AnteAGE MDX Biosome Characterization and Functional Testing

Chi Zhang 1, Rob Knight 1
Orcid ID: 0000-0001-9927-6354

1: Cellese Inc., 1842 Barranca Pkwy, Irvine, CA 92606, USA

Introduction:

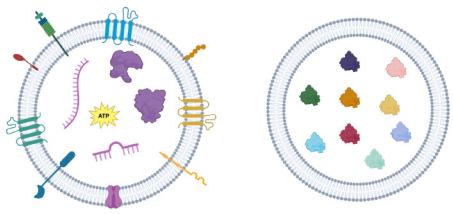
In recent years, there has been a surge in exosome research, focusing on their role in tissue repair and regeneration. This interest extends to the cosmetic industry, which has been exploring the potential of exosomes for skincare and anti-aging solutions. Researchers are leveraging the regenerative properties of exosomes to create innovative cosmetic formulations aimed at enhancing skin health and vitality.

Exosomes are nanoparticles composed of a lipid bilayer. They are produced as a result of the endosomal pathway within all cells and contain a wide array of bioactive components such as proteins, growth factors and RNAs. They are typically 30-150nm in diameter with the lipid bilayer composing a mixture of different lipids (fats), that make up the membrane of the cells which are secreting them [1].

We can take this understanding of exosome biology and synthetically manufacture exosome-like nanoparticles. The synthetic exosomes we create have a controlled size distribution, a lipid bi-layer composed of the same lipids that are found within the exosome membrane, and allow for accurate control of what proteins and growth factors can be encapsulated within the synthetic nanoparticle [2]. Depending on what we are encapsulating, and its polarity, proteins and growth factors can be either loaded into the lipophobic luminal space at the center of the vesicle or within the lipophilic space between the phospholipid tails [3]. The exosome-like lipid nanoparticles we have created to mimic the function of naturally secreted stem cell exosomes are termed Biosomes™ (Figure. 1).

Figure 1:

Schematic Illustration of Biosomes™ demonstrate the structure of Biosome™, highlighting their lipid bilayer composition and encapsulated bioactive components designed to mimic naturally secreted stem cell exosomes.



Exosome Biosome

Manufacturing Process

The process of manufacturing Biosomes can be tailored to produce Biosomes comprising different lipids that preferentially target specific cell types, or to control the stability or protein encapsulation efficiency. AnteAGE MDX Biosomes are manufactured using microfluidic mixing of a solvent containing the lipid mixture and an aqueous mixture of proteins (table 1)) using defined flow rates to precisely control nanoparticle size, Biosomes are engineered with precision and accuracy.

Biosomes are characterized in a similar way to the exosomes. Particle size distribution and concentration are determined by Nanoparticle tracking analysis (NTA). Biosomes are visualized using cryo-Transmission Electron Microscopy or using state of the art super resolution microcsopy (ONI). Finally, Biosomes are tested functionally in vitro using a number of skin relevant assays to determine function.

Table 1:

Growth factors present in Biosomes

Name	Function
TGFb3	Supports collagen production aiding in skin firmness and elasticity
HGF	Supports skin tissue regeneration and improves wound healing
KGF1	Promotes the proliferation and differentiation of keratinocytes
KGF2	Supports growth and maintenance of the epidermis
NGF	Improves skin vitality
SIRT-1	Promotes cellular repair and longevity
SIRT-2	Involved in cell metabolism and skin homeostasis
TRX	Provides antioxidant support
TIMP-1	Maintains extracellular matrix integrity
HBD3H	Supports skin defense against pathogens

Benefits of Biosomes™

- More controlled process and output
- Expedited manufacturing time
- No need for human cells or conditioned media
- Not human derived but contain human proteins that will be recognized by your cells

Structural and Functional Characteristics of Biosomes

Biosomes are lipid-based nanoparticles engineered to deliver specific bioactive components for skin rejuvenation. They possess a lipid bilayer and encapsulate select proteins and growth factors. This targeted composition allows for precise control over their biological activity, enhancing potency by enriching only the active ingredients without extraneous components that might inhibit desired cellular processes.

A key advantage of biosomes is our ability to precisely control their particle size through a combination of specially designed lipid ingredients and optimized production parameters. The specific lipid composition is critical in determining the size and stability of the biosomes. By selecting and formulating lipids with particular characteristics, we engineer biosomes that not only have the desired size but also exhibit enhanced stability and efficient cellular uptake.

Nano Flow Cytometry (NanoFCM) confirms that biosomes have a median size around 78 nm (Figure 2), which is slightly smaller than naturally secreted exosomes. This size is conducive to effective cellular internalization and consistent performance. Additionally, Zeta potential measurements (Figure 3) demonstrate that biosomes maintain a stable and integral structure, indicating their suitability for biological applications and long-term storage.

Figure 2:

Nano Flow Cytometry (NanoFCM) Size Distribution of Biosomes shows the median size of biosomes around 78 nm, indicating a slightly smaller size compared to naturally secreted exosomes, which is favorable for effective cellular uptake.

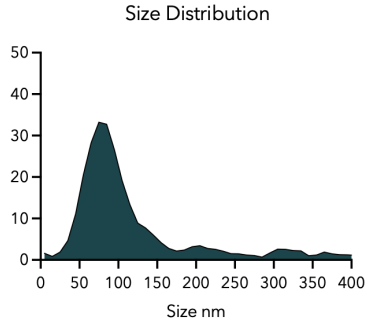
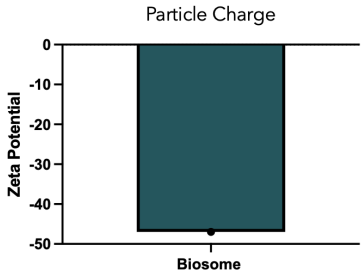


Figure 3:

Zeta Potential Measurements Demonstrating Biosome Stability confirming that biosomes possess a stable and integral structure suitable for biological applications and long-term storage.



Lipid Bilayer Confirmation

The structural integrity of biosomes was further validated using lipid staining analyzed by NanoFCM and Cryogenic Electron Microscopy (Cryo-EM). NanoFCM analysis showed that 93.4% of the particles stained positive for lipids (Figure 4), indicating a high consistency in lipid bilayer formation across the biosome population. Cryo-EM images provided visual confirmation of the intact lipid bilayer structure (Figure 5), underscoring the structural fidelity of biosomes.

Figure 4:

Lipid Staining Analysis of Biosomes by NanoFCM shows that 93.4% of biosome particles stained positive for lipids, confirming high consistency in lipid bilayer formation across the biosome population.

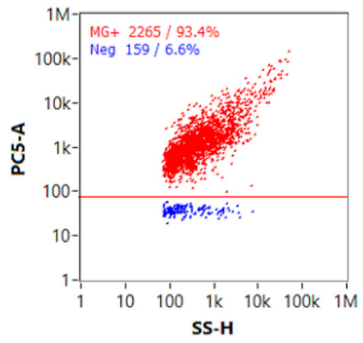
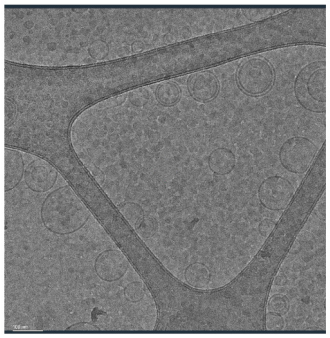


Figure 5:

Cryogenic Electron Microscopy Images of Biosomes provides visual confirmation of the intact lipid bilayer structure of biosomes, underscoring their structural fidelity and similarity to natural exosomes.



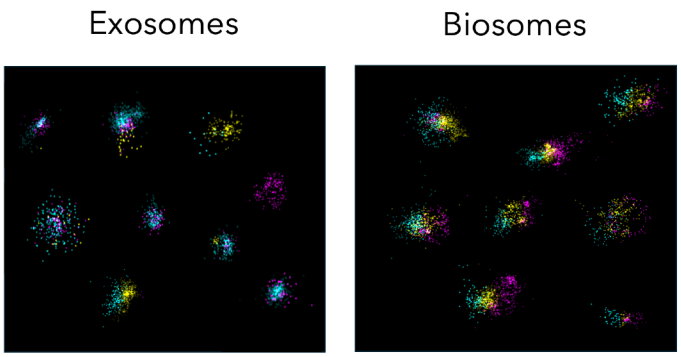
Efficient Cargo Loading

Biosomes are engineered to encapsulate specific growth factors, within their lipid bilayer to confer desired biological functions. We employed super-resolution microscopy (ONI) to verify efficient cargo loading and confirm the structural integrity of our biosomes,

Using fluorescence staining techniques, both the lipid components and the encapsulated growth factors within the biosomes were labeled. The ONI super-resolution imaging allowed us to visualize biosomes at the nanoscale, providing detailed insights into their structure. The images revealed a high degree of co-localization between the lipid membrane and the cargo, indicating that the growth factors are effectively encapsulated within the lipid bilayer (Figure 6).

Figure 6:

Super-Resolution Microscopy (ONI) Images Showing Efficient Cargo Loading in Biosomes illustrates the co-localization of lipid membranes and encapsulated growth factors within biosomes, confirming efficient cargo loading and structural integrity at the nanoscale level.



The ONI analysis demonstrated that biosomes exhibit an integrated structure with efficient cargo loading:

•High Cargo Encapsulation Efficiency: A significant percentage of biosomes showed fluorescence signals for both lipids and growth factors, confirming successful encapsulation of the active components.

•Structural Integrity: The super-resolution images confirmed the uniformity and integrity of the lipid bilayer, which is crucial for protecting the cargo and facilitating targeted delivery.

• Enhanced Potency: Efficient cargo loading ensures that a large proportion of biosomes can deliver the active ingredients to target cells, enhancing their biological effectiveness in vitro and in practical applications.

By confirming both the structural integrity and the efficient loading of growth factors using ONI super-resolution microscopy, we provide strong evidence that biosomes are highly capable nanocarriers. This level of characterization underscores the potential of biosomes to mimic the function of naturally secreted exosomes while offering the advantages of controlled composition and enhanced potency.

Biological Activity Assessment

To evaluate the efficacy of biosomes in promoting skin rejuvenation, we conducted a series of in vitro assays using human dermal fibroblast (HDF) cells. These assays assessed critical cellular functions integral to skin health, including proliferation, collagen production, cell migration, antioxidant activity, and modulation of inflammatory responses.

Biosomes demonstrated a significant enhancement in HDF cell proliferation, as evidenced by increased cell growth compared to the control group (Figure 7). This stimulation of fibroblast activity is essential for skin regeneration and repair. Complementing this finding, biosome treatment led to a substantial increase in collagen production by HDF cells (Figure 8).

Figure 7:

Enhanced Proliferation of HDF with Biosome Treatment demonstrates that biosome-treated HDF cells exhibit increased proliferation compared to control cells, indicating stimulation of fibroblast activity essential for skin regeneration.

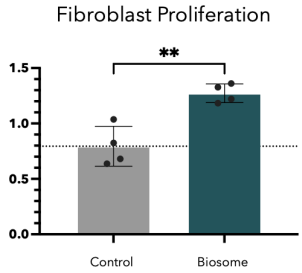
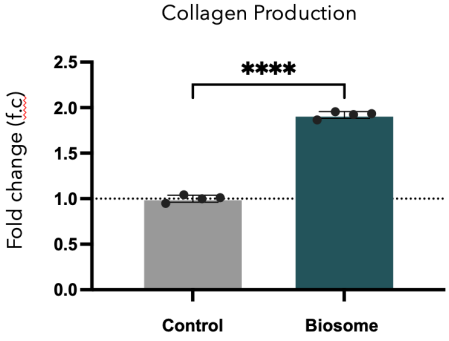


Figure 8:

Increased Collagen Production in HDF Cells Induced by Biosomes shows a substantial rise in collagen synthesis in biosome-treated HDF cells, contributing to improved skin elasticity and firmness.



Collagen is a primary structural protein in the skin, and its enhanced synthesis contributes to improved skin elasticity and firmness. The combined effects on cell proliferation and collagen production suggest that biosomes may contribute to thicker, more resilient skin tissue.

Cell migration is another vital process in wound healing and tissue repair. The scratch wound assay revealed that HDF cells treated with biosomes exhibited markedly improved migration into the wound area compared to untreated cells. Enhanced wound closure was observed in the biosome-treated group (Figure 9A), and microscopic images further confirmed this effect, showing near-complete closure of the wound gap (Figure 9B). By facilitating the movement of fibroblasts to sites requiring repair, biosomes may accelerate wound healing processes and improve skin texture.

Figure 9A:

Quantitative Analysis of HDF Cell Migration Enhanced by Biosomes presents data from scratch wound assays indicating that biosome treatment markedly improves cell migration, facilitating accelerated wound healing.

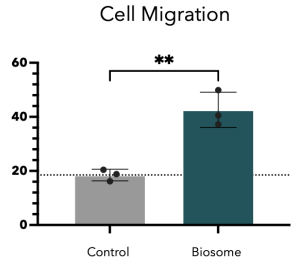
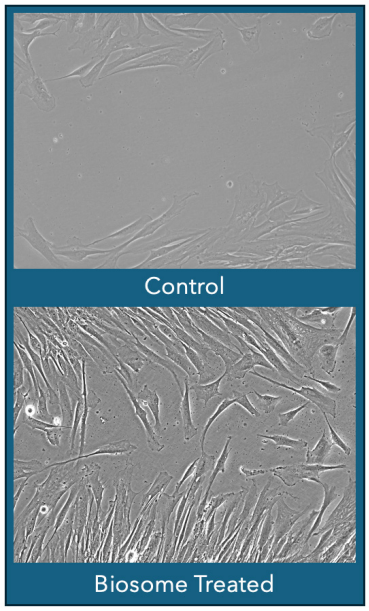


Figure 9B:

Increased Collagen Production in HDF Cells Induced by Biosomes shows a substantial rise in collagen synthesis in biosome-treated HDF cells, contributing to improved skin elasticity and firmness.



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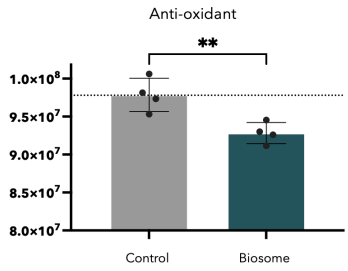
Oxidative stress is a major contributor to skin aging and cellular damage. To assess the antioxidant properties of biosomes, we measured their effect on oxidative stress markers in HDF cells. The results indicated that biosome treatment reduced oxidative stress compared to the control group (Figure 10). This reduction suggests that biosomes possess antioxidant capabilities, potentially protecting skin cells from environmental stressors such as UV radiation and pollution. By mitigating oxidative damage, biosomes may help slow the aging process and maintain skin vitality.

Figure 10:

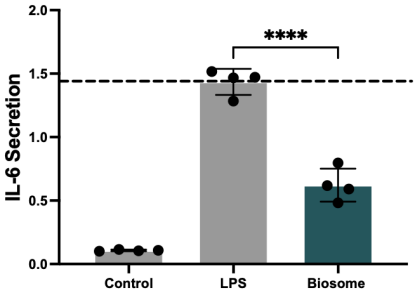
Reduction of Oxidative Stress Markers in HDF Cells by Biosomes depicts the decrease in oxidative stress indicators in biosome-treated cells, suggesting antioxidant properties that protect against cellular damage.



Inflammation plays a crucial role in skin aging and various dermatological conditions. We evaluated the impact of biosomes on inflammatory cytokine secretion in a human monocyte cells line (THP-1). Lipopolysaccharide (LPS) stimulation increased the secretion of interleukin-6 (IL-6), a pro-inflammatory cytokine associated with inflammation and aging. Biosome treatment effectively suppressed LPS-induced IL-6 secretion, reducing it to the control levels (Figure 11A). This anti-inflammatory effect suggests that biosomes can mitigate inflammatory responses in skin cells.

Figure 11A:

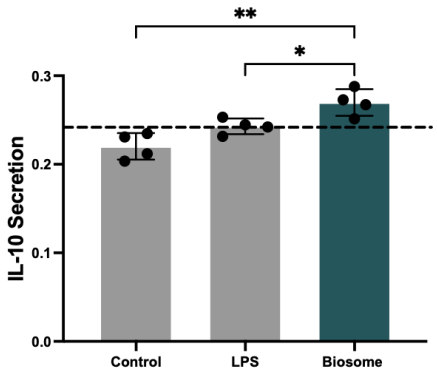
Biosomes Suppress LPS-Induced Interleukin-6 (IL-6) Secretion in THP-1 Cells shows that biosome treatment effectively reduces the secretion of the pro-inflammatory cytokine IL-6 to control levels, indicating anti-inflammatory effects.



Conversely, interleukin-10 (IL-10) is an anti-inflammatory cytokine that promotes healing and modulates immune responses. LPS stimulation increased IL-10 secretion, and biosome treatment further enhanced this effect (Figure 11B). The upregulation of IL-10 indicates that biosomes not only suppress pro-inflammatory signals but also promote anti-inflammatory pathways, contributing to a balanced immune response conducive to skin health.

Figure 11B:

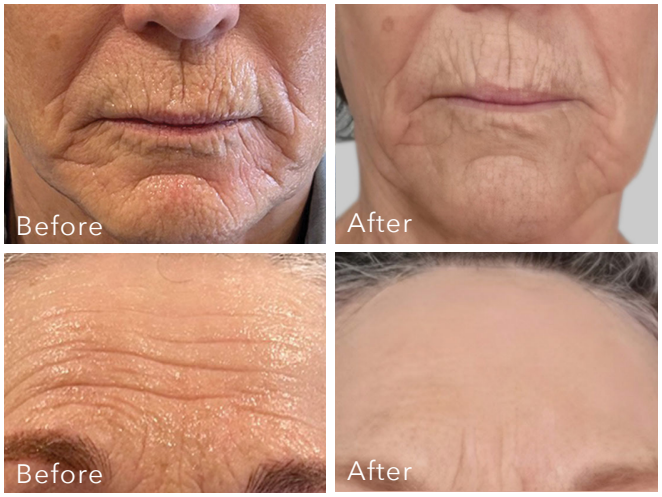
Enhancement of Interleukin-10 (IL-10) Secretion by Biosomes in THP-1 Cells illustrates that biosomes further increase the secretion of the anti-inflammatory cytokine IL-10 following LPS stimulation, promoting healing and balanced immune responses.



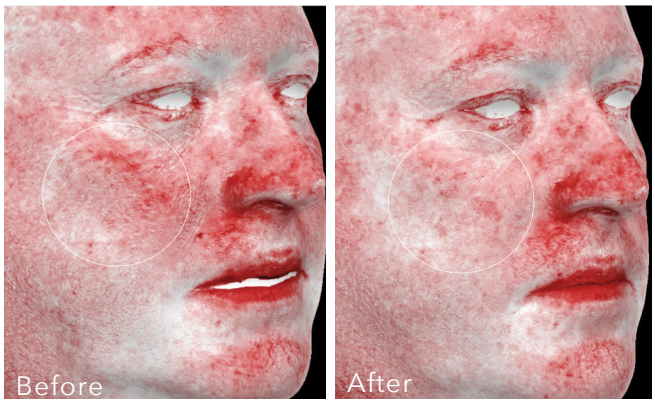
Collectively, these findings demonstrate that biosomes positively influence multiple cellular activities associated with skin rejuvenation. The enhanced proliferation and collagen production strengthen skin structure, while improved cell migration accelerates wound healing. The antioxidant properties protect cells from oxidative damage, and the modulation of inflammatory cytokines suggests a comprehensive approach to maintaining skin homeostasis. By targeting these interconnected pathways, biosomes offer a multifaceted strategy for improving skin health and combating signs of aging. Their controlled composition and potency further underscore their potential as effective agents in advanced skincare formulations.

In vivo application of Biosomes further validates their effectiveness as seen by the reduction in fine lines and wrinkles, improvement in skin firmness and resulting even skin texture (Fig 12).

Figure 12: Before and 2 weeks post Biosome™ application when combined with microneedling.



1 Biosome Microneedling Treatment | 2 Weeks



1 Biosome Microneedling Treatment | 1 Month

Conclusion

Overall these findings highlight the similarities not only in structure but also in the function of Biosomes when compared to MDX exosomes. Biosomes therefore offer a functional, non-human derived nanoparticle that is capable of improving skin function.

References

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