamber emitting artificial ultraviolet light (312 nm) induces cancer to the cultured cells. The fluid cell fits on the atomic force microscope scanner head. The atomic force microscope functions by sensing the surface of the sample it scans with mechanical probes. A beam produced by the atomic force microscope strikes the mechanical probe and bounces onto a photodiode. The photodiode translates the beam intensity to a potential difference which in turn is translated to height differences. The information obtained from the height differences produces images on the nanoscale level. The atomic force microscope's most common usage involves scanning solid samples. The fluid cell allows researchers to scan soft tissue samples in liquid environments while maintaining the liquid static. The resin that makes the fluid cell is transparent.
Key words: Atomic force microscope (AFM), fluid cell, rapid prototyping

1. Introduction

In 1986, Binning, Quate and Gerber a collaboration between IBM and Stanford University[4] developed the atomic force microscope. In cell science, the ground-breaking work using atomic force microscopy was conducted in the early 1990s. Several researchers attempt manipulating soft samples with an atomic force microscope. However, rigidify those samples for scanning remains a challenge.

This research involves using rapid prototyping to design a fluid cell for an atomic force microscope. The fluid cell creates static conditions for liquid samples and allows manipulating cancerous mice fibroblasts on the atomic level. For optimal growth, fibroblasts require sterile conditions and maintenance as monolayer cultures in a nutritive solution called a medium. A humidified environment that is favorable for cell proliferation stores the cells. Simulated ultraviolet light induces cancer to the cultured mice fibroblasts. The diseased cells are immersed in phosphate buffer saline, a solution that keeps cells alive. A fluid cell creates a sealed environment and stationary conditions for the phosphate buffer solution containing the cells. Those cells are used for analysis with an atomic force microscope.

1.1. cell culture: preparation and treatment

The cell's treatment and storage necessitates a sterile hood and an incubator**.** Medium containing 15mM $NaCO_3$,5mL of glutamine, 50U/mL penicillin, 50 mg/mL streptomycin, and supplemented with 10% inactivated (by heat) fetal calf serum (FCS) maintains mice fibroblasts as monolayer cultures and provide proteins[1]. This medium solution, also called complete medium, constitutes a favorable environment for cell growth. Fibroblasts stick to the

bottom surface of the flask they are kept in. The cell cultures are stored in an incubator, which is a humidified environment that mimics physiologically realistic conditions with 5% CO_2 atmosphere at 37ºC. Once the cells have proliferated after a 48 to 72 hour period, they are taken out of the incubator and washed with phosphate buffer. Trypsin is added into the cell flask to detach cells from the flask surface. The obtained solution is placed back in an incubator in order for the trypsin enzyme to be activated. Once activated the enzyme is diluted with 10mL of phosphate buffer saline (PBS). The mixture is centrifuged in order to separate the cells from the phosphate buffer and the enzyme. Cells are then resuspended in complete media dilutions and returned in the incubator.

1.2. cancer induction

An ultraviolet chamber emits artificial ultraviolet light that induces cancer to mice fibroblasts. The ultraviolet light chamber produces two types of ultraviolet lights: ultraviolet A rays and ultraviolet B rays. The ultraviolet chamber contains a filter that only allows ultraviolet B rays to go through and filters out ultraviolet A rays. Ultraviolet B light has been experimentally demonstrated to cause DNA damage[2]. In order to induce cancer, mice fibroblasts maintained as a monolayer in a flask containing medium are removed from the incubator and washed. 1 mL of phosphate buffer is added. The mixture is transferred to petri dishes. Lids are removed from the petri dishes. The petri dishes are placed in the ultraviolet B chamber. A control solution is kept outside the ultraviolet chamber. The time is set for each petri dish. When time is up for each Petri dish; they are removed one by one from the ultraviolet chamber. The cells are then ready for atomic force microscopy analysis.

1.3. ultraviolet light and cancer

Three types of skin cancer occur the most: basal cell carcinoma, squamous cell carcinoma (nomelanoma) and melanoma. Melanoma demonstrates the most fatality. Its occurrence has increased considerably since reliable data was first recorded in the 1930s.[3] Squamous cell carcinoma (Figure 1) and basal cell carcinoma (Figure 2) almost exclusively develops on sun-exposed areas of the skin[3].

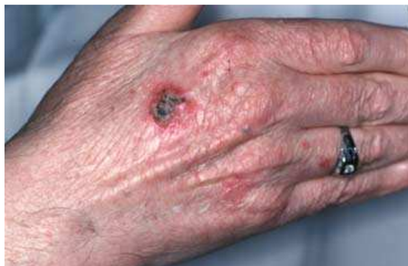

Figure 1. Squamous cell carcinoma[8]

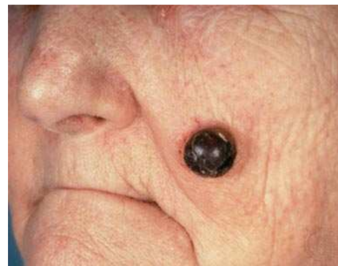

Figure 2. Basal cell carcinoma[7]

Ultraviolet radiation from sunlight damages skin cells genetic material. Ultraviolet radiation can damage many molecules and structures. When ultraviolet and visible radiation reach the skin, one part is deflected and the other part is absorbed in various skin layers. Epidermal cells components, including DNA, mainly absorb wavelengths in the ultraviolet B range. Ultraviolet B range wavelengths can induce mutagenic lesions and alter the DNA. DNA damage can lead to genetic instability and mutations. Mutations affect both the genotype and the phenotype of an organism. Thus, sunlight overexposure increases the risk of skin cancer. DNA constitutes a critical target because its alteration can ultimately lead to skin cancer [2] (Figure 3).

Figure 3. Induction of skin cancer

2. Instrumentation

2.1. atomic force microscope

The atomic force microscope set up consists of a microscale cantilever tip that scans samples by sensing their surfaces. While sensing the surface of the sample, the tip also forms emergent chemical bonds with each atom of the sample. When the tip is brought close to the sample, forces lead to deflection of the cantilever tip (Figure 4). The atomic force microscope comprises three major operational modes: the contact mode, the non-contact mode and the tapping mode. The contact mode functions by simply moving the tip back and forth across the sample surface. The set point controls the amount of cantilever deflection. As the set point increases, the cantilever flexes more and tip-sample forces increase. The non-contact mode functions by oscillating the cantilever .The cantilever tip does not touch the surface of the sample but oscillates above it. The tapping mode functions by vibrating the cantilever tip back and forth across the sample. The tip lightly "taps" on the sample surface during scanning, contacting the bottom surface of the swing[4]. The vibrational frequency produced allows detecting and mapping the structure of the sample that is being scanned. One of the main advantages of the atomic force microscope is its nonintrusive local probe for cells and their dynamics in a biolfluid environment. The atomic force microscope has had a great impact in life science. It can manipulate, visualize and characterize objects and processes on the nanoscale level. Live cells, however, constitute soft objects in the context of atomic force microscopy analysis. A tool is needed to stabilize soft samples for analysis using an atomic force microscope.

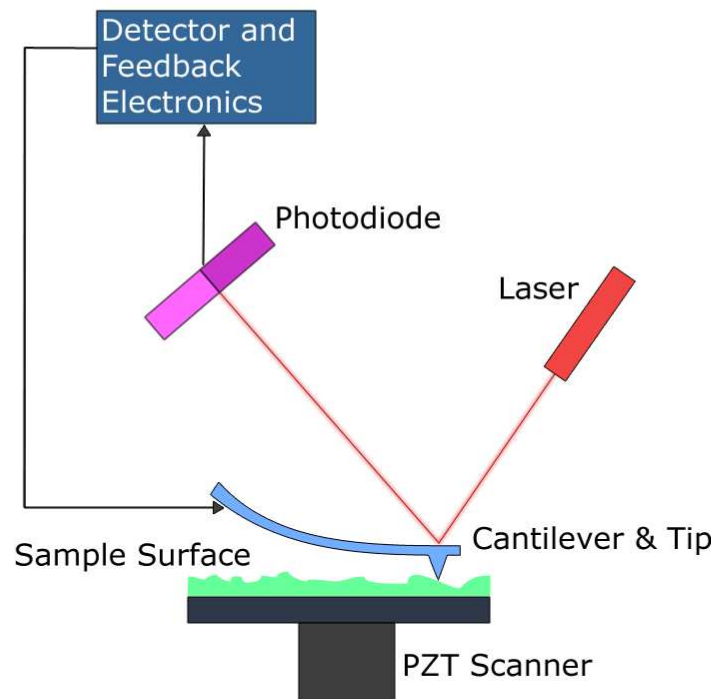

Figure 4. Atomic force microscope diagram[11]

3. Methodology

A fluid cell holds liquids stationary for scanning fluids with an atomic force microscope. Its set up consists of a clear tip holder that allows the atomic force microscope laser spot to pass though and bounce a beam off the cantilever tip. The cantilever tip is supported by a cantilever chip that is mounted on the fluid cell. The laser beam is deflected based upon the sample surface structure.

3.1. materials

The fluid cell is made out of Accura 60. Acurra 60 is a durable and highly transparent resin. It has a fast build speed; high clarity minimizes part cleaning and finishing and produces humidity resistant parts. It allows viewing internal features and passages through parts that. The Accura 60 plastic material ability to look and feel like molded polycarbonate when polished, its reliable and consistent shrinkage behavior and its low viscosity formulation make it one of the best choices for building the fluid cell[10].

3.2. software

The fluid cell design uses Solidworks, a computer aid design (CAD) program that creates 3-dimensional parts and runs on Microsoft Windows. Once the part is drawn with Solidworks it is saved and converted to an STL file, which is a non parametric triangulated surface file. The file is sent to the rapid prototyping apparatus for building.

3.3. rapid prototyping

The rapid prototyping machine employed to build the fluid cell was the stereolithography apparatus. It builds parts at a very high resolution through a layer by layer process. Stereolithography workflow creates plastic parts directly from 3D computer software models by hardening the surface of a liquid photo polymer layer by layer with the help of a laser beam. The laser beam solidifies the resin when it strikes the liquid. Figure 5 illustrates how the elevator drops in the vat as soon as a layer is fully traced. Due to the self-adhesive property of the material, the various layers build up, forming a 3D part in multi-layers. Stereolithography process is the best choice for building parts with high accuracy and smooth surfaces[6].

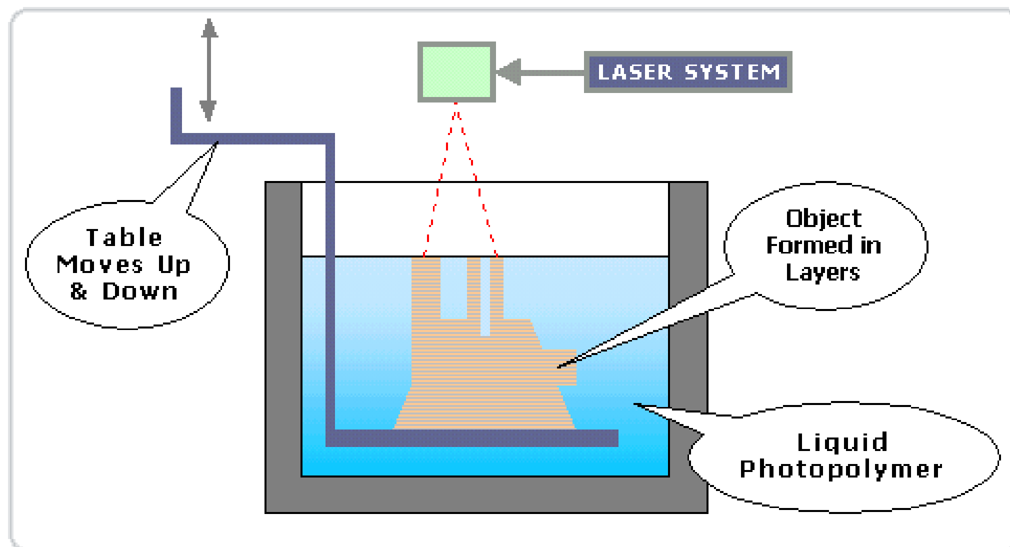

Figure 5. Stereolithography apparatus diagram[6]

3.4. existing fluid cell

The Veeco[12] contact mode fluid cell consists of a closed glass assembly with a wire clip coated with gold that holds the probe. The wire clip is twisted on one side of the fluid cell in order to properly hold the probe. The probe fits in a rectangular groove on the fluid cell bottom. A circular groove that holds an optional O-ring surrounds the rectangular groove (Figure 6). The O-ring holds liquids with low surface tension and forms a reliable seal against the liquid sample that is being scanned. It also prevents fluids from leakage onto the scanner.

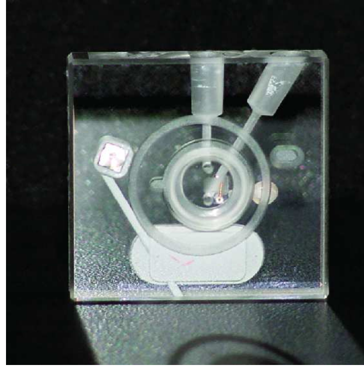

Figure 6. Veeco fluid cell[4]

3.5. fluid cell design features

The prototyped fluid cell created for this research, is an open fluid cell that consists of a small clear plastic assembly. In the center of its surface, lies a circular groove. The circular groove is designed to contain an O-ring that prevents liquids from spilling onto the atomic force microscope scanner. In the center of the fluid cell also lies a rectangular groove designed to hold cantilever chips. The cantilever chip contains the tip that senses the surface of the sample to be scanned. The cantilever chip is held in place with a wire clip. A hole through the fluid cell allows the wire clip to remain secured. The wire clip is bent at a ninety degree angle on the side of the fluid cell that holds the cantilever chip (Figure 7). On the other side of the fluid cell, a spring around the wire allows for lifting and lowering of the bent part of the wire that hold the chip against the rectangular groove of the fluid cell (Figure 8). Two additional grooves also lie on the fluid cell corners. Those grooves fit in the atomic force microscope scanner head screws.

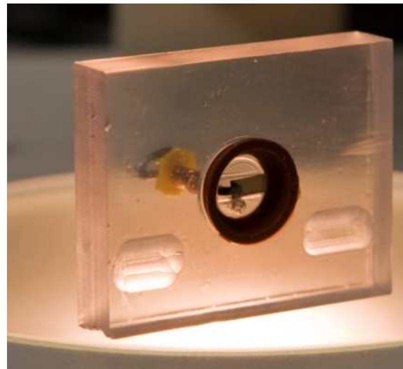

Figure 7. Prototyped fluid cell back view

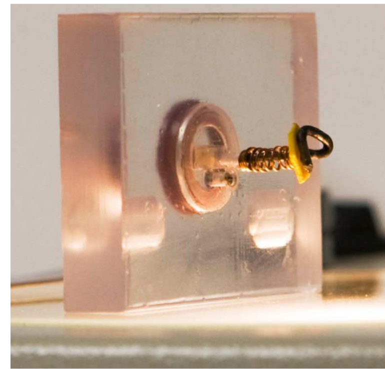

Figure 8. Prototyped fluid cell front view

3.6. fluid cell and atomic force microscope

The fluid cell is placed on the atomic force microscope piezoelectric scanner such that its bottom part, which contains the cantilever tip supported by a cantilever chip and the O-ring, is against the fluid. The liquid sample to be scanned is placed on a glass slide that is placed upon the atomic force microscope scanner head. The fluid cell is seated upon the glass slide. The O-ring creates a sealed environment around the liquid. The cantilever tip connected to the cantilever chip is immersed in the liquid sample and can sense its surface. The fluid cell transparent feature allows the laser beam to pass through and hit the cantilever tip that deflects the beam on the photodiode and creates a voltage difference which is electronically rendered into height information[5](Figure 9).

Figure 9. Fluid cell mounted on the atomic force microscope

4. Operation

The prototyped fluid cell needed revision in order to be tested. Once built the fluid cell, required polishing to make the part as clear as possible in order to avoid any kind of laser beam diffraction. When the fluid cell was ready for testing, it was placed upon the atomic force microscope scanner head. The scanner screws were clamped to the fluid cell for stabilization. The laser beam was turned on. However the clamp obscured the laser beam, keeping it from reaching the fluid cell. The clamp was readjusted and placed in another location on the fluid cell. A hole was mechanically drilled to create another clamp. The new clamp placement was no longer an obstacle to the laser beam. The laser beam was mechanically adjusted both on the vertical and horizontal positions to strike the cantilever tip at the right location .The fluid cell size did not provide enough flexibility to adjust the laser beam. It was mechanically trimmed. The two grooves that were initially designed to fit in the scanner head screws did not provide suppleness to adjust the fluid cell either. They were widened with a driller both horizontally and vertically, such that they created rectangular grooves. They thus provided more room for the fluid cell position to be adjusted, allowing the atomic force microscope laser beam to be properly positioned. The laser was then adjusted such that it could at least locate the cantilever chip surface and be deflected onto the photodiode.

5. Results

The opacity of the fluid cell resin and the loss of laser beam intensity reduced the optimization of the experiment. The fluid cell surface was not smooth enough for the laser beam to go through the fluid cell resin and reach the cantilever tip. The areas of the fluid cell containing the most roughness were so small that polishing them would be a great challenge (Figure 10). These rough surfaces also contributed to scattering the laser beam. The optical microscope connected to the atomic force microscope could not locate the cantilever chip tip that scans the surface of the sample. The regular tip holder that is utilized to scan solid surfaces allowed a voltage value of 7 volts. The fluid cell allowed a voltage value of only 0.3 volts (Figure 11).

Figure 10. Magnified view of rough surfaces

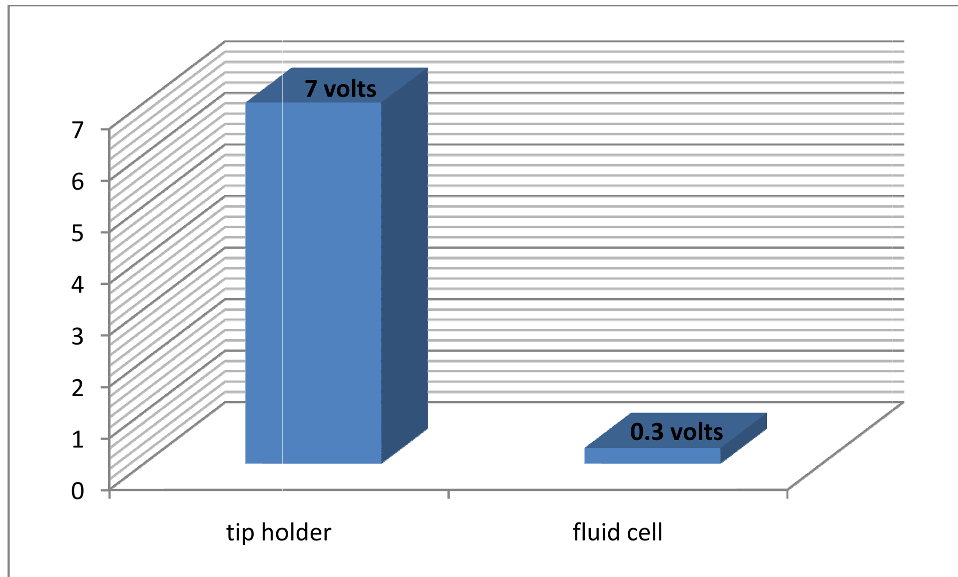

Figure 11. Comparison of voltage measured using fluid cell and regular tip holder

6. Conclusion

Fibroblasts were cultured in sterile conditions. Contamination was avoided and the cells reproduction rate was not altered. Cancer was induced to the cultured skin cell using ultraviolet B light produced by an ultraviolet chamber. The fluid cell was designed using the Solidworks computer software. It was built using the rapid prototyping stereolithography apparatus.

However, due to lack of clarity of the plastic material Accura 60, the laser beam could not pass through the fluid cell with recordable intensity, and the optical microscope could not read through the resin to locate the cantilever tip. The voltage measured with the fluid cell did not permit engagement of the atomic force microscope fluid cell cantilever tip to the sample. Hence, the fluid cell could not be utilized to scan and study cancerous cells morphological changes.

7. Further Work

Further research is needed for creating a clearer fluid cell that will diffract less of the laser beam. One way of getting around this issue can be making molds out of the prototyped fluid cell and casting acrylic or other clearer materials. This will result in higher voltage. Also, a better way of holding the cantilever chip against the fluid cell can be found. Fluid port entries can also be inserted into the fluid cell such that a closed fluid cell is created. The mechanical modifications made on the fluid cell should be incorporated into the Solidworks design as well.

8. Future Applications

The fluid cell can be used for future research purposes such as studying morphological changes in cells that have been exposed to sunscreens and other products suspected to cause side-effects in metabolisms. It can also be used to study chemical solutions atoms interactions, electrolytes, hydrogels and other organic fluids.

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