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Research Article

High Resolution Helium Ion Microscopy of 3T3-L1 Adipocytes

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Abstract

Structural changes at the plasma membrane of adipocytes are critical to proper insulin stimulated glucose uptake and thus glucose homeostasis. Insulin stimulates the fusion of insulin responsive glucose transporters (GLUT4) containing vesicles at the plasma membrane in adipocytes. Previous studies have focused on the molecular and cellular events required for GLUT4 vesicle fusion, however the structural changes occurring at the plasma membrane are less well characterized. In order to investigate the changes that occur at the plasma membrane upon insulin stimulation we first had to visualize the plasma membrane surface at the nanometer scale. Helium ion microscopy (HIM) is a new technology that allows for high resolution visualization of nanostructures without the need for sample coating. In order to visualize adipocytes plasma membrane surfaces, we first had to develop a protocol for sample preparation to maintain the detail and contrast of nanostructures for imaging by HIM. Our findings show that our sample preparation protocol allows for the visualization of plasma membrane textures and nanostructures. We further show nano structural details of the inner surface of adipocyte plasma membranes by imaging plasma membrane sheets. Finally we examined 3T3-L1 preadipocytes at various stages of differentiation to visualize the structural changes occurring in the conversion of fibroblastic preadipocytes into mature adipocytes. Our results show the unique membrane textures and nanostructures present on various cell types. Taken together, we established a protocol for sample preparation of preadipocytes and adipocyte for the HIM that allows for high resolution visualization of nanostructures on both the cytoplasmic and exofacial surfaces of the plasma membrane.

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INTRODUCTION

A critical component of whole body glucose homeostasis is insulin stimulated glucose uptake into adipose tissue and skeletal muscle [1]. Insulin-stimulated glucose uptake requires the coordinated activation of several signaling pathways to stimulate the translocation, docking and fusion of insulin responsive glucose transporters (GLUT4)-containing vesicles to the plasma membranes of adipocytes and skeletal muscle cells [2]. While many of the cellular and molecular aspects of GLUT4 vesicle fusion have been elucidated the structural changes that occur at the plasma membrane upon GLUT4 vesicle fusion are less well characterized. This is in part due to the inability to visualize high resolution images of nanostructures present on the plasma membrane surface. With the recent development of high resolution helium ion microscopy, (HIM), the ultra structural changes occurring at the plasma membrane of adipocytes seem ideal for investigation by this new technology [3-5].

In order to overcome some of the limitations of other electron microscopy techniques, HIM uses a single ion focused beam, longer depth of field and charge neutralization at the surface [3-5]. These features allow for high resolution imaging at the nanometer scale of uncoated samples. Previous methods required metal coating of samples which obscured the detail of surfaces. HIM does not require sample coating and thus has the potential to allow for the visualization of nanostructures that have not been disturbed [3-5]. To visualize the ultra structural changes that occur at the plasma membrane upon insulin stimulation of adipocytes we first had to determine the capabilities of HIM to obtain high resolution images of nanostructures on adipocyte membranes. We also had to develop a protocol for optimizing

sample preparation of biological samples for the HIM. HIM has primarily been used to image non-biological samples at the nanometer scale. More recently, HIM has been used to visualize biological samples such as insect cuticles and collagen [6-9]. The images obtained have significantly greater resolution of nanostructures than that seen by SEM.

In the present study we established a sample preparation protocol for 3T3-l1 adipocyte whole cells as well as plasma membrane sheets for high resolution helium ion microscopy. We also visualized 3T3-L1 pre adipocytes during differentiation into adipocytes. Our studies were focused on maintaining structural integrity of the cell membrane by altering substrates, fixatives and permeabilization parameters. Thus our studies are the first to examine both the cytoplasmic as well as the exofacialsurfaces of adipocyte plasma membranes at high resolution using helium ion microscopy.

MATERIAL AND METHODS

Materials

Tissue culture reagents were purchased from Gibco (Grand Island, NY). Dexamethasone, 3-isobytyl-1-methyl-xanthine and poly-d-lysine were obtained from Sigma (St. Louis, MO).Insulin was from Roche Diagnostics Corporation (Indianapolis, IN).

Cell culture

3T3-L1 preadipocytes were cultured in 10% Calf Serum (CS)/ Dulbecco's Modified Eagle Media (DMEM) till confluent. Two day post confluent preadipocytes were induced to differentiate in 10% Fetal Bovine Serum (FBS)/DMEM with 0.52 mM 3-isobutyl 1-methyl-xanthine (MIX), 1.7 µM insulin, and 1 µM dexamethasone (DEX) for 2 days. Media was then changed to 10%FBS/DMEM plus 0.425 µM insulin. The media was replaced with 10%FBS/DMEM every other day [10].

Substrate and sample preparation optimization

3T3-L1 pre adipocytes were cultured on glass coverslipsor gold coated coverslips. Coverslips were gold sputter coated for 60-90 seconds at 10 mA. Pre adipocytes were cultured and differentiated on either uncoated coverslips or gold coatedcoverslips. Day 7 adipocytes were washed with 0.1M cacodylate buffer, pH 7.3, and fixed with 2.5% glutaraldehyde, 2% formaldehyde in 0.1M cacodylate buffer, pH 7.3 for either 30 minutes or 2 hours. Cells were then washed with deionized water. Cells were permeabilized with 0.1% triton X-100 for 15 minutes. Cells were washed with deionized water and then subjected to a secondary fixation of 4% osmium tetroxide (OsO_4) for 30 minutes or 1 hour. Cells were washed in deionized water and then dehydrated using acetonitrile, at the following concentrations: 25%, 50% 75%, 95%, and 100% twice at 5 minutes each with the second 100% remaining on the sample overnight to evaporate. Cells were then imaged by helium ion microscopy.

HIM

A Zeiss Orion plus Helium Ion Microscope (Carl Zeiss, Oberkochen, Germany) was used to visualize high resolution images of 3T3-L1 preadipocytes and adipocytes. The working distance for the HIM was 7-10 mm and the accelerating voltage was 30-35 kV with a blanker current ranging from 0.4 to 2.8 pA. During imaging the surface charging was neutralized by use of a low energy electron flood gun that was applied to specific regions of the sample.

Plasma lawn assay for HIM: 3T3-L1 adipocytes were grown on gold coatedcoverslips. Cells were washed with 0.1M cacodylate buffer, pH 7.3. Cells were fixed in 4% formaldehyde, 2% glutaraldehyde in deionized water for 45 minutes. We then rinsed cells in deionized water. Cells were incubated in poly-d-lysine at 0.5 mg/mL for 1 minute. The cells were rinsed three times in hypotonic buffer, containing 23 mMKCl, 10 mMHepes, 2mM $MgCl_2$, and 1mM EDTA, pH 7.5. Next, cells were incubated in sonication buffer (70 mMKCl, 30 mMHepes, 6 mM $MgCl_2$, 3 mM EGTA, 1 mMdithrothreitol, 0.1 mMphenylmethylsulphonyl fluoride, pH 7.5) [11]. The cells were sonicatedfor 5 seconds and then washed with deionized water. Next, a dehydration series was performed with acetonitrile at 25%, 50%, 75%, 95%, 100% (5 minutes each) and a final 100% overnight. The plasma lawns were then visualized using helium ion microscopy.

HIM sample preparation for pre adipocytes during differentiation: Pre adipocyteswere grown to confluence and then maintained for two days. Cells were then induced to differentiate as described previously. Pre adipocytes (day 0) and day 2, 4, and 7 adipocytes were washed with 0.1M cacodylate buffer, pH 7.3. Then the cells were fixed overnight in 2.5% glutaraldehyde, 2% formaldehyde in 0.1M cacodylate buffer at pH 7.3. The coverslips were washed with deionized water. Cells were permeabilizedwith 0.1% Triton x-100 for 15 minutes, followed by a wash with deionized water. The cells were then secondarily fixed with 4% osmium tetroxide, (OsO_4) for 1 hour. Finally, cells were washed with deionized water, followed by a dehydration series in acetonitrile: 25%, 50%, 75%, 95%, 100% (5 minutes each) and in a final 100% solution overnight.

RESULTS AND DISCUSSION

Optimization of substrate for adipocytes for HIM

In order to examine the nanostructures on the plasma membrane surface of adipocytes, we first had to be able to determine a suitable substrate for the cells to be cultured on and on which optimal differentiation would occur. Previous analysis of adipocytes by SEM used glass coverslips for the culturing and differentiation of 3T3-L1 adipocytes [12,13]. Our initial attempts at examining adipocytes on glass coverslips by helium ion microscopy showed low resolution and contrast (Figure 1A and B). To achieve greater resolution we coated the coverslips with gold particles in order to reduce the effects of secondary electrons. The thin layer of gold coating on the coverslips did not alter the proliferation or differentiation properties of the adipocytes. As shown in (Figure 1C and D) the resolution of the adipocytes was significantly improved. Individual cells as well as ultra structural details were visualized by helium ion microscopy. In addition, the contrast was also significantly improved. While previous studies using SEM used coated samples which obscured the nanostructures which resulted in reduced resolution, HIMallows for visualization of uncoated adipocytes which provides greater resolution of the native surface. These studies demonstrate that gold coating the coverslips, not the sample allowed for greater

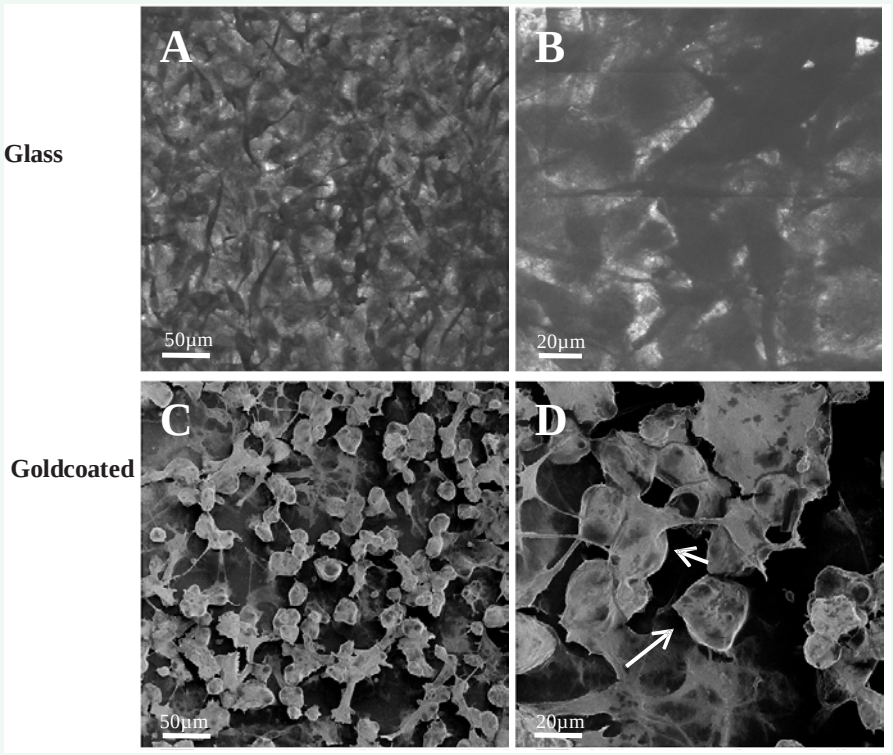

Figure 1 Optimization of substrate surface for 3T3-L1 adipocyte visualization by helium ion microscopy. 3T3-L1 pre adipocytes were plated on (A, B) glass cover slips or (C, D) gold-coated glass cover slips. Two day post confluent pre adipocytes were induced to differentiate as described in the Materials and Methods section. Day 73T3-L1 adipocytes were visualized by HIM. (A and B),Cells grown and differentiated on glass were difficult to image due to the surface charging; (C and D), however, cells grown and differentiated on the gold sputter coated glass cover slips can be clearly seen. Note the number of round cells indicating fully differentiated adipocytes (arrows).

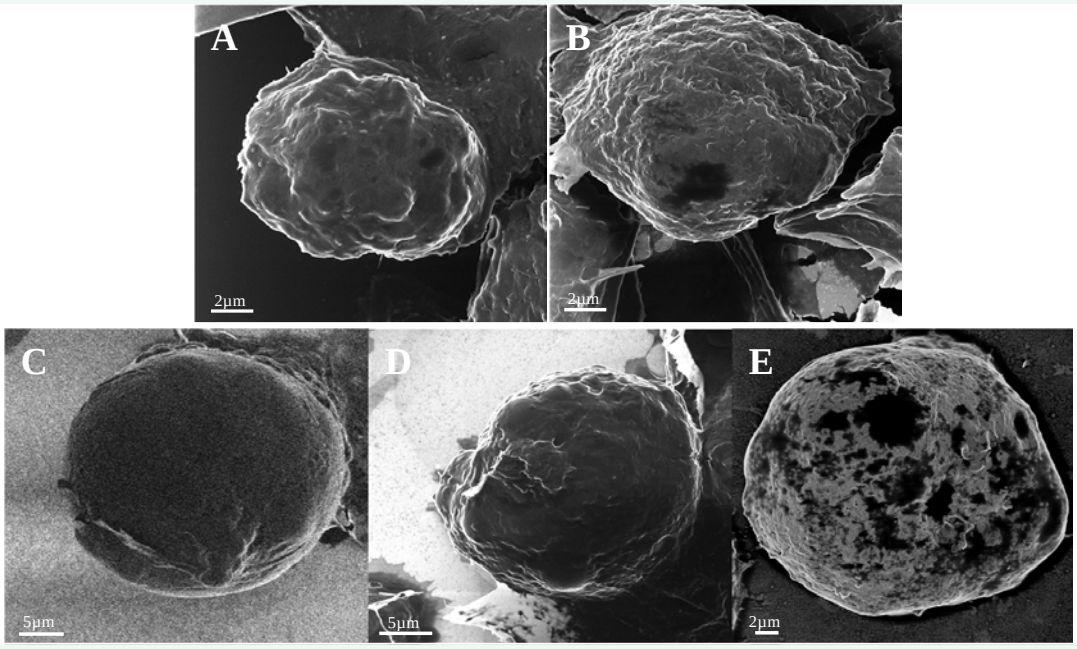

Figure 2 Optimization of biological sample preparation for helium ion microscopy. 3T3-L1 preadipocytes were plated on gold-coated glass cover slips and induced to differentiate. Day 7 adipocytes were fixed with formaldehyde/glutaraldehyde for (A) 30 minutes or (B) overnight or fixed with formaldehyde/glutaraldehyde and OsO4 for (C) 30 minutes or (D) 1 hour or (E) fixed with formaldehyde/glutaraldehyde/OsO_4 and treated with Triton-X 100 for 30 minutes. Adipocytes were visualized by HIM. Fixation without OsO4 resulted in a wrinkled looking cell (A and B). The addition of OsO4 improved the preservation of cellular morphology presumably via oxidation and cross linking of the lipids within the lipid storage droplets.

resolution and contrast of adipocytes by HIM but did not alter the properties of adipocyte differentiation.

Optimization of biological sample preparation for HIM

In order to obtain high resolution images proper sample preparation is critical. Adipocytes have a unique architecture in that unlike most cells, adipocytes have very little cytoplasm [14]. Adipocytes generally can have many lipid droplets of varying sizes as well as one large unilocular lipid droplet that can occupy most of the cytoplasmic area. Thus sample preparation for adipocytes poses unique challenges. We initiated our investigation with a standard electron microscopy cross-linking fixative mixture that contained both glutaraldehyde and formaldehyde. This has been shown to work on many cell types: formaldehyde based fixation quickly permeates (1mm/minute) and stabilizes protein structures but lacks the cross-linking potential demonstrated by glutaraldehyde fixation which has a slowerpermeation of the sample. Adipocytes were fixed with 2.5% glutaraldehyde, 2% formaldehyde in 0.1M cacodylate buffer, pH 7.3 for either 30 minutes or overnight (Figure 2A and B). We incubated the samples in our fixation solution for two times: a short 30 minute incubation which has shown to work efficiently in other cells and a longer over night incubation. We also post-fixed the cells in osmium tetroxide (as well as without). Since adipocytes are lipid-laden cells, the idea was to test whether this step was necessary not only to preserve the membrane and cellular lipid structure, but also provided an electron dense labeling of these structures for imaging as well.

Our findings show that overnight fixation of adipocytes with both the formaldehyde/glutaraldehyde fixative resulted in higher resolution of nanostructures and greater detail of membrane surface textures than cells fixed for 30 minutes.The staining of the large lipid volumes provides contrast to the cell surface as well as preserving the shape of the adipocytes, which are essentially large reservoirs of lipid. The cells without osmium tetroxide appeared collapsed and lacked any structure.We first fixed adipocytes with formaldehyde and glutaraldehyde overnight and then treated cells with OsO_4 for either 30 minutes or 1 hour (Figure 2C and D). Our results demonstrate that greater contrast and visualization was achieved with an OsO_4postfixationtreatment for 1 hour, which probably is a result of the large volume of lipid present in adipocytes.

Next we tested whether we could determine qualitative changes in the plasma membrane using HIM. To do this we permeabilized the plasma membrane with a strong detergent, Triton-X 100 that would remove some or all of the lipid bilayer. One goal wastodetermine whether this procedure would allow for visualization of structures (cytoskeletal and/or transport) just beneath the surface of the plasma membrane. As shown in Figure 2E, Triton-X 100 treated cells had regions that appeared to be differently electron dense, perhaps revealing areas on the cell surface where the plasma membranehad been removed. The OsO_4staining of lipids provided greater contrast for visualization of the plasma membrane. Collectively, these studies have established a protocol for preparing adipocytes for HIM. These protocols will allow for the investigation of ultra structural changes at the plasma membrane upon insulin stimulated GLUT4 vesicle fusion with the plasma membrane in future studies.

High resolution imaging of adipocyte plasma membranes by HIM

Insulin stimulation of adipocytes results in activation of an array of coordinated cellular signaling pathways that result in the translocation and fusion of GLUT4 containing vesicles to the plasma membranes. The structural changes that occur at the membrane that are associated with GLUT4 fusion are less well characterized. In order to elucidate the changes occurring at the plasma membrane upon GLUT4 vesicle fusion we first had to determine if high resolution images of nanostructures could be visualized by our sample preparation for HIM.Therefore,day 7 adipocytes were prepared as described above and visualized by HIM. Our results demonstrate that uncoated adipocytes can be visualized at high resolution at both low and high magnification (Figure 3A-F). Our protocol for adipocyte sample preparation allowed for the visualization of detailed plasma membrane textures (Figure 3F) and nano protrusions (Figure 3E and F) on adipocytes by HIM. While similar structures have been visualized by SEM the images did not have the resolution of the HIM at high magnification. The Triton-X 100 treatment provided significant contrast of the plasma membrane from the underlying lipid droplets. Proteins present on the nanoprotrusion and the pattern of protein localization can now be determined by the use of gold-labeled antibodies. Previous studies in adipocytes as well as other cell types have used gold-labeled antibodies to identify specific proteins by both SEM and HIM [15,16].

High resolution visualization of the cytoplasmic surface of adipocyte plasma membranes

In adipocytes, insulin stimulates the translocation of cytoplasmic GLUT4 containing vesicles tothe plasma membrane to facilitate glucose uptake. Proper insertion of GLUT4 vesicles is critical for glucose uptake. Previous studies have shown that protein associated with the cytoplasmic face of the plasma membrane in adipocytes can be more clearly identified by examining plasma membrane lawns [17]. Our studies established a protocol for the preparation of plasma membrane lawns for HIM. Our results show detailed nanostructures present on both the cytoplasmic face (Figure 4D) as well as the exofacial surface(Figure 4E and F) of the plasma membranes of adipocytes by HIM.The nanostructures imaged on the cytoplasmic surface are similar to those identified by SEM and provide further support that our sample preparation and visualization are identifying structures previously imaged. In SEM studies these structures were present near gold-labeled GLUT4 [18]. The nanostructures that project from the surface outward maybe structures involved in cell attachments to the substrate. Antibody labeling of GLUT4 and cytoskeletal proteins such as actin and myosin are required to determine the role these structures play in the topology of the plasma membrane. Our previous studies using confocal microscopy have shown that GLUT4, F-actin and myosin IIA are present on plasma lawns [19,20]. The interactions between there protein complexes may elucidate the structural changes visualized by HIM.

HIM high resolution images during 3T3-L1 preadipocyte differentiation

Finally we wanted to determine whether our adipocyte sample preparation would work for other cell types. We choose

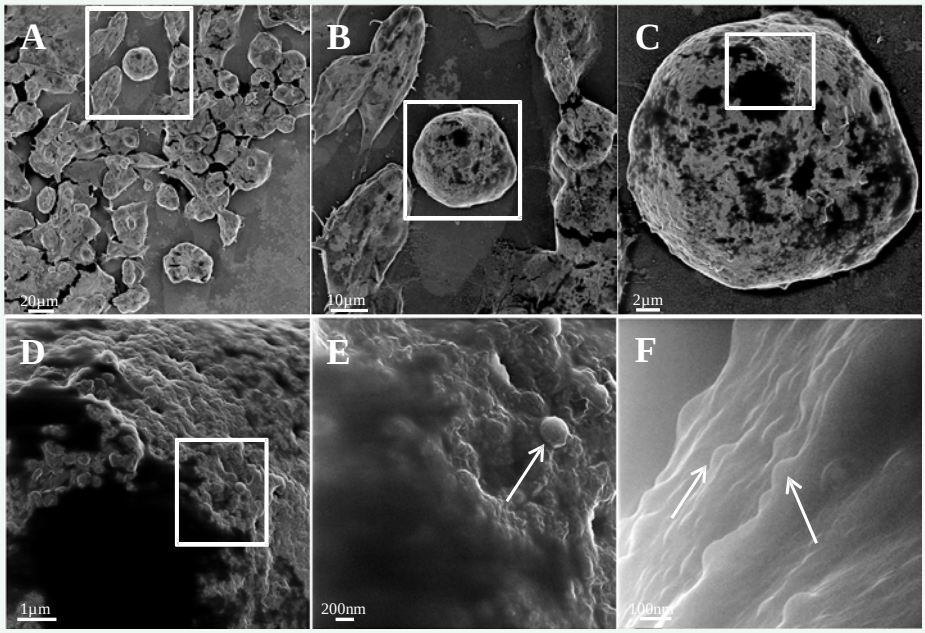

Figure 3 Visualization of the exofacial surface of the plasma membrane of adipocytes. Day 7 3T3-L1 adipocytes were fixed and treated with Triton-X 100 and the plasma membrane surface was visualized by HIM. The images are shown in order of increasing magnification A-F and demonstrate HIM depth of field, as the entire field of view remains in sharp focus regardless of the magnification. The images explore the surface of a differentiated adipocyte, shown in box in A at low magnification (457X) and in a slightly higher magnification B (1447X). Note the round shape of the differentiated adipocyte is preserved among the more trapezoidal shaped undifferentiated fibroblastic pre adipocytes. These cells have been treated with a detergent to extract some of the lipid from the plasma membrane. C) The surface appears mottled from this extraction revealing underlying cytoplasmic lipid stores which have been heavily oxidized by the OsO4 treatment. At higher magnifications D) 14,000X, E) 38,000X, and F) 114,000X, more detailed features are clearly observed. Numerous round vesicular structures can be seen (E and F, arrows). While the composition of these structures is unknown, the presence, size, and stability suggest that these may be multimeric protein aggregations, nascent vesicles beneath the plasma membrane, or lipid organizations such as lipid rafts.

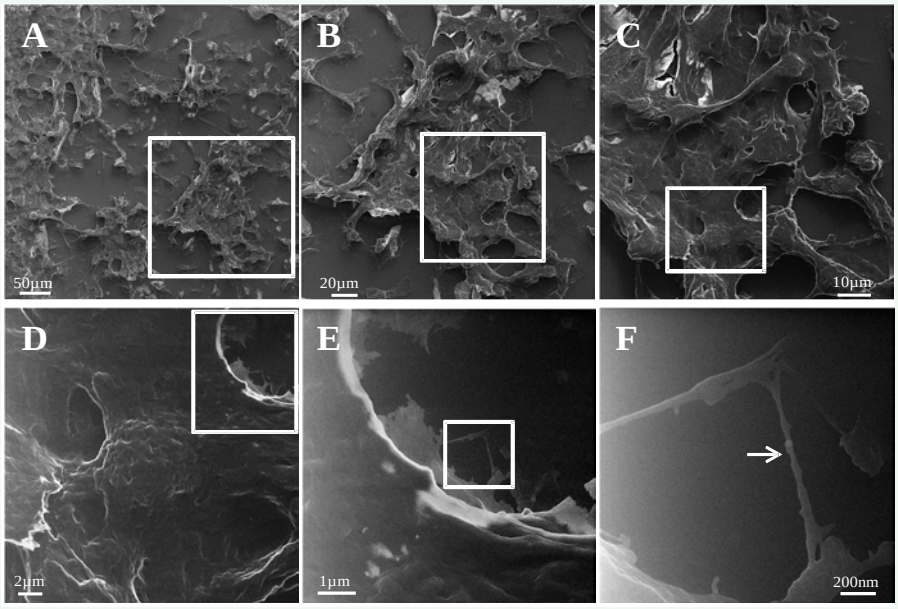

Figure 4 Visualization of the cytoplasmic surface of adipocyte plasma membranes. 3T3-L1 adipocytes (Day 7) were differentiated and plasma membrane lawns were prepared as described in the Materials and Methods. Plasma lawns were fixed and then the cytoplasmic surfaces of the plasma membranes were visualized at high resolution by HIM. The images are shown in order of increasing magnification A-F. (A) Low magnification (457X) and in a slightly higher magnification B (1447X), show the intracellular connections to the surface and the outline of cells can be clearly distinguished (arrow). (F) At the highest magnification, 63,000X, individual fibers that are between 20 and 30 nm wide can be clearly seen on the surface substrate (arrow).

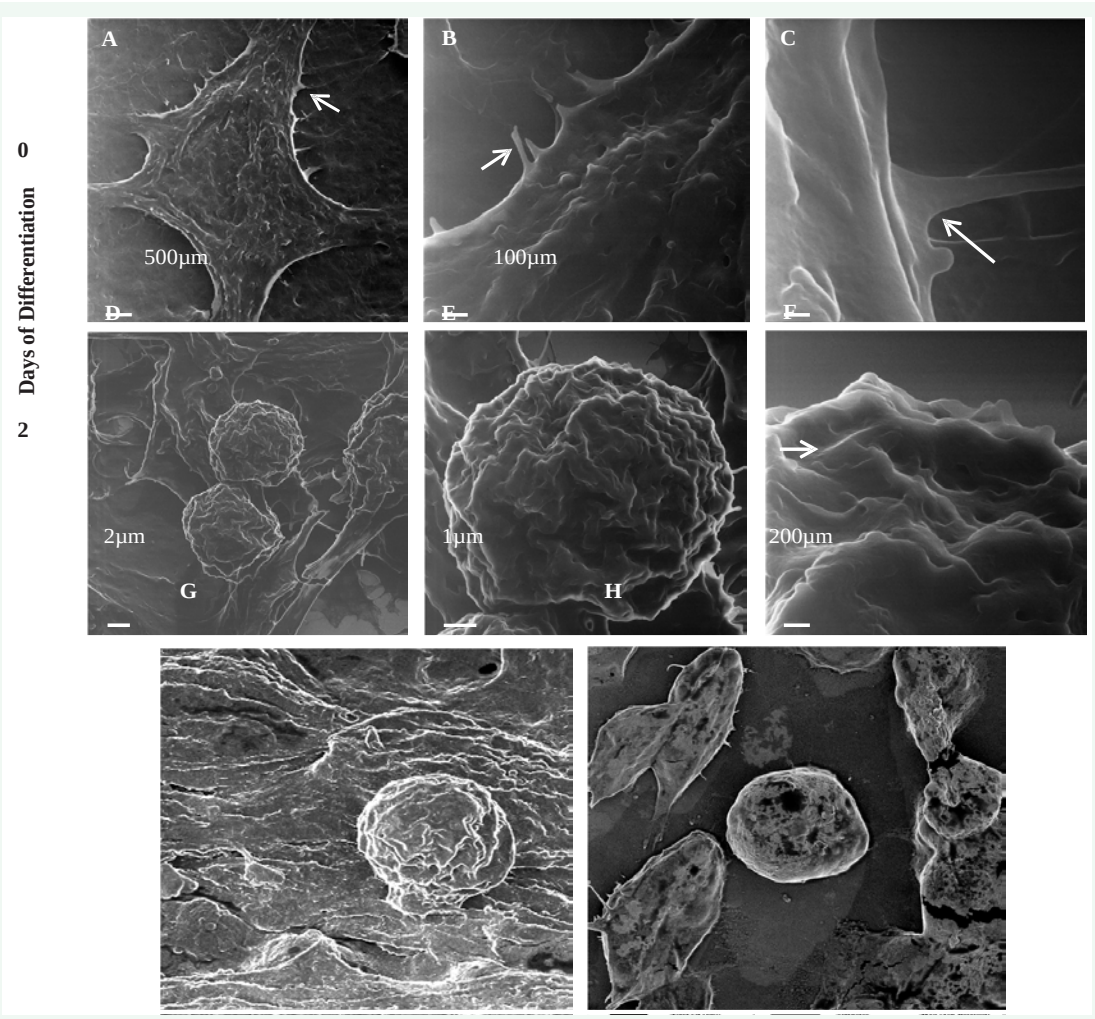

Figure 5 Visualization of cells at different stages of 3T3-L1 preadipocyte differentiation. (A-C) Two-day post confluent 3T3-L1 preadipocytes (Day 0) were induced to differentiate as described in Materials and Methods. Cells were visualized on Days 2, 4 and 7 (D-F) of differentiation by HIM. (A) Visualization of a standard preadipocyte which has the classic trapezoidal fibroblastic morphology; note the numerous cellular extensions in contact with the substrate. (B), (C), At higher magnification, individual cellular extensions show as projective directly from the cellular surface (arrows). (D) and (E) Major morphological changes can be seen by the second day of differentiation. As the cell accumulates lipid, the surface becomes more irregular and the cell become rounded. (F) the cell surface is rough (arrow) but lacks any surface projections.

the 3T3-L1 preadipocyte cell culture model to examine the plasma membrane by HIM because the stages of preadipocytes differentiation are well characterized and morphologically distinct [21]. 3T3-L1 preadipocyteswere grown to confluency (day 0) and theninduced to reenter the cell cycle and proliferate for 2 days and then differentiate. Cells were prepared from different days of preadipocyte differentiation and visualized by HIM (Figure 5A-H). Although day 0 preadipocytes are a fibroblastic cell type the sample preparation we established for adipocytes was able to preserve the morphological features of fibroblasts (Figure 5A-C). HIM allowed for high resolution and contrast to reveal detailed plasma membrane nanostructures. Day 2 proliferating preadipocytesare cells that are in either G1 of the cell cycle or dividing. The HIM images of day 2 dividing preadipocytes reveal complex membrane texturesand nanometer scale projections that are between20-30nm wide (Figure 5D-F). These structures may represent cytoskeletal elements such as bundles of actin filaments or microtubules [22]. After two rounds of proliferation preadipocytes start to express adipogenic transcription factors and adipocyte specific genes that result in the adipocyte phenotype (Figure 5G and H). Day 4 adipocytes show more of the adipocyte architectural features of mature day 7 adipocytes. The membrane features present on day 7 adipocytes were present on day 4 adipocytes.

In summary, our studies provide a protocol for the preparation of preadipocytes and adipocytes for visualization by HIM. We also showthat HIM can image detailed surface textures and nanostructures present on the plasma membranes of adipocytes. We were also able to image significant detail on the cytoplasmic surface of the plasma membranes of adipocytes by HIM.We also used HIM to visualize plasma membrane changes occurring at various stages during 3T3-L1 preadipocyte differentiation. Collectively, our findings are the first to image adipocyte plasma membranes by high resolution HIM. These studies provide the foundation for detailed studies examining the expression and localization of proteins at the plasma membrane during insulin

stimulated glucose uptake. In a larger context these studies could be applied to a variety of cell types to examine changes at the plasma membrane due to exocytic and endocytic processes.

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REFERENCES

1. Cushman SW, Wardzala LJ. Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. J Biol Chem. 1980; 255: 4758-4762
2. Stöckli J, Fazakerley DJ, James DE. GLUT4 exocytosis. J Cell Sci. 2011; 124: 4147-4159.
3. Fujiyoshi Y. Electron crystallography for structural and functional studies of membrane proteins. J Electron Microsc (Tokyo). 2011; 60: 149-159.
4. Tobias CA. Failla Memorial lecture. The future of heavy-ion science in biology and medicine. Radiat Res. 1985; 103: 1-33.
5. Joy DC, Griffin BJ. Is microanalysis possible in the helium ion microscope? Microsc Microanal. 2011; 17: 643-649.
6. Boseman A, Nowlin K, Ashraf S, Yang J, Lajeunesse D. Ultrastructural analysis of wild type and mutant Drosophila melanogaster using helium ion microscopy. Micron. 2013; 51: 26-35.
7. Dwiranti A, Hamano T, Takata H, Nagano S, Guo H, Onishi K, Wako T, Uchiyama S, Fukui K. The effect of magnesium ions on chromosome structure as observed by helium ion microscopy. Microsc Microanal. 2014; 20: 184-8.
8. Rice WL, Van Hoek AN, Paunescu TG, Huynh C, Goetze B, et al. High resolution helium ion scanning microscopy of the rat kidney. PLoS One 2013; 8: e57051.
9. Vanden Berg-Foels WS, Scipioni L, Huynh C, Wen X. Helium ion microscopy for high-resolution visualization of the articular cartilage collagen network. J Microsc. 2012; 246: 168-176.
10. Student AK, Hsu RY, Lane MD. Induction of fatty acid synthetase synthesis in differentiating 3T3-L1 preadipocytes. J Biol Chem. 1980; 255: 4745-4750.
11. Kanzaki M, Pessin JE. Insulin-stimulated GLUT4 translocation in adipocytes is dependent upon cortical actin remodeling. J Biol Chem. 2001; 276: 42436-42444.
12. Lange K, Brandt U. Insulin-responsive glucose transporters are concentrated in a cell surface-derived membrane fraction of 3T3-L1 adipocytes. FEBS Lett. 1990; 261: 459-463.
13. Lange K, Brandt U, Zimmermann B. Relationship between insulin stimulation and endogenous regulation of 2-deoxyglucose uptake in 3T3-L1 adipocytes. J Cell Physiol. 1990; 142: 1-14.
14. Poulos SP, Dodson MV, Hausman GJ. Cell line models for differentiation: preadipocytes and adipocytes. Exp Biol Med (Maywood). 2010; 235: 1185-1193.
15. Osumi M, Konomi M, Sugawara T, Takagi T, Baba M. High-pressure freezing is a powerful tool for visualization of Schizosaccharomyces pombe cells: ultra-low temperature and low-voltage scanning electron microscopy and immunoelectron microscopy. J Electron Microsc (Tokyo). 2006; 55: 75-88.
16. Maruyama Y, Ebihara T, Nishiyama H, Suga M, Sato C. Immuno EM-OM correlative microscopy in solution by atmospheric scanning electron microscopy (ASEM). J Struct Biol. 2012; 180: 259-270.
17. Sweeney G, Somwar R, Ramlal T, Volchuk A, Ueyama A, et al. An inhibitor of p38 mitogen-activated protein kinase prevents insulin-stimulated glucose transport but not glucose transporter translocation in 3T3-L1 adipocytes and L6 myotubes. J Biol Chem. 1999; 274: 10071-10078.
18. Guilherme A, Soriano NA, Bose S, Holik J, Bose A, et al. EHD2 and the novel EH domain binding protein EHBP1 couple endocytosis to the actin cytoskeleton. J Biol Chem. 2004; 279: 10593-10605.
19. Fulcher FK, Smith BT, Russ M, Patel YM. Dual role for myosin II in GLUT4-mediated glucose uptake in 3T3-L1 adipocytes. Exp Cell Res. 2008; 314: 3264-3274.
20. Steimle PA, Fulcher FK, Patel YM. A novel role for myosin II in insulin-stimulated glucose uptake in 3T3-L1 adipocytes. Biochem Biophys Res Commun. 2005; 331: 1560-1565.
21. Lane MD, Tang QQ. From multipotent stem cell to adipocyte. Birth Defects Res A Clin Mol Teratol. 2005; 73: 476-477.
22. Dawicki-McKenna JM, Goldman YE, Ostap EM. Sites of glucose transporter-4 vesicle fusion with the plasma membrane correlate spatially with microtubules. PLoS One. 2012; 7: e43662.

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