Development of Best Suited Media for MSC Exosome Production

White paper

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INTRODUCTION

Exosomes are small membrane-bound vesicles released by cells into the extracellular space, playing a crucial role in intercellular communication and molecular transfer. With their involvement in various physiological processes and disease progression, exosomes have given new insight to the field of therapy, drug delivery, diagnostics, and regenerative medicine. The exosome market is expected to grow exponentially, calling in more researchers and investors each year.

For studies involving cells, it is essential to grow cells outside the body. As the blood stream is essential to deliver nutrients in the body, cell culture media provides the necessary nutrients for cell growth in vitro. As the cells grow, exosomes will be released and accumulated in the media to serve its original purpose as autocrine or paracrine signals. However, the isolation of these exosomes face some critical challenges due to the source of these medium components.

When supernatants from the cell cultures were harvested, exosomes produced by the cells are contaminated by other exosomes. The nutrient, in which most cases is fetal bovine serum (FBS), was unsurprisingly also found to be rich in bovine exosomes, similar in size and function. As the field of exosomes grew, these challenges were in part overcome, by depleting the FBS with high speed centrifugation.

We must know that the research for exosomes all carry one purpose, which was to be applied back to the human body. Using bovine serum for cellular growth in this process caused a natural discrepancy, and therefore human serum was used as an alternative. Using human serum caused new concerns, such as its price and insufficient supply.

The paradigm for this medium is now shifting to chemically defined medium (CDM), which is a cutting-edge approach that offers a serum-free formulation with a specific defined composition of known materials, free from animal or human-originating components. CDM provides researchers with a high level of control, consistency, and reproducibility in cell culture experiments. The importance of suitable media in exosome research cannot be overstated. The choice of media formulation is crucial in maintaining cell-specific characteristics and optimizing exosome production.

In this white paper, we introduce the development of CellCor™ EXO CD, in response to the demand for safer and more traceable alternative cell medium. CellCor™ EXO CD was revealed to be pure and was shown to produce more exosomes. Resulting exosomes were also shown to have superior function in wound healing assays and angiogenesis assays.

Development of CellCor™ EXO CD

Serum-derived media, particularly FBS-containing media, present risks of contaminant exosomes and non-extracellular vesicle (EV) nanoparticles. These impurities can confound the analysis of exosomes and affect the accuracy and reliability of research studies. Completely depleting exosomes from culture media while maintaining essential growth factors and nutrients still remains a challenge. Effective removal of impurities, including small non-coding RNA (ncRNA) contaminants, is crucial for reliable and valid cell culture-based studies focused on exosomes.

We developed CellCor™ EXO CD which addresses these challenges by offering an exosome-specific serum-free chemically defined media that ensures the production of pure exosomes with minimal contamination. This innovative media formulation provides researchers with a reliable platform for exosome-related studies. Chemically defined medium are known to have less impurities to start with, but CellCor™ EXO CD were reassured this by passing through 0.22um pore filters in our efforts to remove any possible impurities FIGURE 1 . Of note, in this graph, depleted FBS (d-FBS) seems purer than CellCor™ EXO CD, but its proliferative function as a medium was sacrificed as a result FIGURE 4 .

FIGURE 1

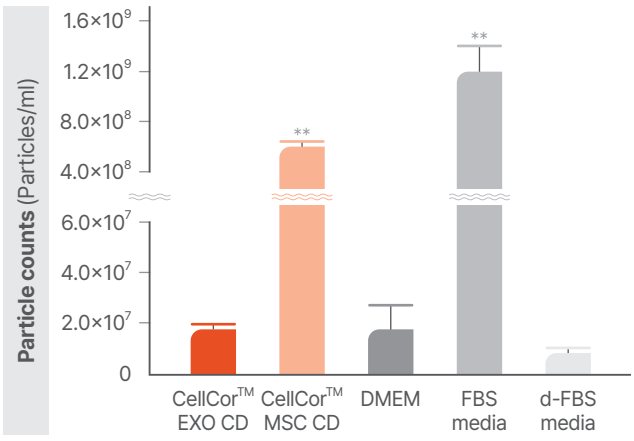

Figure 1 | Comparison of background particles from CellCor™ EXO CD and other indicated mediums. (** p<0.01)

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CellCor™ EXO CD offers several key advantages over traditional media formulations. First, it eliminates more than 95% of impurities, ensuring the production of pure exosomes with minimal contamination. This purity is crucial for downstream applications and accurate characterization of exosome cargo. Second, CellCor™ EXO CD provides convenience and standardization in culture, allowing for ease of use and simplified workflows. Researchers can apply CellCor™ EXO CD to various sources of MSCs, including adipose-derived MSCs, bone marrow-derived MSCs, and umbilical cord-derived MSCs, and potentially other cells without compromising its performance. The stability, safety, and homogeneity of CellCor™ EXO CD promote stable cell growth and proliferation, providing a consistent and reliable supply of exosomes for research and therapeutic purposes. Data from FIGURE 2 shows that the CellCor™ EXO CD is free of bovine impurities, and rich and pure in exosomes, as demonstrated by their markers, human CD9 and CD81.

FIGURE 2

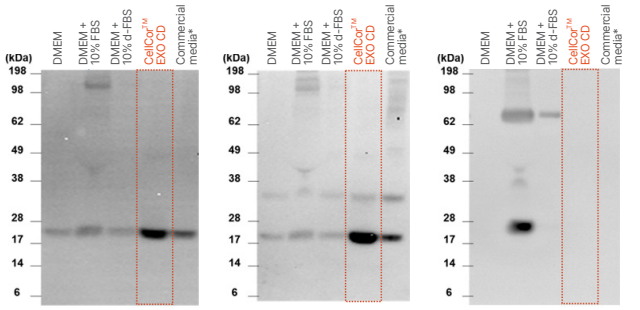

Figure 2 | Western blot images of human and bovine exosome markers in isolated exosomes. Cells were grown under conventional environments for 3 days and their exosomes were isolated by tangential flow filtration. (* This medium is xeno-free grade media for MSCs and requires media change in the exosome collect stage.)

For practical application, we've optimized the protocols for changing the original medium to CellCor™ EXO CD, and have obtained data for these testings. We also tested a commercially available chemically defined media for exosome isolation as a comparison. Media were tested for change in three different time points in the order from the latest to the earliest. Medium change was done during the collection process, during the seeding process (which is after the expansion) and from the initial point of the cell thawing process FIGURE 3A . The longer the cells were exposed to the CellCor™ EXO CD medium, the better the results FIGURE 3B . Impressively, all the results outperformed that of the currently available commercial medium.

FIGURE 3

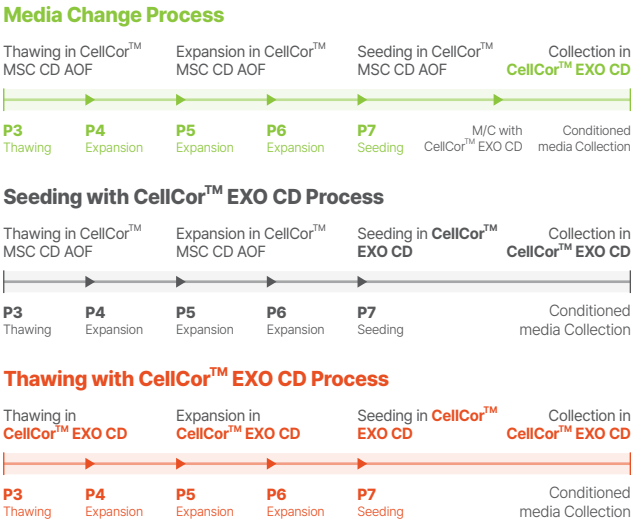

Figure 3A | Medium change to CellCor™ EXO CD was done during collection (top), cell seeding (middle), and cell thawing (bottom) process.

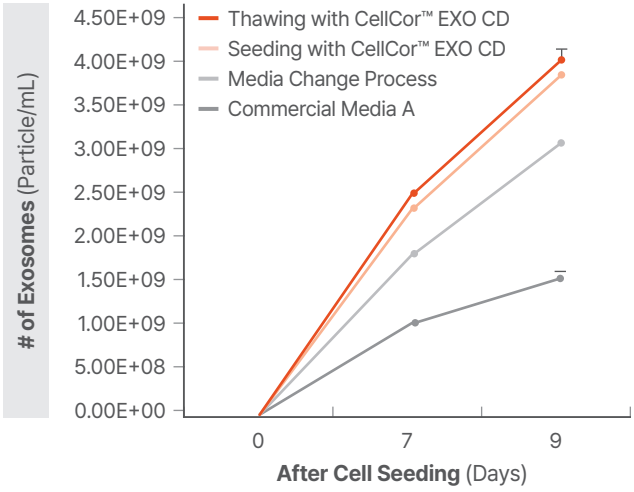

Figure 3B | Number of exosome particles harvested 7 and 9 days after cell seeding.

Optimized for Stem Cell Culture

Culture medium plays a vital role in supporting stem cell growth and maintaining their stemness. In the context of MSCs, CellCor™ EXO CD demonstrates its superiority in promoting MSC proliferation compared to other media formulations, including FBS-containing media and commercial media. With approximately 3.8 times better proliferation rate than FBS media and 1.4 times better than commercial media, CellCor™EXO CD provides a favorable environment for robust stem cell growth and culture FIGURE 4 . From this robust growth comes elevated exosome harvests. Furthermore, its unique formulation eliminates the need for media change during MSC cultivation FIGURE 5, simplifying the workflow and facilitating the isolation of pure exosomes at desired endpoints. This will greatly enhance the efficiency and convenience of exosome production.

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FIGURE 4

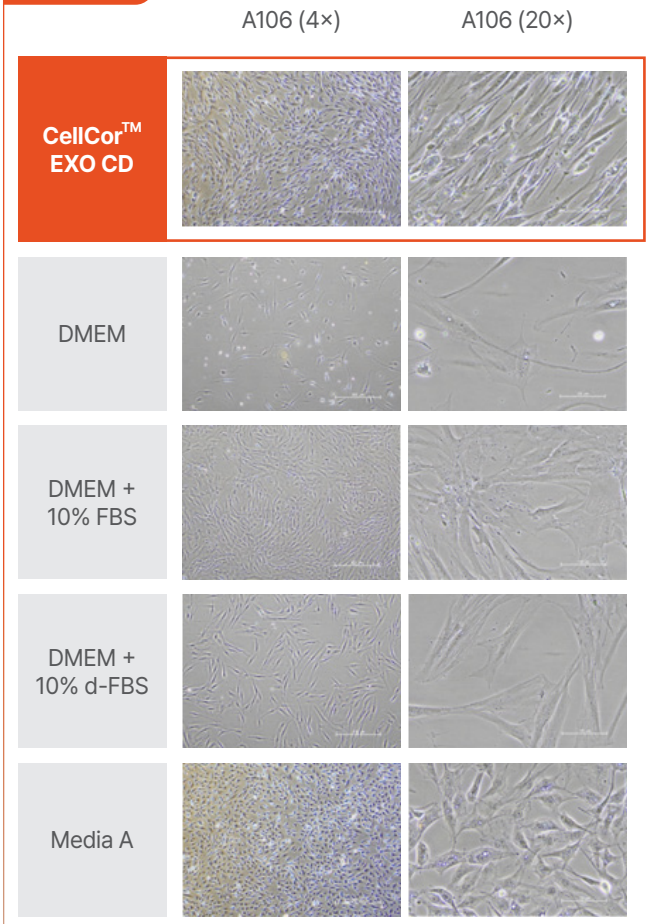

Figure 4A | 4× and 20× phase morphologies of MSCs with initial seeding of 1 x 10^5 cells grown for 5 days.

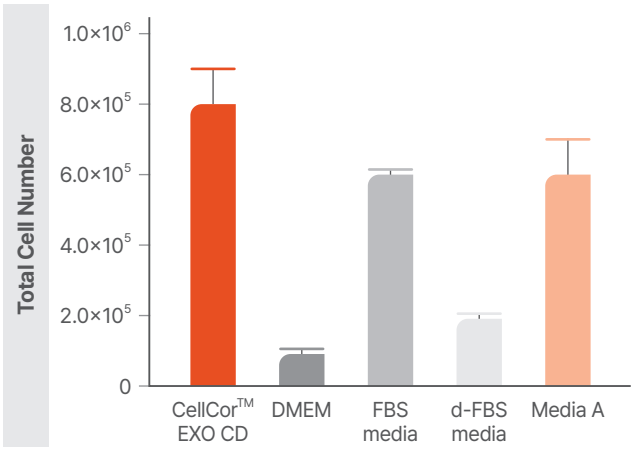

Figure 4B | Total cell numbers of MSCs from culture media, respectively.

FIGURE 5

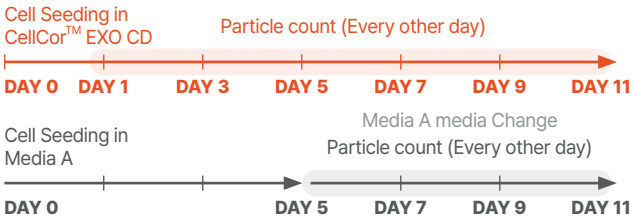

Figure 5A | Comparison of culture schedule for CellCor™ EXO CD and commercially available medium.

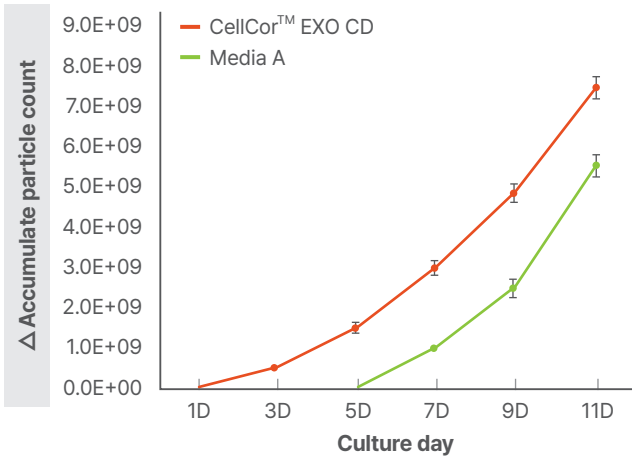

Figure 5B | Particle counts for exosomes grown as shown in culture schedule, respectively. 1x10^5 cells were seeded for both methods. Particle counts were measures every 2 days after day 1.

MSC-Derived Exosomes

Mesenchymal stem cells (MSCs) have emerged as a promising source of exosomes with therapeutic potential. The efficiency of exosome production from MSCs depends on the culture media used. Comparative analysis of media formulations reveals that CellCor™ EXO CD outperforms other media formulations, enabling approximately 2.5 times greater exosome production FIGURE 6 . These findings underscore the superior performance of CellCor™ EXO CD in obtaining high-quality MSC-derived exosomes for research and therapeutic applications.

FIGURE 6

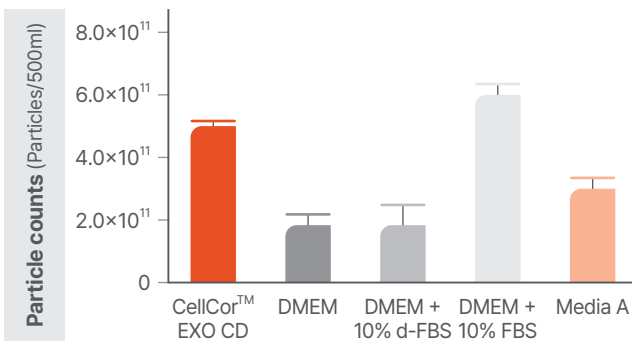

Figure 6 | Exosome particle counts obtained from 1×10^5 MSCs cell culture for 5 days harvested from each indicated medium.

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Wound healing and Angiogenesis Assays

CellCor™ EXO CD demonstrates its potency in producing MSC-derived exosomes with regenerative potential. Wound healing assays using exosomes obtained from CellCor™ EXO CD-cultured MSCs show significant potential in the proliferation and regeneration of wounds FIGURE 7 . This highlights the therapeutic potential of MSC-derived exosomes in promoting wound healing processes. Furthermore, angiogenesis assays reveal the angiogenic potential of exosomes produced using CellCor™ EXO CD, emphasizing their ability to promote blood vessel formation and tissue regeneration FIGURE 8 . These findings further support the application of CellCor™ EXO CD in therapeutic contexts.

FIGURE 7

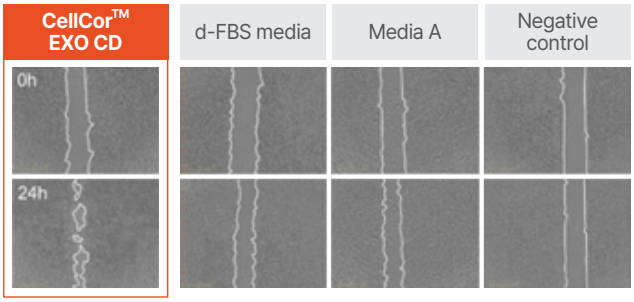

Figure 7 A | Wound healing assay of HaCaT cells 24 hours after treatment with exosomes harvested in each indicated medium.

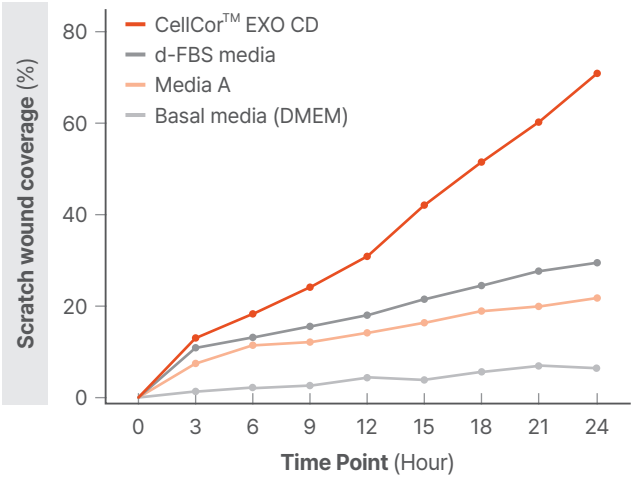

Figure 7 B | Graph of wound coverage in every 3 hour time points.

FIGURE 8

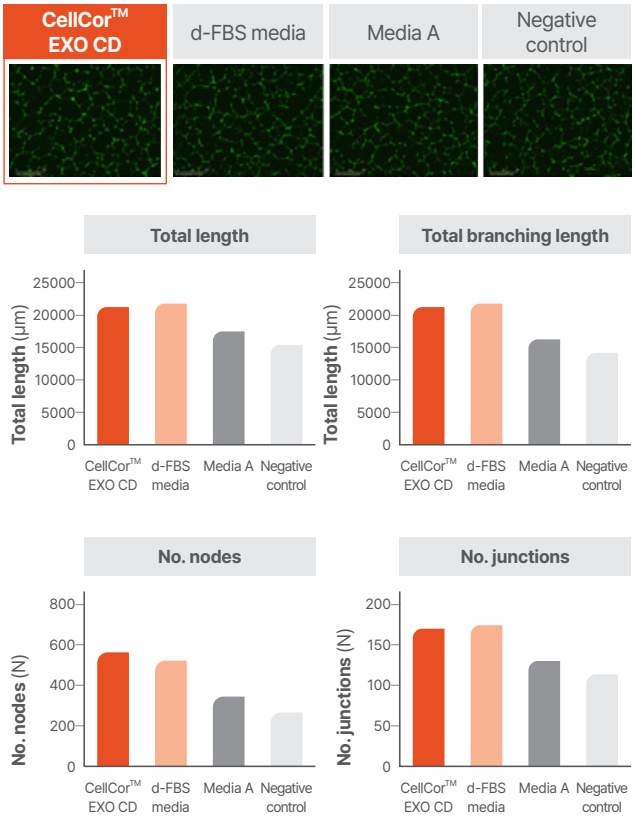

Figure 8 | **Top** Calcein AM stained images of HUVEC cells 17 hours after treatment with 1x109 particle/ml of exosomes harvested in each indicated medium. **Bottom** Angiogenic index.

Discussion

The use of chemically defined media provides significant advantages in exosome research. By ensuring excellent consistency and traceability, chemically defined media offer researchers a high level of control and reproducibility. CellCor™ EXO CD minimized impurities in culture media to obtain pure and controlled environment to harvest exosomes, eliminating potentially confounding factors in downstream analyses. It also outperformed competitors in cell proliferation, exosome production, and in functional assays, highlighting their potential in regenerative and therapeutic applications. CellCor™ EXO CD is an exosome-specific serum-free chemically defined media, offering a controlled and effective baseline for exosome production. By adopting CellCor™ EXO CD, researchers will be able to obtain pure and potent exosomes ready for clinical applications.

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