Transmission Electron Microscopy

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Tradename: AC ExoTone

Code: 60194

CAS #: 7732-18-5 & 85251-63-4 & 123465-35-0 (or) 8002-43-5

Test Request Form #: 10450

Lot #: N230613C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principle Investigators: *Daniel Shill and Hannah Duckett*

Test Performed:
Freeze Fracture
Transmission Electron Microscopy

Introduction

Liposomes have been a standard delivery system of the cosmetic and personal care industry as the artificial spherical vesicles can be loaded with lipophilic and hydrophilic actives. Despite this versatility, the instability, large size, and variable dimension range (15 – 3,500 nm) of liposomes limits performance of this delivery system, as transfer of the encased actives to intended cells is significantly reduced, restricting bioavailability. As a result, exosomes have gained attention as a technology in the cosmetic and personal care industry to enhance the transport of actives to specific sites. These extracellular vesicles are a diverse group of small (< 500 nm) cell-derived membranous structures participating in homeostatic processes as a critical mechanism of intercellular communication. Extracellular vesicles are characterized by a phospholipid bilayer embedded with surface proteins that enclose genetic material, lipids, and proteins. The biology of extracellular vesicles prevents degradation, enhances the delivery of encapsulated cargo to target recipient cells, and increases bioavailability, constituting a superior delivery system to liposomes. Specifically, exosomes dock at the plasma membrane and activate surface receptors that initiate intracellular signaling cascades to internalize the cargo. Although most extracellular vesicles are derived from human or animal stem cells, the bioauthentic exosomes in **AC ExoTone** are naturally synthesized and extracted from *Pyrus malus* (apple) fruit.

Accordingly, freeze fracture transmission electron microscopy was utilized to confirm the presence and size of bioauthentic exosomes within **AC ExoTone**.

Assay Principle / Methodology Overview

Freeze fracture transmission electron microscopy (TEM) is a technique to examine small structures of rapidly frozen biological samples via TEM. The exosomes were sandwiched between two small blank metal disks (planchets) then plunge-frozen in liquid ethane, sealing the exosomes between the two planchets. The planchets are then loaded into the freeze-fracture instrument and fractured open under liquid nitrogen temperatures into two separate disks. The freshly cleaved surfaces are coated with platinum and carbon to make a cast, which is then cleaned to remove any biological material. The fully cleaned cast is placed on a grid and examined by a transmission electron microscope.

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Exploiting the wave-particle duality of electrons, a transmission electron microscope accelerates a beam of electrons through the platinum-carbon cast, which modifies the sample, and imprints an image that is detected. In summary, the images are not of the exosomes themselves, but of casts made from the exosomes. The images obtained in this report were captured via freeze fracture transmission electron microscopy in the Analytical Instrumentation Facility at North Carolina State University.

Results

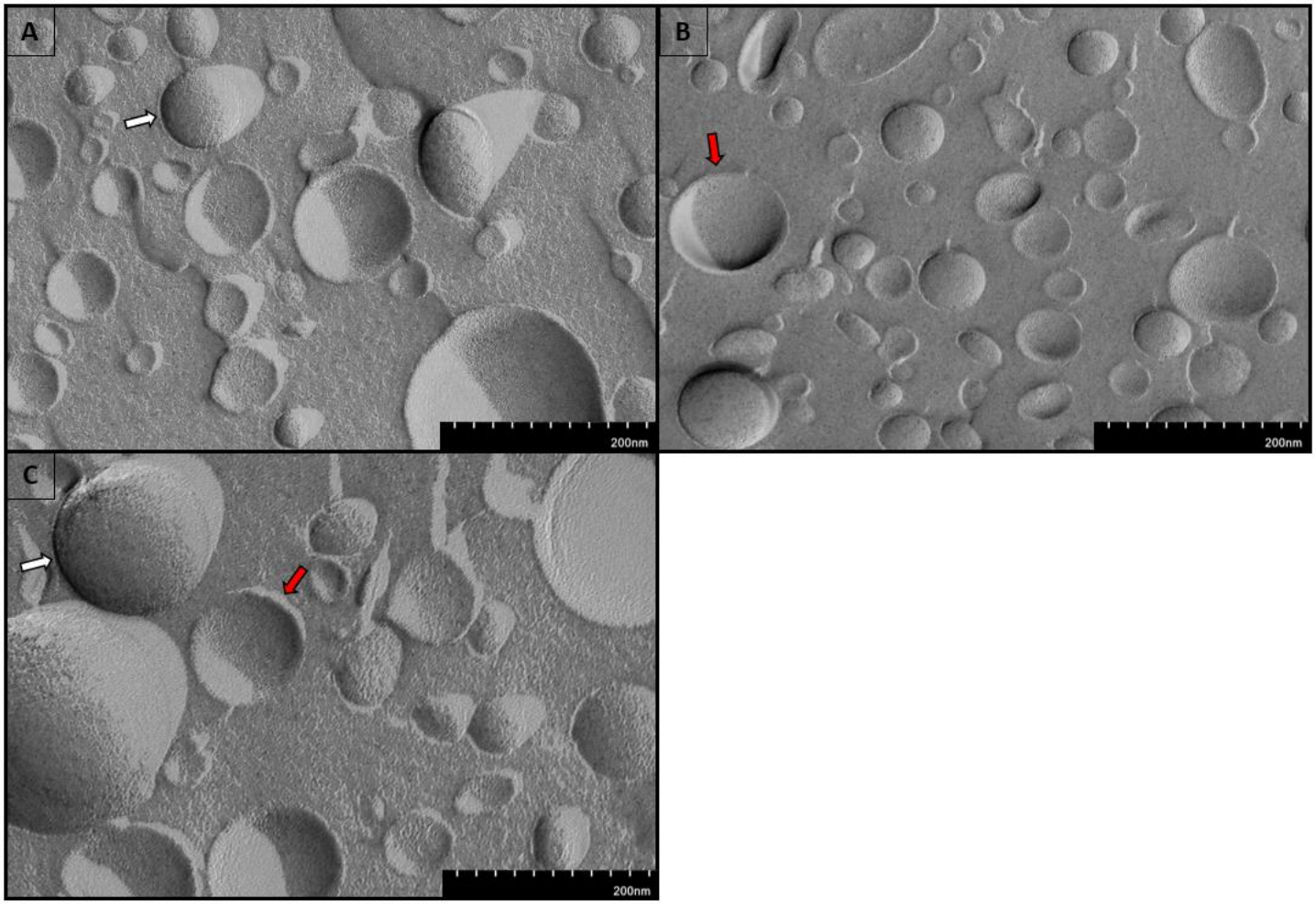

Figure 1. TEM images of **AC ExoTone** at A) 80x B) 60x, and C) 100x magnification. The red and white arrows, respectively indicate examples of 'craters' and 'bubbles' in the platinum-carbon cast, demonstrating the presence, size, and structure of bioauthentic exosomes in **AC ExoTone**.

Discussion

As shown in Figure 1, the 'craters' and 'bubbles' in the platinum-carbon cast demonstrate the presence, size, and structure of bioauthentic exosomes in **AC ExoTone**. Importantly, the extracellular vesicles in **AC ExoTone** exhibited sizes less than 250 nm in diameter. The naturally synthesized and extracted exosomes from *Pyrus malus* (apple) fruit present in **AC ExoTone** demonstrate the morphology of a phospholipid bilayer vesicle, similar to liposomes. However, the bioauthentic exosomes in **AC ExoTone** remain a superior delivery system, compared to liposomes, as the embedded surface proteins inhibit phospholipid inversion and enhance cell-to-cell communication via delivery of cargo to target recipient cells. In summary, the above freeze fracture TEM images confirm the presence, size, and structure of exosomes in **AC ExoTone**.

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.