

Tradename: AC ExoVitalize

Code: 60193

CAS #: 7732-18-5 & 8016-20-4 & 90244-99-8 & 123465-35-0 (or) 8002-43-5

Test Request Form #: 10450

Lot #: N230612B

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 ExoVitalize** are naturally synthesized and extracted from *Citrus paradisi* (grapefruit) fruit and *Citrullus lanatus* (watermelon) fruit.

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

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.

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

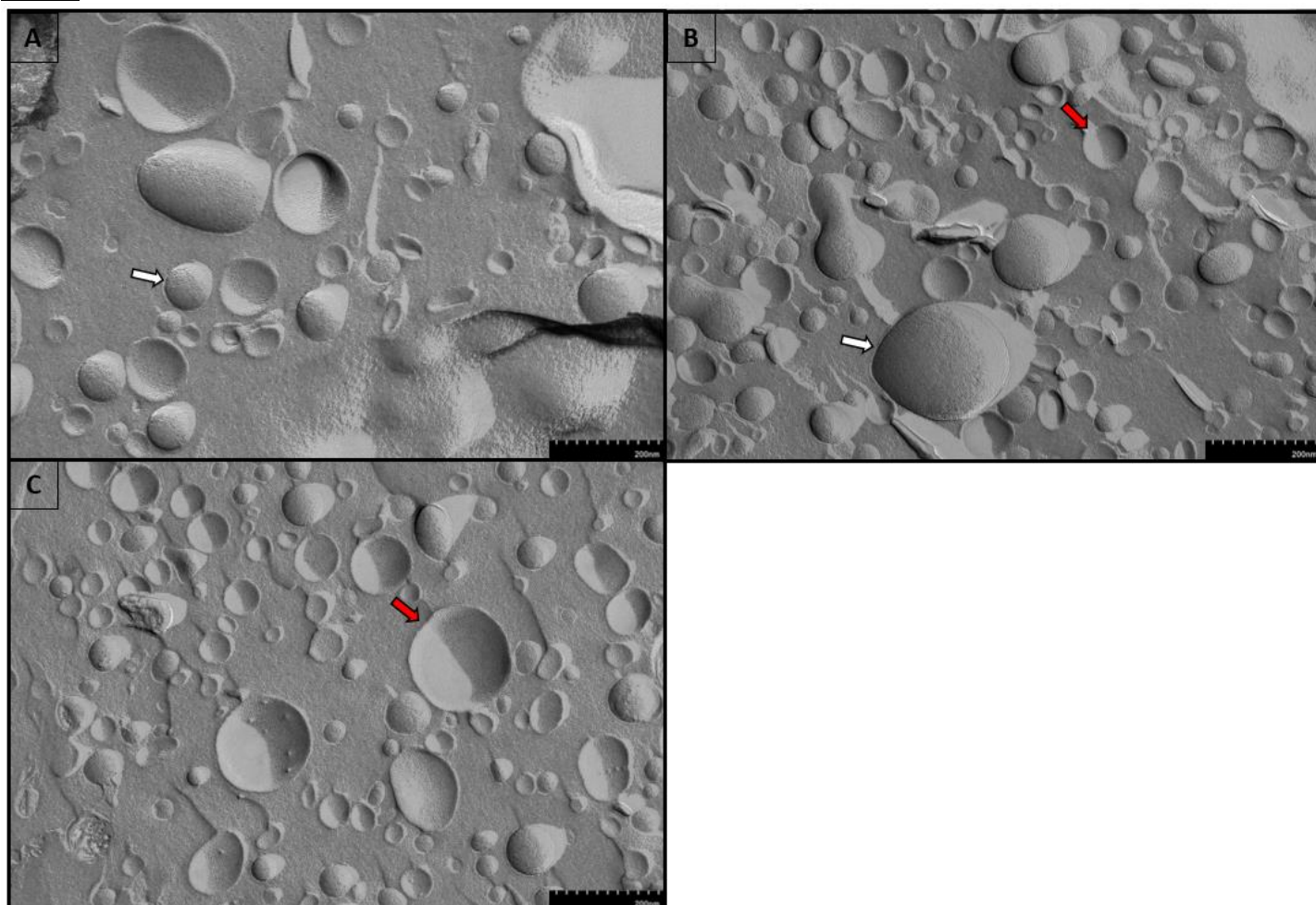


Figure 1. TEM images of AC ExoVitalize at A) 60x B) 60x, and C) 60x magnification. The red and white arrows, respectively indicate examples of 'craters' and 'bubbles' in the platinum-carbon cast, demonstrating the presence, size, and structure of bioauthentic exosomes in AC ExoVitalize.

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 ExoVitalize. Importantly, the extracellular vesicles in AC ExoVitalize exhibited sizes less than 250 nm in diameter. The naturally synthesized and extracted exosomes from *Citrus paradisi* (grapefruit) fruit and *Citrullus lanatus* (watermelon) fruit present in AC ExoVitalize demonstrate the morphology of a phospholipid bilayer vesicle, similar to liposomes. However, the bioauthentic exosomes in AC ExoVitalize 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 ExoVitalize.