Electro-adhesive forceps for tissue manipulation

Alexander Vankov[2], Philip Huie[1,2], Mark Blumenkranz[1], Daniel Palanker[1,2]

[1] Department of Ophthalmology, School of Medicine, Stanford University, Stanford, CA;
[2]W.W. Hansen Experimental Physics Laboratory, Stanford University, Stanford, CA 94305-4085

ABSTRACT

Capturing, separation and removal of thin, evasive, and often transparent membranes attached to the underlying tissue is typically a very difficult task in vitreoretinal surgery. The most challenging part of such procedures is in initial separation of the membrane, which then allows for a strong grip of the micro-tweezers holding it from two sides. Attempts of performing this procedure often lead to piercing and otherwise damaging the underlying tissue. Accordingly, there is a need for devices that could attach to tissue in a minimally-traumatic manner approaching it from only one side. It is desirable that such a device would attach to a tissue on a push of a button and release it on demand. We developed a technique that allows for strong attachment of an electrode to tissue with a single electrical pulse, and disconnection of it from the tissue with a different pulse. Adhesion does not require any electrical support after the pulse, and the adhesive forces generated on a wire electrode of 50 μm in diameter are sufficient for manipulation of all types of cellular and non-cellular intraocular tissues. To reduce electroporation-related tissue damage the bipolar train of pulses is applied with burst duration 50–200μs. At optimal pulse parameters the tissue damage is limited to a single layer of cells adjacent to the surface of electrode. Electrically-induced adhesion is very convenient for lifting and manipulation of vitreoretinal membranes. It can also be used for attachment of a needle to a membrane for injection of liquid into the sub-membrane space, thus separating the membrane from the underlying tissue without peeling. Similarly, injection of medication into small retinal blood vessels can be performed without insertion of the needle into it.

Keywords: vitreoretinal surgery, electrosurgery, adhesion, forceps, tissue manipulation

1. INTRODUCTION

Mechanical forceps are widely used for manipulation of tissue in microsurgery in general and in ophthalmology in particular. Capturing a thin and evasive membrane is a difficult task since such membranes easily escape the grip of the forceps due to even a minor flow of water introduced during closure of the forceps. Another difficulty is in grasping a thin membrane strongly attached to the underlying tissue. The most difficult part of such procedure is in initial separation of the membrane, which will then allow for a strong grip of the microtweezers holding it from two sides. Attempts of performing this procedure often lead to piercing and otherwise damaging the underlying tissue. It would be very desirable to have a micromanipulator that could attach to a tissue on a push of a button and release it on demand. In this case manipulation of tissue would be possible even though the tissue is accessible only from only one side.

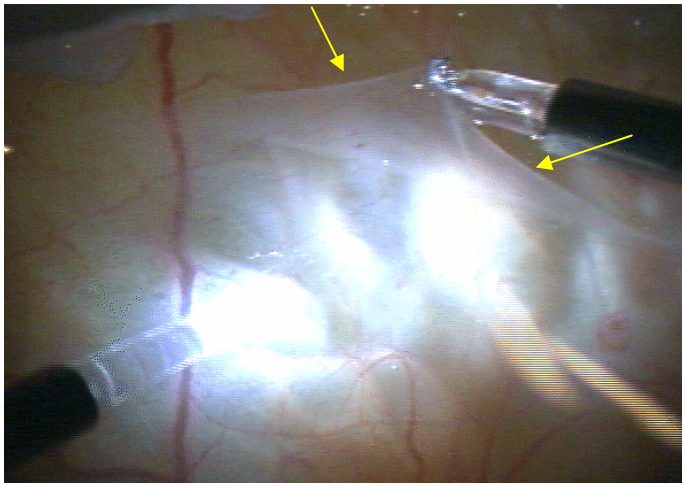

Figure 1. A membrane attached to electrode is elevated. The electro-forceps probe is in the right upper corner. Illumination probe is in the left lower corner. The edges of the membrane are shown by the arrows.

2. METHODS AND RESULTS

To address these problems we developed a new technique, called Electro-Adhesive Forceps. The device is composed of an insulated probe with a protruding metal electrode (active electrode). The second (return) electrode is typically much larger than the active electrode and its location in the operation field is not critical. The device is activated by electric pulse applied between the active and the return electrodes. Pulse duration can vary from 30 microseconds to several milliseconds. Pulse energy should be below the threshold energy required for formation of a complete vapor cavity around the electrode.

Application of such a pulse (or a few pulses) to the probe kept in contact with tissue induces strong adhesion of tissue to the metal surface, such that the tissue can be lifted and manipulated, as shown in Figure 1. For detachment of tissue from the electrode a stronger pulse can be applied, such that it creates a transient vapor cavity surrounding the whole probe, thus disconnecting the tissue from metal. The same pulse will clean the surface of the conducting element from biological debris.

As a model of live soft tissue we applied the chorioallantoic membrane (CAM) of the white leghorn chicken embryos 14-18 days into incubation. A piece of CAM was manipulated inside the open egg, or was excised and pinned down in a Petri dish. For detection of permeabilization of the cellular membrane we used Propidium Iodide (PI) at concentration of 4 μM. The dish with tissue was loaded onto a fluorescent inverted microscope (TE2000U, Nikon) and the PEAK probe [7] was positioned at 45^{o} to the tissue surface using XYZ micromanipulator. The size of the fluorescent zone was measured using CCD camera (NL/CCD-512, Princeton Instruments) with WinView-32 software.

Tissue damage during electrosurgery is generated by three primary mechanisms: heating, electroporation, and mechanical damage due to explosive vaporization and collapse of the bubbles. To minimize the size of the heated zone around the electrode the diffusion of heat should be restricted. To limit the heat diffusion length in water by cellular size (i.e. 10 μm) pulse duration should not exceed 100 μs.

Damage associated with electroporation (increased permeability of the cellular membrane induced by high cross-cellular voltage) critically depends on the temporal structure of the waveform. Monopolar pulses produce large damage zone (see Figure 2a). Due to low electrical conductivity of the cell membrane the electric potential inside the cell equalizes within a short time after application of electric field. The cellular relaxation time constant t ~ 0.5 μs is the product of membrane capacitance (~ 5pF [5]) and cytoplasm resistance (~ 100kΩ[5]). Thus with pulses longer than 1μs

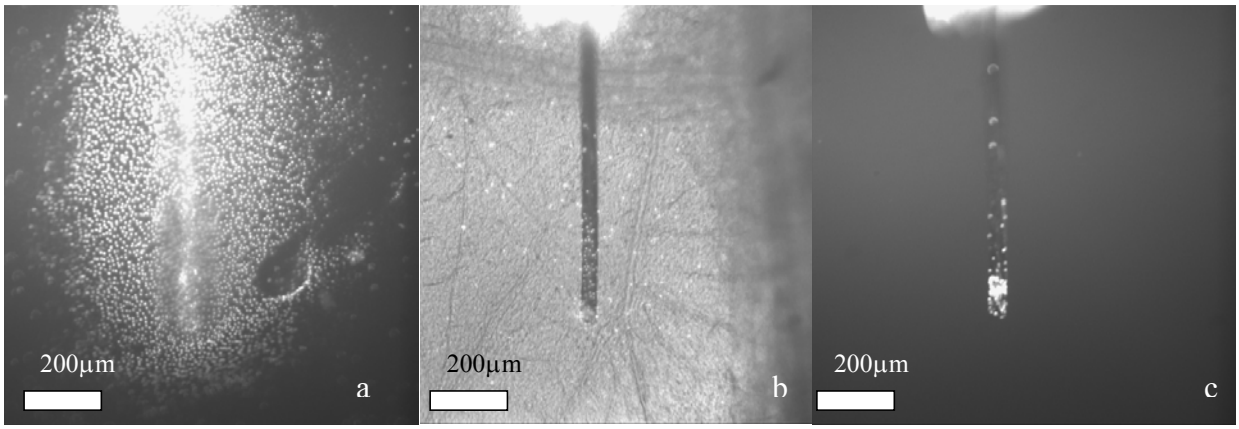

Figure 2. Propidium Iodide staining indicates damaged cells in a chorioallantoic membrane attached to a wire electrode. Pulse parameters: Amplitude 150V, frequency 1MHz, duration 100μm. **(a)** Monopolar (positive) pulse, **(b)** Voltage-balanced burst, **(c)** Electrode after mechanical detachment.

the cross-cellular voltage is actually applied mostly across the cellular membranes. High trans-membrane electric field (above 900 kV/cm [1]) can damage the ion gates and pumps – proteins controlling the flow of ions across the cellular membrane. Electroporation can be reduced by either (a) alternating the voltage faster than intracellular relaxation time

(i.e. above 1 MHz) and/or by (b) decreasing the amplitude of electric field. Effect of the first approach is demonstrated in Figure 2.a. Voltage-balanced square burst of alternating polarity at 1 MHz was applied to the probe. Voltage amplitude was +/-150V and total burst duration 100 μs. Voltage balance was maintained at the level of 1%, while duration of the positive and negative phases was balanced no worse than 4%. As one can see in Figure 2 the width of the damage zone was drastically reduced and became comparable to the cellular size.

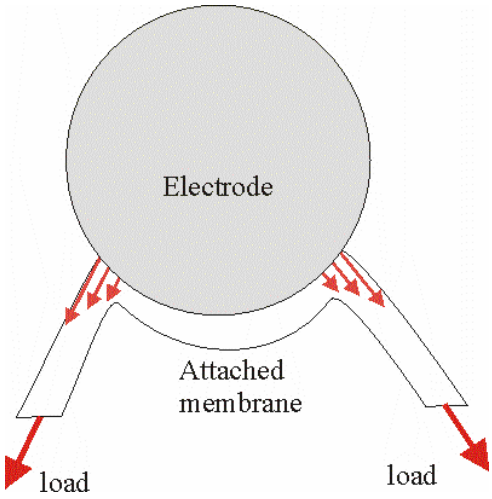

Figure 3. Flexible membrane attached to the wire probe. Load is applied along the tear line.

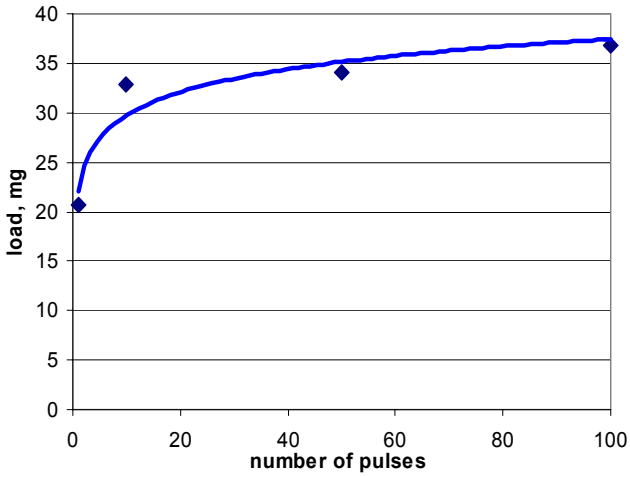

Figure 4. Attachment force increases with number of pulses. Pulse parameters: Amplitude 150V, frequency 1MHz, duration 1ms, probe length 0.2mm, diameter 50 μm.

For quantitative assessment of the adhesion forces we were measuring elastic extension of a thin membrane (CAM) attached to the probe. Elastic forces were calibrated with dynamometer for each specimen. It was found that the elastic force increases linearly with the membrane extension with accuracy better than 6% in all specimens. Probe 0.2mm in length was first brought into contact with tissue using an XYZ translation stage under the surgical microscope. Upon introduction of the adhesion with one (or a few) pulses the probe was lifted vertically (see Figure 3) until a complete detachment from the tissue. The maximal tissue extension was measured and the force was calculated based on the calibration performed with the same sample. To maintain a reproducible adhesion of the probe to tissue the debris were removed from the electrode after each measurement by application of a few pulses in the plasma-mediated regime. To avoid tissue damage during this procedure the probe was withdrawn from tissue.

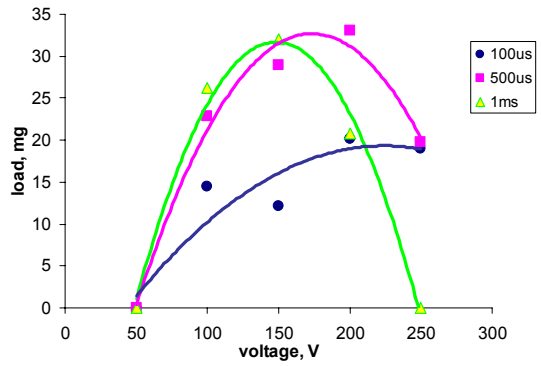

Figure 5. Adhesion strength measured as a function of voltage with three pulse durations: 0.1, 0.5 and 1 ms. Weakening of adhesion with higher voltages is associated with formation of the vapor bubbles rupturing and disconnecting the tissue from the electrode.

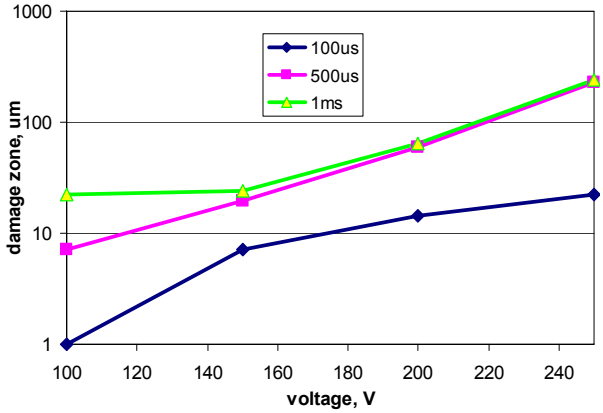

Figure 6. Damage zone as a function of voltage measured with bursts of various durations. Pulse duration less than 0.5ms results in the damage zone of cellular size.

Adhesion force was found to increase with application of additional pulses until it was reaching saturation at the level of about 170 mg/mm (see Figure 4) after a few tens of pulses. No significant widening of the damage zone with number of pulses was observed.

Adhesion force critically depends on amplitude of the pulse: it starts at the threshold of about 50V, reaches its maximal value, and then declines, as shown in Figure 5. Voltage at which the maximal force is achieved depends on pulse duration - it increases with shorter pulses. Weakening of adhesion with higher voltages is associated with formation of the vapor bubbles rupturing and disconnecting the tissue from the electrode.

Damage zone was also found to rapidly increase with voltage, as shown in Figure 6. The optimal settings appear to be 100-150 V with pulse duration 0.1-0.5 ms, when the force is close to its maximum, while the damage zone is still on the order of the single cellular width.

In addition to the measurement of the damage zone in CAM using Propidium Iodide staining the damage was also assessed on corneal samples using histological sectioning and light microscopy. A section of a rabbit cornea with a 25 μm gold wire attached is shown in Figure 7. In a 1 μm-thick histological section the thin gold film folded on itself, thus appearing as a dark half-circle. The wire was attached to the cornea with 10 pulses of 150 V and duration of 150 μs. Corneal tissue appears darker in a close proximity to the point of contact (indicated by an arrow in Figure 7). The size of the damage zone does not exceed 10 μm.

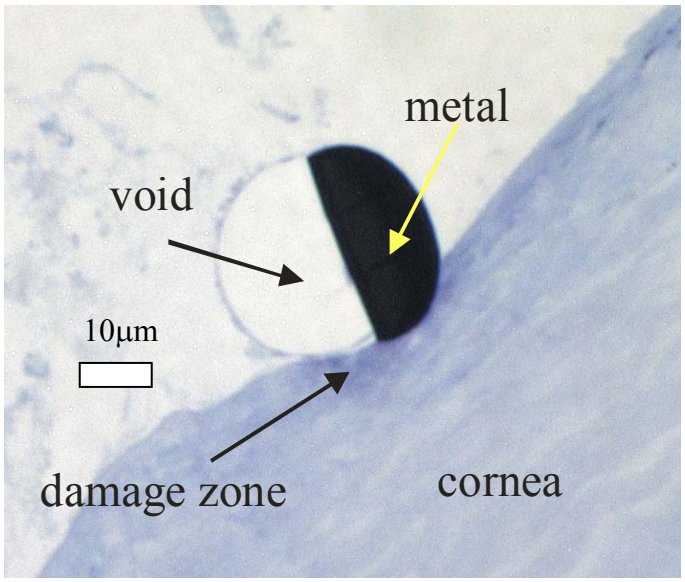

Figure 7. Histological section of the 25 μm gold wire probe attached to a rabbit cornea. 10 pulses of 150 μs were applied at 150 V.

3. DISCUSSION

Adhesion may be established by the physical anchoring or chemical bonding produced by heat or chemical reactions. Physical anchoring may occur with penetration of the denatured tissue into the porous surface of the probe. For this to occur the scale of the surface roughness must be larger than the size of molecules of the adhesive substance. Denaturated beta-sheet of the rabbit cornea consists of the aggregated molecules of a least 300nm in length (see Figure 8). This mechanism cannot be responsible for adhesion to gold wire in our experiments since its surface roughness was below 5nm (measured with the high resolution TEM).

Observation of adhesion to a metal probe that was heated by non-electrical means indicates that adhesion is induced by heating, and does not have to involve high electric fields and electrochemical reactions.

To explore the nature of adhesive forces between the biological tissue and the metal probes we dispersed nanoparticles of led at the electrode-tissue interface during heating with electric pulses. The tissue was then processed for histology and the thin sections photographed with the transmission electron microscope. As one can see in Figure 8 the led nanoparticles can be seen attached to the collagen fibrils in a regular pattern with periodicity of about 60nm (indicated by arrows in Figure 8). This distance correlates very closely with the periodic structure of the collagen fibrils, which is 64nm in length [2]. This observation allows one to assess the density of the bonds which can be created at the metal-tissue interface during adhesion. Based on TEM observations of native collagen in cornea, the distance between fibrils is about 150nm, which results in the bonds density of $1.1 \cdot 10^8$ mm^{-2}. During our force measurements with 50 μm wire, about 200 μm of its length was attached to tissue. The total number of the adhesive bonds in that area will be $1.6 \cdot 10^6$. With the adhesive force of about 0.3 mN measured in that experiment, the strength of each bond is about 200

pN. Assuming the length of the molecular link being 1-2 Å, the energy of such adhesive bonds will be 12-24 kJ/mol. This value is much lower than the energy level of the covalent bonds (60-700 kJ/mol [5]), and fit well within the range of hydrogen bonds, dipole interactions and Van der Waals forces (10 – 40 kJ/mol [3, 4]).

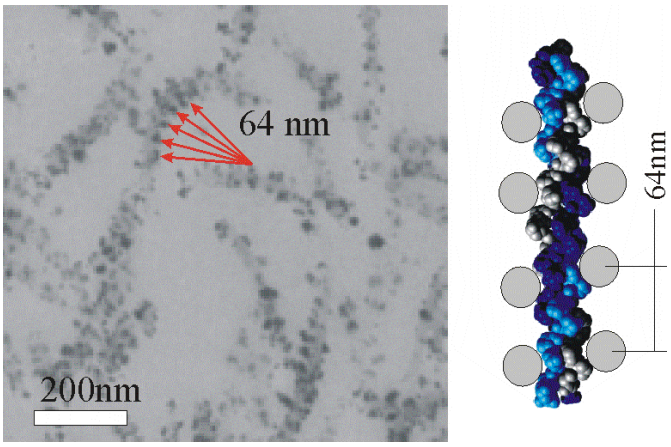

Figure 8. TEM micrograph of the rabbit cornea. Led nanoparticles can be seen attached to the collagen fibrils in a regular pattern with periodicity of about 60nm (indicated by the arrows).

4. CONCLUSIONS

Application of electric pulse (or a few pulses) to the probe brought in contact with tissue induces strong adhesion of biological material to the metal surface, such that the tissue can be lifted and manipulated. The tissue can then be released by application of a pulse producing a transient vapor cavity. To avoid the heat diffusion beyond the single cellular size the pulse duration should not exceed 100 μs. Dominant mechanism of damage appears to be electroporation, and the size of the associated damage zone can be reduced to about 10 μm by alternating the polarity faster than the intracellular relaxation time (i.e. above 1 MHz). Adhesion of metal wires to biological tissue can be performed with collateral damage zone not exceeding one cellular layer (10μm). The electro-adhesive interactions appear to be within the energy range of hydrogen bonds, dipole interactions and Van der Waals forces.

Acknowledgements

Authors would like to thank Roopa Dalal for help with histological processing of the samples.
Funding was provided by the National Eye Institute (NIH) R01 Research grant.

REFERENCES

1. R. C. Lee, M. Capelli-Schellpfeffer, K.M. Kelley, *Electrical Injury*, Annales of the NY Academy of Sciences Vol. 720, NY 1994.
2. Weaver, J.C. and Y.A. Chizmadzhev, *Theory of Electroporation: a Review.* Bioelectrochemistry and Bioenergetics, 1996. **41**(2): p. 135-160
3. A.L. Leninger, *Principles of Biochemistry*, Worth Publishers, Inc., 1985.
4. Sharple, L.H. and Schonhorn, H. Adv. Chem. Ser.1964, 43, 189
5. Kinloch, A.J. , J.Mater. Sci., 1980, 15, 2141
6. J. Malmivio, R.Plonsey, *Bioelectromagnetism*, Oxford Univ. Press, 1995, p. 97
7. Palanker, D., A. Vankov, and J. Miller, *Effect of the probe geometry on dynamics of cavitation.* SPIE, Laser-Tissue Interaction XIII, 2002. **4617**: p. 112-117.