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Paper ID #44744

Characterization of Physiochemical Surface Properties in Neural Cell Fates

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Session 44744

A Novel Method to Categorize Live, Dead, and Differentiated Neural Stem Cells Using Hydrophobicity and Electrophoretic Mobility Measurements

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Abstract

The physiochemical membrane properties of cellular electrophoretic mobilities and hydrophobicities of live, dead, and differentiated ReNcell VM Neural Stem Cells were experimentally characterized using zetasizer and goniometer measurements, respectively. The study's comparative analysis of neural stem cells (NSCs) indicates significant differences in contact angle measurements measured in water and diiodomethane (CH_2I_2, DIM). Dead undifferentiated NSCs contact angles decreased 54% compared to live undifferentiated NSCs in water and increased 13.52% in diiodomethane. When live differentiated NSCs were considered, a 44% and 3% decrease in contact angles was quantified in water, and DIM compared to live undifferentiated cells. Further, our results showed that the electrophoretic mobilities of undifferentiated dead and live ReNcell VMs were following the expected rising and then plateauing profile as a function of increased ionic strengths. As the ionic strength increased from 0.01M to 0.15M NaCl, the electrophoretic mobilities percentage differences ranged from 4.64% to 29.01%.

These observations imply that alterations in membrane integrity, composition, and surface protein expression expected between differentiated and undifferentiated cells as well as between live and dead cells impact the surface charge and hydrophobicity of NSCs, serving as potential markers for cell viability and classification. Insights gained from studying hydrophobic properties and surface potential of NSCs can be extended to other cell lines, assisting in improving live classification and predicting a cell’s differentiation potential. Enhanced understanding of cellular physiochemical properties can open new avenues for potential therapeutic medicine.

Introduction

Neural stem cells (NSCs) are an immortalized human neural progenitor cell line that is unique in their capability to differentiate into distinct neural lineages *in vitro* and have shown electrophysiological activity. This makes them pivotal in understanding and treating neurodegenerative diseases [1]. The differentiation pathways of NSCs and responses to stimuli are closely related to their cell membrane surface properties [2] including membrane composition of surface proteins. Changes to composition of cell membrane are expected to result in changes to physiochemical properties including cell surface potential and hydrophobicity [3]; the properties that can be measured experimentally. Balance of interaction forces governed by the cellular charge and hydrophobicity impact many cellular functions including cell adhesion, migration, and interactions with biomaterials. Therefore, categorizing NSCs by their physiochemical properties will lead to new understanding of NSC biology and is necessary for the advancement of therapeutic applications of NSCs [3].

Current popular methods to classify and quantify the viabilities of NSCs such as immunohistochemistry are end-point assays and cannot provide live cell categorization and isolation. Therefore, real-time classification techniques of live NSCs with label-free and non-disruptive methods are needed. The complex physiological dataset including both surface charge and hydrophobicity of NSCs is ideal for the understanding and classification of differentiation potential and developmental stage of cells.

The surface charge of a cell can be indicated by its surface potential. Cellular surface potential can be quantified from electrophoretic mobilities of cells measured by dynamic light scattering (DLS) using a zetasizer in a range of ionic strengths. This technique is a non-invasive and efficient method to measure the mobility of cells under a DC electric field [4]. Previously, it has been shown that zeta potential of plasma membranes is quantifiable and can be used to classify mononuclear cells, erythrocytes, and various cell lines [5]. Cellular zeta potential has also been used as biomarkers for profiling diabetic conditions in red blood cells [6]. These principles can be applied to various types of neural cells as well. For example, since glia do not directly engage in electrical signaling, it is our expectation that they will have different surface potentials compared to their progenitor states and dead cells. The insights gained from quantifying these cell's surface potentials will lead to a better understanding of their role in neuronal regeneration and can lead to methods to predict the differentiation potential of cells.

The surface hydrophobicity of cells can be modeled in terms of the van der Waals Surface tension form contact angle measurements using three different solvents. Briefly, a goniometer is used to measure the contact angle of a drop of solvent on a monolayer of cells. Using this technique, it was reported that polydimethylsiloxane nanocomposites loaded with titanium dioxide nanoparticles repelled SW 480 human colon cancer cells, which could have potential applications in many biomedical devices [7].

This study examines the electrophoretic mobilities and contact angles of mammalian neural RenVM

cells. The data collected will enable us to compute the surface potentials and hydrophobicities of differentiated and undifferentiated as well as live and dead neural RenVM cells. Data generated will then be used as basis for design of tools to detect neurodegenerative diseases, assessing head trauma, and supporting research on neural stem cell regeneration therapy.

Methods

Cell Culture for Goniometer:

To prepare live undifferentiated cells, ReNcell VM Neural Stem Cells were cultured on laminin coated 8-well chamber slides in a DMEM medium supplemented with B-27™ Supplement (50X), 2mg/mL Heparin, 10,000 U/mL, 20ug/mL Penicillin-Streptomycin, 20ug/mL of Human Recombinant EGF Protein, and 10ug/mL of Human Recombinant FGF-basic Protein – known as proliferation medium – to a confluent monolayer. All cells during the culture were maintained at 37°C in a humidified atmosphere of 95% air/5% CO_2. Media was then removed from each chamber and left inside a biological safety cabinet to evaporate for 1 hour at room temperature.

To prepare dead undifferentiated cells, cells were cultured on laminin coated T75 cm^2 tissue culture flasks in the proliferation medium described above and grown to a concentration of 1.0×10^6 cells/mL. Cells were then deprived of oxygen by sealing the flask for 5 days. Dead cells, which detached naturally from the flask, were collected. This suspension was subjected to centrifugation at 300g for 5 minutes. The resulting pellet was resuspended in fresh proliferation medium. The resuspended cells were deposited onto 8-well chamber slides and were then placed in a biological safety cabinet, allowing for a controlled and sterile environment. The cells were left to evaporate over a 24-hour period at room temperature.

To prepare live differentiated cells, cells were seeded at 25,000 cells/well on laminin coated 8-well chamber slides. To initiate differentiation, media was changed to that without growth factors. Following 7-10 days of differentiation, the medium was removed, and cells were left to evaporate at room temperature for 1 hour. All cultures were prepared in triplicates.

Cell Culture for Zetasizer:

The preparation of the live undifferentiated cells consisted of the cells being cultured upon laminin coated T75 cm^2 tissue culture flasks in the proliferation medium to a concentration of 1.0×10^6 cells/mL and then suspended in the proliferation medium. To prevent damage, the live cells were introduced to the sodium chloride buffers' respective concentrations *via* pipette titration and slowly mixed with the pipette before their electrophoretic mobilities are measured.

The dead undifferentiated cells were grown to a concentration of 1.0×10^6 cells/mL and deprived of oxygen for 5 days by a sealed flask. The suspension of the dead cells was collected and subjected to centrifugation at 300g for 5 minutes and the resulting pellet was resuspended in fresh proliferation media. Once complete, sodium chloride concentrations were added at a normal rate to dead cells and vortexed right before being quickly loaded into the zeta sizer to prevent cell settling.

Contact Angle Measurements:
A Goniometer (Kruss Drop Shape Analyzer 100S) was employed to conduct contact angle measurements using polar (water) and nonpolar (diiodomethane) solvents. Chamber slides, each accommodating a monolayer of cells, or approximately 25,000 cells per well, were prepared for experimentation. Each droplet, ~2 μL in volume, was subjected to at least three distinct measurements, with three drops analyzed in each instance [8]. Data points were collected for the contact angle measurements on both sides of the droplet where the drop contour intersects with the surface projection (baseline) [9]. The contact angle measurement process was repeated three times for three separate experiments, resulting in 3 trials. The real-time measurement and recording of the left contact angle (CA(l)), right contact angle (CA(r)), and mean contact angle (CA(m)) of each droplet were facilitated by these executed experiments [9].

After analysis of the cells' contact angles, chamber walls were placed back onto the slide and fresh media was added to rehydrate the cells. Cells were placed in a humidified 37°C incubator for 24 hours and imaged with brightfield microscopy to analyze the morphological changes of these cells when exposed to evaporation and solvents.

Electrophoretic Mobility Measurements:
The ReNcell VMs were diluted to the desired concentration of 2.0×10^5 cells/mL. Once attained, the concentration was separated into five 1 mL samples in Eppendorf Polypropylene PCR Tubes. The samples were centrifuged at 300g for 5 minutes. The pellet was then resuspended in a respective sodium chloride concentration immediately before running the sample through the zetasizer to avoid settling of cells or disruption of the cell membrane. The sodium chloride concentrations included 0.15 M, 0.10 M, 0.075 M, 0.04 M, and 0.01 M. Sodium chloride is used to neutralize the charges of the cells. While cells have an overall net charge, their charge varies at different points on their surfaces. Since the sodium chloride dissociates into anions and cations, it can then bind to the cell different charge domains to neutralize it. By using a range of sodium chloride, different amounts of ions are present in the solution and therefore can affect the electrophoretic mobility.

The zetasizer (Malvern Zeta Sizer Nano Series (Worcestershire, UK))) assessed electrophoretic mobilities of the prepared live and dead undifferentiated ReNcell VMs. This was achieved through the utilization of Dynamic Light Scattering (DLS). The zetasizer system examined particle electrophoretic mobility by employing Electrophoretic Light Scattering (ELS). 30 measurements were performed for 3 runs for each sample and averaged [10]. The average of the three trails of the measured electrophoretic mobility was taken and plotted as a function of the sodium chloride's ionic strength. This process was repeated for both the live and dead undifferentiated cells. The surface potential of the cell can be calculated by modeling the electrophoretic mobilities measured as a function of ionic strength using the soft-particle analysis of the Derjaguin-Landue-Verwey-Overbeek (DLVO) theory of colloidal interactions [11].

Results and Discussion

The experimental results reveal numerical differences in hydrophobicity between live and dead neural stem cells (NSCs). Dead undifferentiated NSCs demonstrated contact angle measurements ranging from 32.95° ± 1.723 (water) to 73.30 ± 4.56° in CH_2I_2, while live undifferentiated NSCs exhibited a different range of 50.816° ± 3.164 (water) to 63.392° ± 4.202 (CH_2I_2). Live differentiated NSCs displayed mean contact angle measurements ranging from 32.455 ± 2.64 (water) to 61.81 ± 1.835 in CH_2I_2 (***Figure 1***). Consistent with literature-defined conditions, hydrophobicity is indicated when the contact angle θ is >90°, while hydrophilicity is indicated when θ is <90° [12]. According to the determined parameters, all experimental NSC samples are found to be hydrophobic. Although the glass chamber slide used for cell culture inherently possesses a hydrophobic surface, there is a noticeable variation in the extent of hydrophobicity among the NSC samples.

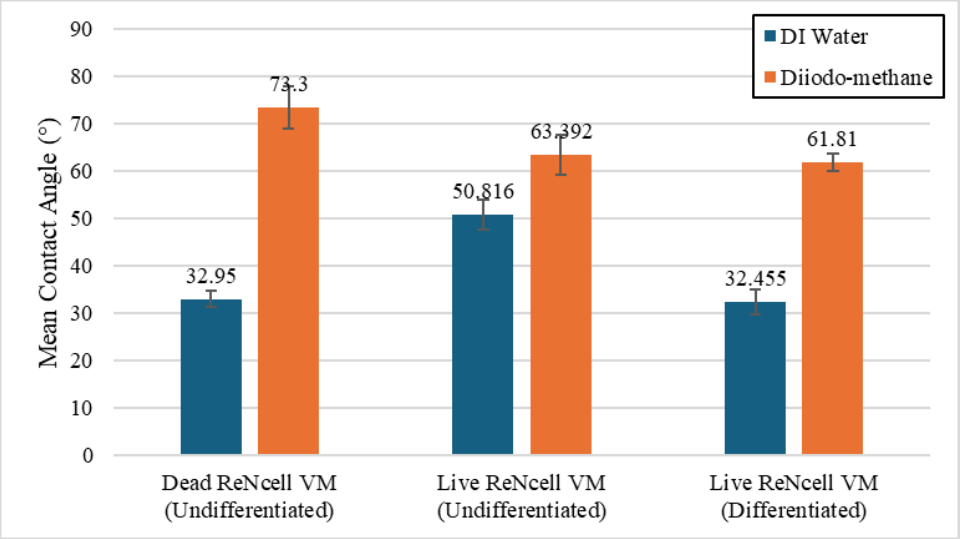

Figure 1. Mean experimental contact angle measurements of ReNcell VM cell experiment.

Comparative analysis of characteristics reveals significant differences in contact angle measurements. Dead undifferentiated NSCs exhibited a mean contact angle value that is 54.22% less hydrophobic than live undifferentiated NSCs (water) and 13.52% more hydrophobic than live undifferentiated NSCs (CH_2I_2). Among the varied live differentiated NSCs, the water solvent exhibited a hydrophobicity value with a 44.01% decrease compared to live undifferentiated, while live differentiated NSCs showed a 2.53% decrease hydrophobicity in CH_2I_2. These specific numerical values provide additional support for the validity of our findings in characterizing NSCs. Brightfield imaging was conducted to further analyze the morphology of NSCs when exposed to these solvents.

Undifferentiated and Dead ReNcells exhibit visible differences when exposed to water and diiodo-methane droplets. Dead ReNcell VM stem cells appear spherical, lacking visible axons (***Figure 2A***). Applying water alters the cells to a lighter appearance (***Figure 2B***), while diiodo-methane is not absorbed, remaining on the surface shown by the dark area on the left (***Figure 2C***).

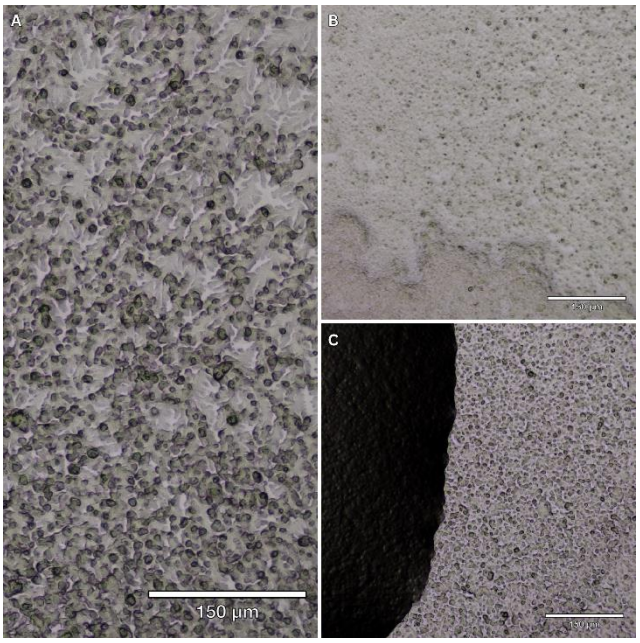

Figure 2 A-C. Brightfield images of dead RenVM progenitor cells. A) Evaporated dead ReNcell VM cells B) Dead ReNcell VM cells after exposure to H_2O droplet C) ReNcell VM cells after exposure to CH_2I_2 droplet. Scale bars represent 150µm.

Live, undifferentiated ReNcells display axonal growth (***Figure 3A***). Evaporation for one hour alters the morphology of the cells, elongating and appearing more densely packed, likely due to the extreme environmental stress from dehydration (***Figure 3B***). It is possible that, due to the dehydration of NSC, alterations of the cell's surface properties took place and, in return, affected the overall hydrophobicity of the cells.

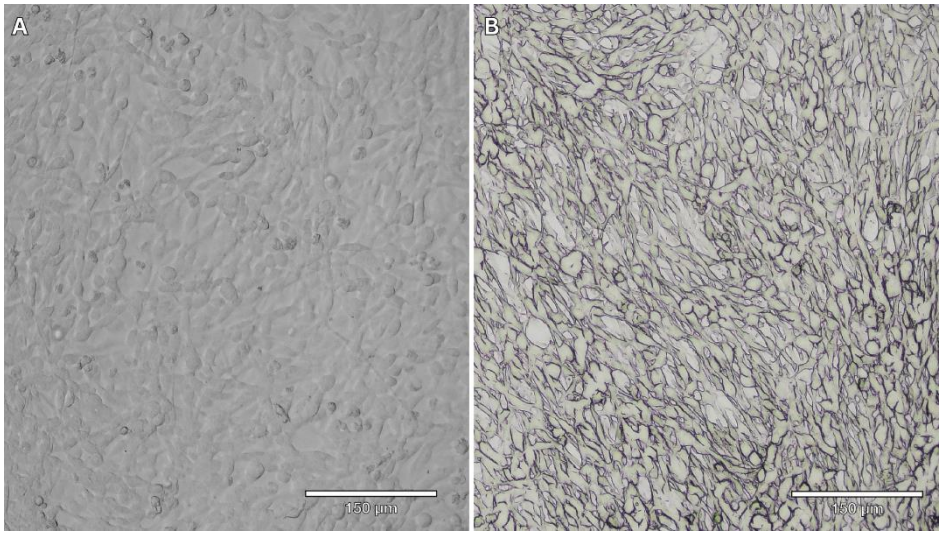

Figure 3 A-B. Brightfield images of live progenitor cells. A) Live progenitor cells B) Live cells after 1hr evaporation. Scale bars represent 150µm.

The introduction of solvents to live, evaporated stem cells reveal noticeable variations in absorption by the cells and induces changes in cell morphology and density. A water droplet creates a distinct boundary between exposed and non-exposed cells, causing cells to resemble those in proliferation media (***Figure 4A***). This results in some detachment and removal of cells, with partial recovery observed upon adding media to the cells. There is still a distinct line where cell detachment took place (***Figure 4B***). When a droplet of diiodo-methane is added, the substance does not absorb into the cells but sits on the surface (***Figure 4C***). Upon adding media back to the cells, a smaller amount of recovery is evident compared to cells exposed to water droplets, possibly indicating cytotoxicity (***Figure 4D***).

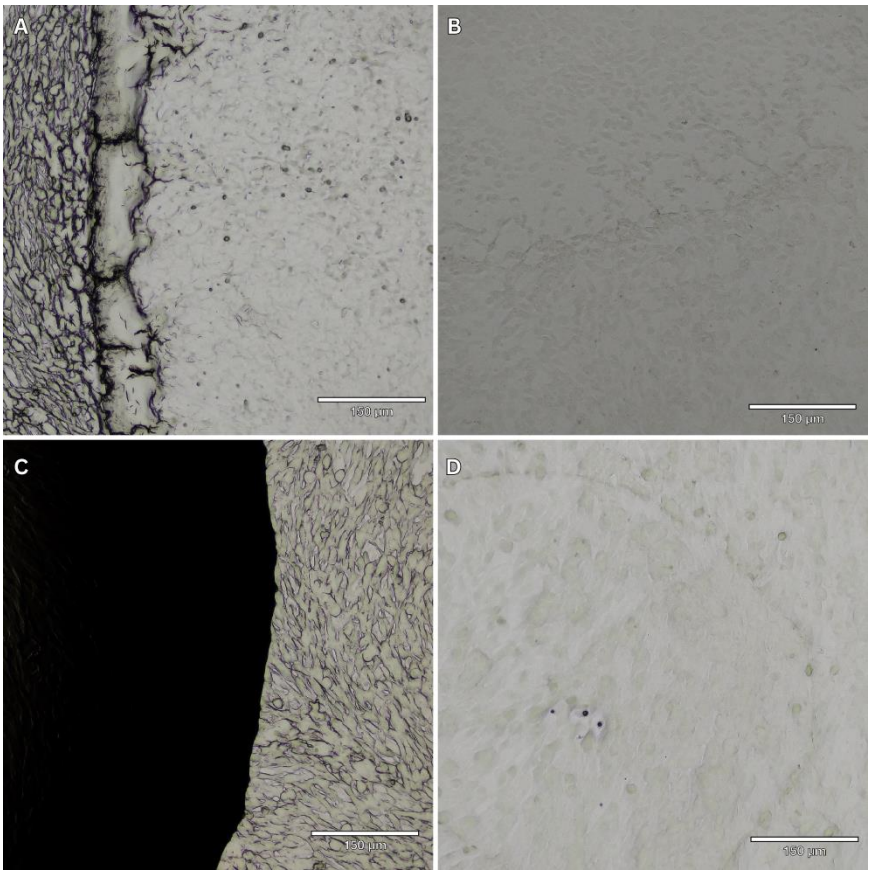

Figure 4 A-D. Brightfield images of live progenitor cells after exposure to liquids and rehydrated in proliferation media. A) Live ReNcell VM cells exposed to H_2O droplet B) Rehydrated H_2O exposed cells. C) Live ReNcell VM cells exposed to CH_2I_2 droplet D) Rehydrated CH_2I_2 exposed cells. Scale bar represents 150µm.

As RenVM cells undergo generalized differentiation, the cell surface becomes heterogeneous likely due to the altering cell morphologies, surface proteins, and ion channels. Hydrophobicity values exhibit a wider range, while still maintaining values closer to live undifferentiated cells than dead cells (***Figure 1***). The addition of media after the experiment appears to have kept the cells viable based on the brightfield images. However, distinct remains of the drop site are visible, indicating alterations to

these cells and a potential cause of cell death due to osmotic shock or cytotoxic shock. Performing fluorescence imaging would further validate this observation and provide additional insights into the effects of conducting this experiment on live cells.

The electrophoretic mobility measurements for dead ReNcells are greater than those for live progenitor cells across all NaCl concentrations. The standard error for each datapoint reflects the heterogeneity of cells (***Figure 5)***. Dead ReNcells are spherical, displaying little heterogeneity in morphology. Since these NSCs are dead before the buffer washing process, surface proteins and ion channels are similar across these dead cells which is reflected by the smaller standard error bar. As for live progenitor cells, they exhibit a higher standard error due to the environmental stress they experience during buffer washes with NaCl concentrations.

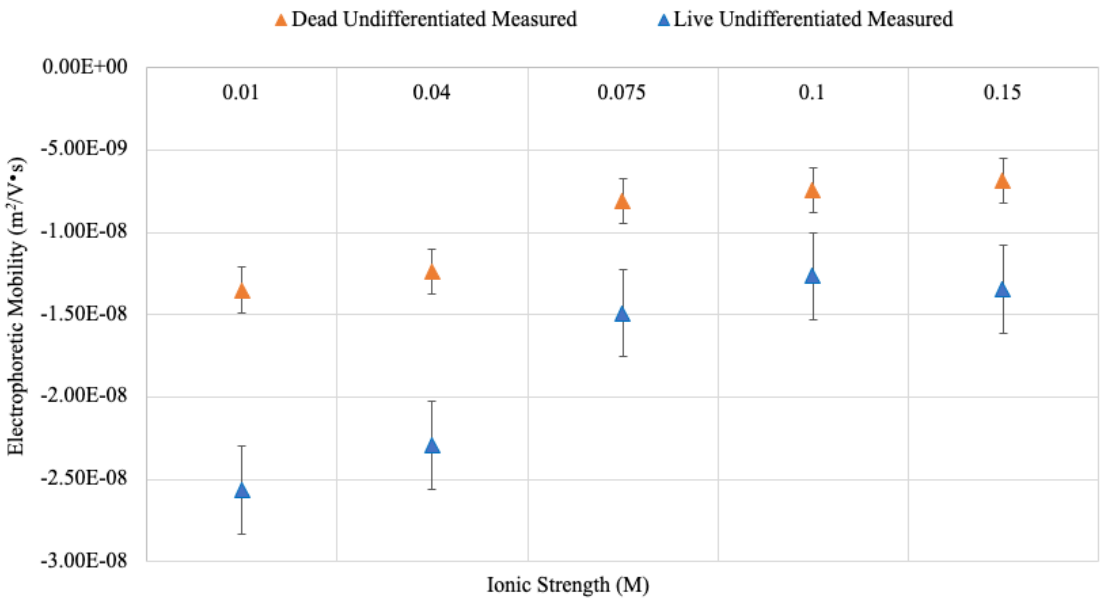

Figure 5. Electrophoretic mobility measurements of Live Undifferentiated ReNcell VMs and Dead Undifferentiated ReNcell VMs as a function of added salt concentration. The symbols represent the average of three experimental measurements.

To further examine the morphological changes in cells after being placed in NaCl concentrations, brightfield imaging was conducted (***Figure 6***). The lower cell density and change in cell size and shape between adhered stem cells and cells in a 0.04 NaCl concentration shows that performing buffer washes and resuspending live stem cells in NaCl concentrations alter the morphology and surface properties of cells. Some cells also experienced osmotic shock from this process. Future experiments utilizing streaming potential or microelectrophoresis could provide a better option to gather data on these cells, causing less stress response and minimizing heterogeneity amongst cells. Quantification

of the electrophoretic mobilities of differentiated ReNcells will provide a more complete dataset to further classify these cells.

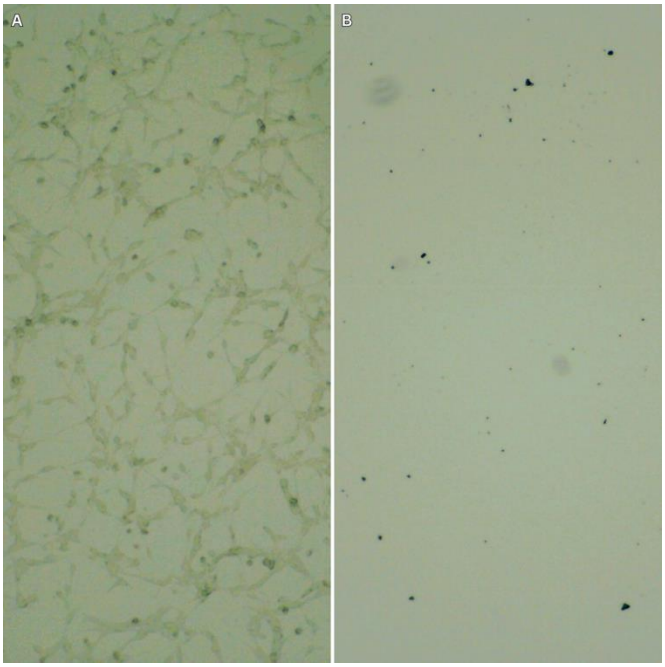

Figure 6 A-B. A) Live undifferentiated cells after proliferation B) ReNcell VMs after buffer wash in 0.04 NaCl solution

Conclusions

Quantifying live, dead, and differentiated neural stem cells using colloidal techniques of goniometry and zetasizer measurements showcase differences in hydrophobicity and electrophoretic mobility between cells. These values imply that the differences between membrane composition, surface protein expression, and membrane integrity affect the interfacial forces on the cell membrane. The process of using the goniometer and zetasizer likely influences these surface properties due to exposure of different stresses. The multidimensional dataset makes it possible to categorize neural stem cells based on their surface physiochemical properties in a label-free manner using various advanced machine learning algorithms including Random Forests and Support Vector Machines.

Future experiments will focus on the imaging of differentiated cells using fluorescence microscopy and enhancements in the hydrophobicity and zeta potential study. Atomic Force Microscopy (AFM) will be implemented to gain further insight into the mechanical properties of the cells' surface. To minimize stress responses to cells, aqueous based solvents such as aqueous two phase systems (ATPS) will be used to measure the contact angle formed by the liquid meniscus and provide information for development of cell separation techniques [13]. Further research will include using these techniques on a variety of cell lines.

Acknowledgements

We acknowledge the support from the UTSA GREAT seed funding and the UTSA TechPoP fund to G.S. and N.I.A., and UTSA's startup funding to G.S. The authors extend their gratitude to Joel Salinas, for insightful discussion throughout the course of this work, and to Sean Tritley for assistance with the ReNcell VM culturing.

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Isabella Treviño is currently pursuing a major in Biomedical Engineering, with a concentration in Biomaterials and Tissue Engineering, along with a minor in Criminology and Criminal Justice Law at the University of Texas at San Antonio. Drawing on a wealth of knowledge gained from both industry and academia, she actively participated in the development of various medical devices, contributing to research in product development and manufacturing. Isabella's interest in the medical device field originates from her role as a medical assistant, where she observed the transformative impact of medical technologies on patient well-being. This firsthand encounter acts as a motivating force, inspiring her active involvement in the advancement of innovative healthcare solutions.

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Brooke McGill is pursuing a Bachelor of Science, directed in Chemical Engineering, at the University of Texas at San Antonio. She wishes to specialize in biomedical and medical science within the Chemical Engineering field, which is why she takes her job as Dr. Nehal Abu-Lail's Research Assistant, very seriously. Passionate about the cause, she observes the cellular surface protein makeup and physiochemical properties of chondrocytes and other cells alike so her family members with Arthritis may be at ease one day.

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