



Introducing DNA Precipitates Adhesion to Normal Eukaryotic Cells Potential Application in Cancer Cells

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Abstract

Introduction: The data herein presented was observed in previous experiments; and now reported in this manuscript. It entails a demonstration of the sticky property of Ethanol precipitated DNA fibers when in contact with organic material such as eukaryotic cells, lipid droplets, as well as inorganic metal iron filings. Adherence is defined as the quality or process of sticking fast to an object or surface. External magnets were used in an attempt to dislodge trapped paramagnetic iron filings adhered to DNA fibers. Microphotographs and video recordings are introduced. Possible application of cellular membrane penetration in Gene Therapy is proposed.

Materials and Methods: From prior published experiment by this author, cheek cells intracellular material, including DNA strands and lipid droplets were ethanol precipitated inside a test tube by following written instructions on DNA precipitation published online by The University of Michigan. The sticky DNA precipitate, resembling white mucous was transferred onto a clean glass slide by a thin wooden stick. Iron filings were then sprinkled onto the precipitate and covered by a similar slide; the assembly was dubbed a sandwich (SDW). A small round magnet was taped to one end of a six-inch long thin wooden stick. Under microscopy control, the magnet was manually moved to dislodge the iron from the DNA fibers. Video Images were obtained via a video-microscope Celestron, LCD model # 44348 Torrance California, USA; and digitally stored in an Apple Mac Book computer with apple Inc. iPhoto 8.1.2. Application.

Results and Discussion: Microphotographs and video recordings obtained show the precipitated DNA strongly adhering to cheek cells, lipid droplets, iron filings and debris floating in the same laminar flow within the SDW. Notably, the smoothness of intracellular lipid droplets surface inhibited *in toto* adhesion to the DNA precipitates. It could be theorized that the engulfed cells metabolism could be altered by transmembrane transfer of exogenous coacervates molecules. The question arises: Can the DNA precipitates that bind to eukaryotic or cancer cells be associated with activation of genes that may lead to mitophagy and autoimmune disease?

Keywords: DNA Precipitates; Coacervates; Fluid Laminar Flow; Cell Membrane Adhesion; Gene Therapy; Cancer Cells Adhesion

Introduction

The data herein presented was observed albeit unpublished by this author in previous published papers [1]. The purpose of his manuscript is to demonstrate the sticky property of DNA precipitate by showing via video recordings of the precipitate attaching to organic (eukaryotic cells) and inorganic material (metal iron filings) and its implications in cell biology. The role of cells "sticki-

ness" in cancer progression and metastases has been stated and demonstrated "Cells' stickiness comes from proteins that sit on their surface. These proteins can make contact with similar proteins on other cells, holding together and keeping our bodies and organs in the right shape, and place" [2]. Basic research performed 58 years ago demonstrated that "It was found that the normal epidermal cells were strongly adhesive but not very sticky, whereas

the cancer cells were extremely sticky but poorly adhesive. Thus, it was concluded that the two properties are different and independent” [3]. Microphotographs and video recordings will demonstrate the DNA precipitates adhering or perhaps sticking with eukaryotic cells membrane and iron particles.

Materials and Methods

From prior published experiment by this author, cheek cells intracellular material, in the form of DNA strands and lipid droplets were precipitated in a test tube by following written instructions on DNA precipitation published online by The University of Michigan (Figure 1) [4]. The DNA precipitate was transferred onto a clean glass slide, iron filings were sprinkled onto the precipitate and covered by a similar slide and dubbed a sandwich (SDW). A small fragment of a rubberized magnetized material was attached to the end of a wooden stick and used to generate motion of the iron particles trapped in the SDW. Images of intracellular DNA precipitate, lipid droplets and moving iron particles were obtained via a video-microscope Celestron, LCD Digital Video Microscope model # 44348 Torrance California, USA; and digitally stored in an Apple Mac Book computer with apple Inc. iPhoto 8.1.2. Application.

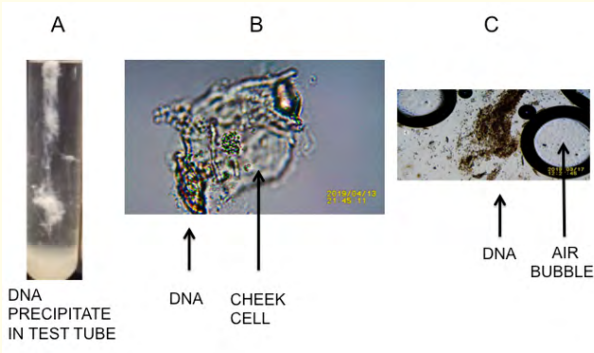


Figure 1: Panel showing. A= Ethanol precipitated DNA fibers in test tube. B= Black arrows pointing covered cheek cell by DNA precipitates. C= Black arrows: Denser clump of DNA precipitate and air bubble (not adhering to DNA).

Results

Microphotographs and video recordings obtained show the precipitated DNA strongly adhering to cheek cells, lipid droplets, iron filings and debris floating in the same fluid laminar flow with-

in the SDW. Notably, the smoothness of the intracellular lipid membranes (droplets) did not allow to be adhered *in toto* by the DNA, although malleability to physical compression was shown (Figure 2, 3 + video), It could be theorized that the engulfed eukaryotic cells metabolism could be altered by the adherence of the sticky precipitate; also allowing for passive or chemical altered liquid DNA molecules (coacervates) penetrating the cell membrane.

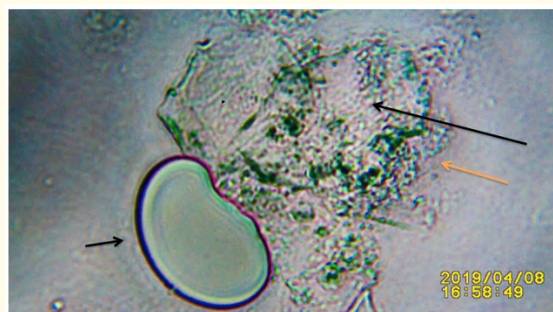


Figure 2: Short black arrow= Lipid droplet mechanically deformed by clump of DNA precipitate. Orange arrow= DNA precipitate. Long black arrow= DNA precipitate covering and adhering to eukaryotic cell (Cheek Cell).

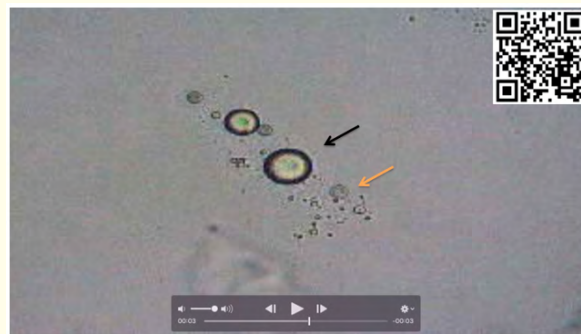


Figure 3: Demonstration of DNA precipitates carrying lipid droplets and moving as a cohesive unit. Black arrow= Lipid droplet. Orange arrow= DNA precipitate. For details please visit <https://youtu.be/uvTmPBSNkrU> or scan QR Code in top right corner.



Figure 3A: DNA precipitate adhered to inorganic material (metal iron filing). Microphotograph of video frame showing Orange arrow= Metal iron filing. Black arrow= DNA precipitate adhering to iron filing- The iron filing moved by a small external magnet. The video shows the strong adhesiveness (sticky) property of the DNA.

For details please link to: <https://youtu.be/9GBIPWNPBwE> Or scan QR Code on top right of image.

Discussion

The findings herein introduced are as result of basic research done with minimal equipment; therefore the conclusions are inferred from graphic static and moving images obtained via an optical video microscope. Inferred conclusions are widely used in science, one example is in where deviation from the norm in motion by celestial bodies ie: stars differs from the actual observations in the majority [5]. In this manuscript the inferred conclusions were rationalized from observing the unexpected interaction of clumps of white sticky DNA precipitates with cheek cells, lipid droplets and iron particles (Figure 4, 4A) [6].

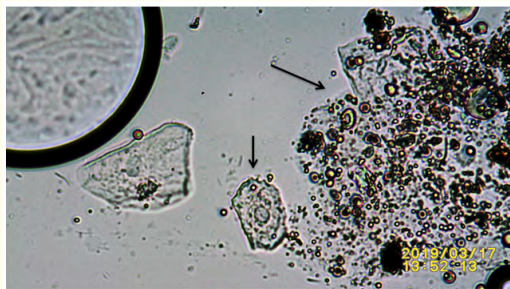


Figure 4: Slide showing sticky property of DNA precipitates adhering/sticking to eukaryotic cells. Left corner= Air bubble. Black Arrows= Sticky DNA precipitate adhering to cheek cells.

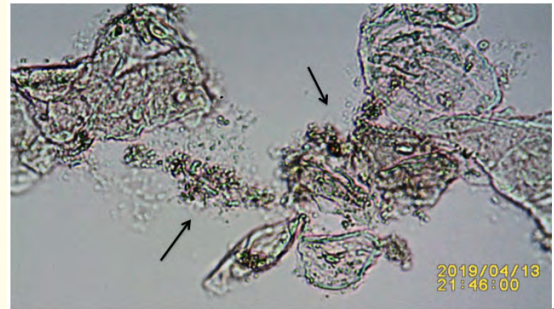


Figure 4A: Sticky DNA precipitate adhering/sticking and covering cheek cells (Eukaryotic Cells). Black arrows= Pointing at DNA precipitate.

Provocative maneuver showing DNA attachment to cells and inorganic material (Iron particles)

The white mucous DNA precipitate (as shown in Figure 1) was extracted via a wooden stick and placed on a clean 1mm thick glass slide. Three drops of the author's saliva were placed on top of the DNA; additionally metal Iron filings were then onto the precipitate/saliva mix and covered by a similar slide; the assembly was dubbed a sandwich (SDW). A small round magnet was taped to one end of a six-inch long thin wooden stick. Under microscopy, control experiments and DNA precipitated samples were subjected to external magnetic forces. A small magnet was manually moved demonstrate the sticky properties of both DNA precipitates and cells in normal fluid saliva samples. Video Images were obtained. Is important to note that the purpose of covering the iron filing/saliva/DNA mix is necessary to maintain the paramagnetic iron filing within the slide assembly. Video recordings are shown as follows: Control (Figure 5 + video recording).

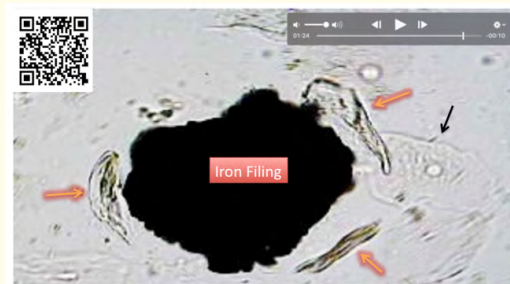


Figure 5: Demonstration of Metal Iron Filing in SDW (25x75x-1mm slide covered by cover glass) causing cells deformations. Iron filing externally controlled by small round magnet attached to wooden stick. Orange arrows= As the iron filing rotates hydrodynamic forces cause cells deformation. Black Arrow= Cheek cell in different vertical plane (laminar flow) unaffected by the forces. For additional details link to : https://youtu.be/wz_Uo_ab10Q or Scan QR Code in upper left corner of image.

Control provocative maneuver using plain saliva on slide the laminar flow specificity

An external magnet was used to move a paramagnetic iron particle placed in a liquid saliva smear, the hydrodynamic shear forces applied are seen to causing eukaryotic cells deformations. This shearing phenomenon is seen in blood smears when prokaryotic red blood cells are rapidly spread onto a glass slide. Figure 5 + video below demonstrate the shear effect; this effect is only seen in the laminar flow (vertical plane) corresponding to the floating iron filing.

Provocative Maneuver Using DNA Precipitate on slide

The figure and video recording below, also shows using an external magnet to move a paramagnetic iron particle placed in the DNA precipitate smear. The hydrodynamic shear forces applied are seen to causing eukaryotic cells deformations. This phenomenon is seen in blood smears when prokaryotic red blood cells are rapidly smeared onto a glass slide. Figure 6 + video below demonstrate the shear effect; this effect is only seen in the iron particle laminar flow present in the liquid DNA precipitate.



Figure 6: DNA precipitate with imbedded externally controlled iron particles showing: Greater hydrodynamic effect on cells and particles in same laminar flow as the iron particles (notice blurred image). Cells in deeper laminar flow not affected by the forces.

For details link to: <https://youtu.be/Yc2ddDJpxxA> Or Scan QR Code in right upper side of image.

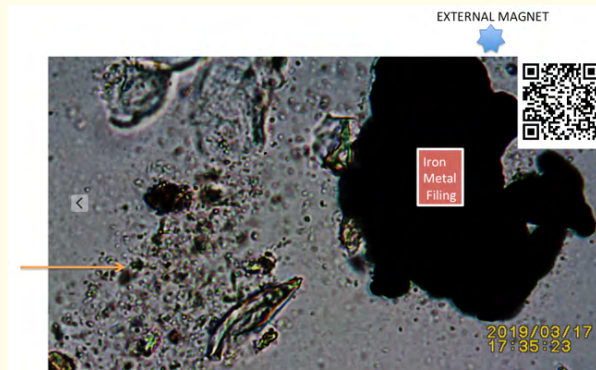


Figure 7: Another precipitated DNA clump- iron particle remotely controlled by external magnet, unable to detach from precipitate. Orange Arrow= DNA precipitate. For motion details Scan QR Code in upper right corner.

Conclusions

New findings

Laminar flow individuality

Experiments of provocative maneuvers demonstrate the sticky property of DNA precipitates are limited to a specific laminar flow. This is noticed in both controls, where plain saliva was used and in repeated experiments where DNA precipitates were tested (Figure 5, 6 + videos).

Lipid droplets and iron particles adhering to DNA precipitates

Intracellular lipid droplets and iron particles were shown adhering to DNA precipitates (Figure 2, 3, 3A + videos).

As a point of interest, research also shows that ethanol precipitation of DNA has two distinct phases: The first is the display of liquid free-floating colloids (coacervates). By the process of hybridization the coacervates merge to form double stranded DNA fibers. Is the first phase that perhaps because the small molecular size that could penetrate the bilayer lipid membrane of eukaryotic cells.

Implications in gene therapy

Several question arise: Once the DNA precipitates stick to the cell. Can DNA coacervates colloids cross the cell membrane?

Can the DNA precipitates that bind to eukaryotic or cancer cells be associated with activation of genes that may lead to mitophagy and autoimmune disease?

Supporting the above queries are published data supporting in the development of Therapeutic Nucleic Acids (TNA) [6-8]. Additionally mitochondrial dysfunction has been linked to chronic diseases [9]; and recognition of Cytoplasmic DNA instigates cellular defense [10].

Would the use of transmembrane gene therapy lead to programmed cell death? [11]. The methodology herein introduced may help researchers to hone in on key points and correct inadvertent errors relevant to anti-cancer research.

Conflict of Interests

None to declare.

Funding Sources

None.

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