Chapter

Development of Exosomes for Esthetic Use

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Abstract

While there are thousands of peer-reviewed papers on exosomes, most of the work has been done in the medical field. Studies and clinical trials on exosome-related products for the esthetic industry have just begun to be a regular occurrence. One of the reasons for this is a lack of regulatory approval for any exosome use. The FDA does not regulate topical cosmetic use, while only a few exosomes are registered on the International Cosmetic Ingredient Dictionary (ICID) of the Personal Care Product Council (PCPC), so most esthetic providers are utilizing exosomes in this manner. Clinical uses for exosomes in esthetic practice include the treatment of burns, active acne, atopic dermatitis, and chronic skin irritations. When used in combination with energy-based device treatment, exosomes reduce inflammation and redness, improve the rapidity of healing for laser and microneedling patients, and reduce the tendency for fibrosis and thick hypertrophic scar formation when used topically. Byong Cho is the CEO & CTO of ExoCoBio, one of the four largest exosome companies globally. He has developed a large research, development, and GMP manufacturing facility just south of Seoul, Korea. His topic, the development of exosomes for clinical esthetic use, will take us through the process of developing a safe and cost-effective biological regenerative product while staying in line with regulatory limitations.

Keywords: regenerative, esthetic, exosome, secretome, scar treatment, resurfacing, hair restoration, burn treatment

1. Introduction

I first learned about exosomes in early 2016. I heard the word "exosome" for the first time from a close friend and professor of biology. After that, for several months, I did basic research and study on papers and patents related to exosomes [1]. Through this, I found that exosomes can develop a completely new biotech technology, and in particular, I became very interested in "stem cell-derived exosomes" for regeneration and dermatological applications. During 6 months in 2016, I was able to secure basic knowledge about it. In addition, it was identified that stem cell exosomes have great potential as a next-generation regeneration plus anti-inflammation technology. Further, I became very interested in Dr. Sai Kiang Lim, and her brilliant discovery, who discovered stem cell-derived exosomes for the first time in the world [2].

Additional factors that made me interested in exosomes in 2016 include my background. I had more than 15 years of experience in investing in biotechnology and commercialization in venture capital. After putting together 2 biotech startups in early 2002, I was the first venture capital investor in Asia who supported the commercialization of botulinum toxin technology. I have continued to have a deep interest and experience in the field of medical esthetics since then, and I thought that I found "something new" about the commercialization potential of stem cell-derived exosomes. I have been continuously trying to find and commercialize new technologies in the field of "regenerative medicine or aesthetics" for more than 10 years before knowing about exosomes.

My vision was to fill an unmet need for our global aging population. While there are botulinum toxins, dermal fillers, & energy-based devices, not all of them would be appropriate for very senior people. We would need something new—a regenerative esthetics or regenerative medicine treatment option that might be able to reverse certain aspects of aging. Also, stem cell-derived exosomes can be used to treat incurable or very difficult skin diseases like dermatitis, psoriasis, scleroderma, skin fibrosis, and so on, based on the dual and synergistic function of regeneration and anti-inflammation. This premise of exosome therapy could certainly expand the field of regenerative dermatology.

2. As a pioneer in the field, what were your first steps in developing exosomes?

After learning about exosomes, the most important tasks for me were (1) establishing a business plan, (2) licensing and developing exosome technology, (3) financing to create a successful exosome startup, (4) registering adipose stem cell-derived exosomes as a cosmetic ingredient, and (5) finding a partner for marketing and sales in the US.

While exploring numerous scientific papers and patents on exosomes around the world [3, 4], I sought what kind of business to do with exosomes. In particular, the biggest challenge was to determine whether stem cell exosomes would become a major technology in the field of "regenerative medicine or regenerative aesthetics" in the future. And, since then I had no exosome technology on my own, and to license stem cell exosome technology, I read all the publications of Dr. SK Lim and directly contacted her to discuss technology licensing and to have her as a scientific advisor for 6 months in 2016. While studying other scientists' technologies and patents [5, 6], I was able to build a solid business plan at the time of ExoCoBio's establishment. Of course, since exosome technology is in its infancy, and I thought there would be many changes in the future, I planned to develop various types of exosome-based esthetic technologies and products in my 5-year business plan (**Figure 1**).

In the commercialization of new technology such as exosomes, the most important thing was a series of financing to support the business plan. With my early ideas, I made lots of calls to my VC friends to pre-market the business plan for 6 months. At that time, I had about 15 years of experience in the venture capital industry and successful commercialization and IPO (Initial Public Offering) of two biotech companies. With this background, I felt I could incorporate ExoCoBio in Jan 2017. Just after 3 months, I was able to raise about USD 12 million in March 2017 through Series A financing and angel financing from multiple venture capital firms and individuals. It was the biggest financing as Series A funding for a biotech startup in South Korea.

Our first office was small. After setting up my business plan and team building, ExoCoBio was incorporated in a tiny office of about 270 SqFt outside Seoul, South

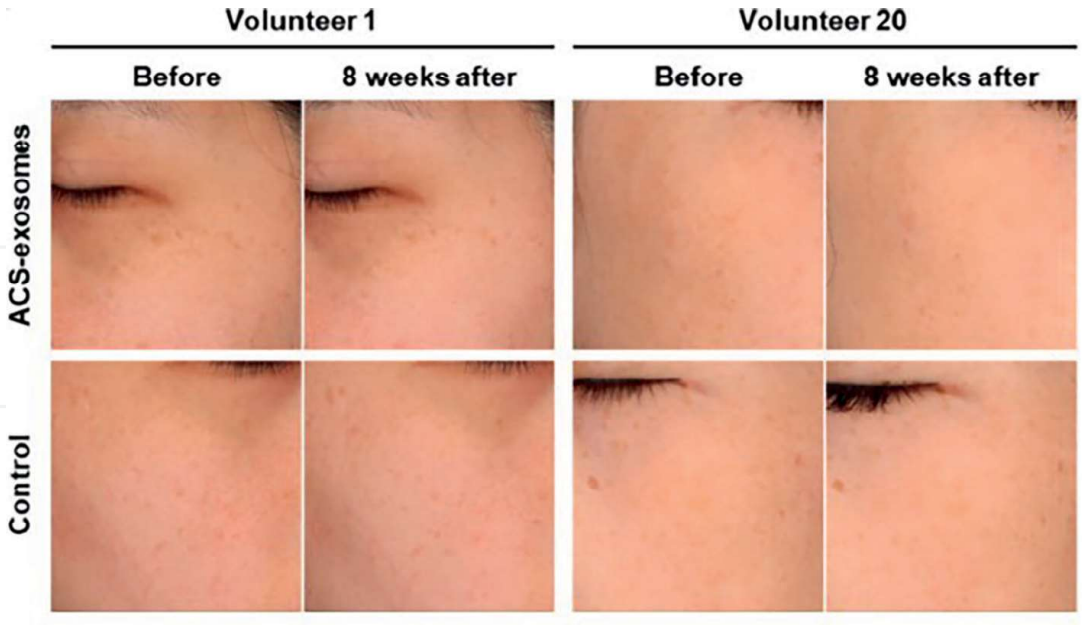

Figure 1.
Clinical evaluation of the skin brightening effect of ASC-exosomes.

Korea, in Jan 2017. The company successfully raised about $2 million from individual investors to get the first office and ExoCoBio got a small and humble laboratory in a university to start to develop our own manufacturing process of cell culture, TFF process, in vitro tests, and others.

One of the most significant jobs in 2017 was to register our own exosomes based on adipose stem cells (ASCE) to the ICID of the PCPC in the US. Since I knew that cosmetic registration was critical to commercialize this new exosome technology, and, I wanted to be the first in the world, the registration process was started immediately after the financing around April 2017. It took about 9 months which was longer than expected, because there was no predicate ever of cosmetic exosomes. With all the efforts, ExoCoBio became the first to have the International Nomenclature Cosmetic Ingredient (INCI) name and, still now, ExoCoBio has proudly the biggest number of exosome registration in the ICID.

The last job to develop a successful exosome business in 2017 was to find a partner who can do market this new technology and sell our products even before we have an actual product of ASCE 2 years ago. So, I was introduced to BENEV Inc. in California, US, which had about 20 years of experience in growth factor-based esthetic products worldwide. When I first met Mr. Ethan Min at his office in May 2017, in Mission Viejo, CA, we did talk a lot about our experiences and business plan including the product concept for several hours. Very fortunately, we had the same mind! He gave me important insight into the future of regenerative esthetics based on exosomes and then we initiated to collaborate to be the first and to create a new industry.

3. Were there any sources (say bone marrow) that you tried and discarded? Why choose ADSCs for cosmetic use?

This is a very good question. It seems that many scientists and doctors have a misunderstanding about the source and quality of exosomes, especially about adipose stem cell-derived exosomes (ASCE).

So far, ExoCoBio has been focusing on adipose stem cell-derived exosome technology.

In 2019, ExoCoBio conducted an internal comparison of three types of stem cell exosomes. In other words, we cultured three types of adipose tissue-derived stem cells, umbilical cord-derived stem cells, and Wharton Jelly-derived stem cells and isolated exosomes from each. Then, when in vitro efficacy tests were done, there was no significant difference in efficacy. Through this series of internal studies, it was determined that adipose stem cell exosomes are the most commercially superior in terms of efficacy and cost.

Adipose stem cell exosomes produced by ExoCoBio have been proven with 8 scientific publications [7–12] and 48 patents including 8 US patents, that they will be the most effective in the esthetic field. For example, our clinical trial demonstrated that skin with acne scars was improved by the combined use of exosome derived from adipose tissue-derived mesenchymal stem cells with fractional CO_2 laser would provide synergistic effects on both the efficacy and safety of atrophic acne scar treatments [11].

As of March 11, 2023, there are 5609 "stem cell exosome" papers searched in Pubmed, including 625 "adipocyte stem cell exosome" papers, which is a significant proportion. I believe absolutely that ASCE has been scientifically validated. ExoCoBio is the only company that has performed a double-blinded, randomized, and split-face clinical study based on any kind of stem cell exosomes in the world. Also, according to a paper published by Xu, H. et al. in 2019 (41), it is known that adipose stem cell exosomes have the highest regenerative effect and contain the most growth factors for cardioprotection and anti-apoptotic effects than other exosome sources (**Figure 2**).

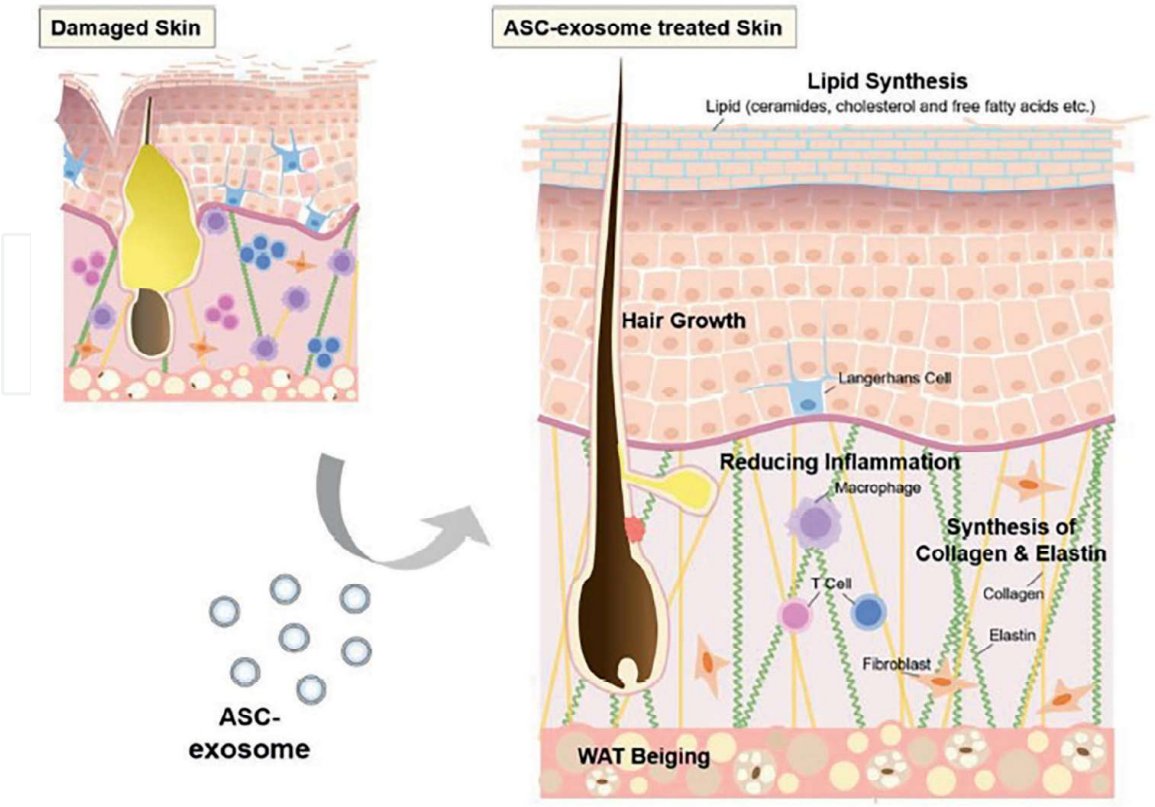

Figure 2.
Effects of ASC-exosomes on skin.

Some important discoveries made in the field of stem cells over the past 20 years, and as a result, several pros and cons were found. Exosomes derived from stem cell has been studied and the results of published articles demonstrated that exosome can overcome the cons of stem cell [13]. Stem cells and exosomes are different in many ways. In addition, adipose stem cell exosomes have the following advantages:

- Stem cells and stem cell exosomes are fundamentally different. Stem cells are live cells, but stem cell-derived exosomes are not live. In terms of the risk of adverse effects, since exosomes are not living cells, there is a much lower risk of adverse effects, such as immune rejection, tumor formation, and infection, which can occur with stem cell transplantation.

- There are a variety of stem cells, but each seems to have different characteristics. In particular, in terms of anti-inflammatory effect, adipose stem cells and their exosomes are judged to be the most excellent [14]. According to our unpublished findings, ASCE contains a huge number of miRNA-let-7, as the most abundant miRNA among about 200 types of miRNAs inside exosomes. Actually, miRNA-let-7 is the most well-known anti-inflammatory as well as an anti-cancer miRNA [15].

- The largest organ in the human body is the "skin" [16]. The skin is the most important organ that protects us from the external environment. In addition, "subcutaneous fat" is the organ with the biggest number of stem cells in our body [14]. Therefore, adipose stem cells, as one of the most critical parts of our skin, constantly secrete exosomes to protect us and keep our skin healthy and young.

- In addition, when researchers compare and analyze the three types of stem cells, adipose stem cells are usually compared after being collected from adults. In this case, of course, the cell passage number, which is the number of times that the culture has been sub-cultured, is high, so it is not fair to compare these with younger stem cell populations. If we compare these with umbilical cord-derived stem cells, for example, at passage 5, we think we should compare adipose stem cells with the same passage number. However, since most papers compare stem cells with different passage numbers, it is presumed that there are significant errors in comparative data.

- Moreover, exosomes seem to have significantly different efficacy depending on the way they are produced. ExoCoBio has been continuously developing and upgrading the patented ExoSCRT™ technology (KR Patent No. 10–1,895,916) for mass manufacturing of exosomes at the highest and consistent quality in compliance with the Good Manufacturing Practice (GMP), with 4 master cell banks created which store autologous adipose tissue-derived stem cell at passage 2 with stem cell positive markers and without negative markers.

- Lastly, adipose stem cells are the most accessible cell source of the lowest cost. Through this, it is possible to produce exosomes at the most affordable price.

4. Were there any processes production wise that you tried and changed?

In my view, there are a few important decisions to make or still to develop something to have better exosomes. ExoCoBio has tried to improve some parts in the last few years.

First, we need to think about the purity and impurities of exosomes. This is also related to regulatory affairs. During the last two decades or more, multiple clinical studies [17, 18] have shown that stem cells, including allogeneic stem cell treatments, are generally safe. So, it is very natural that stem cell-derived exosomes are believed to be generally safe, even though we need to do an extensive toxicological evaluation in the future. How many impurities can a product contain and retain safety? For example, any blood-derived exosomes may naturally contain lots of lipoproteins or proteins aggregate during the isolation process. Though not toxic, the presence of these may dilute the efficacy of the exosome product.

Second, the choice of a storage buffer is very important to maintain the potency of exosomes in the long term. For many companies and scientists, exosomes including stem cell exosomes are produced and stored in a phosphate-buffered saline (PBS) or similar simple buffer, which can easily degrade the quality of exosome in a short time, as published in the Journal of Extracellular Vesicles by Dr. Samir Andaloussi et al. in 2022. Dr. Andaloussi discovered that exosomes are drastically fractured at all temperatures of +4 ~ −80 degrees in Celsius tested. This may be a reason why many liquid exosome products currently available on the market have limited or less efficacy in the field. ExoCoBio has been focused on developing its proprietary formulation for long-term stability.

Third, lyophilization is the best process for the long-term storage of exosomes at present. Combined with a specific formulation of storage buffer, lyophilization can extend and keep the quality of exosomes shelf stable for 2 years. Two different groups of scientists have proven that they could produce exosomes in compliance with the GMP procedure a few years ago [19, 20]. Liquid or frozen exosomes are stable only for a limited time or only in a special formulation. A challenge in clinical practice is the storage of exosomes in a cryo tank or a special freezer that generates temperatures of −80 degrees Celsius. If the cryo tank runs out of nitrogen, or the power is off during the weekend, the costly exosomes are no longer usable. Stability on the shelf at room temperature or in a standard refrigerator can help a clinic maintain safety standards more easily than with demanding specialized laboratory equipment.

Fourth, pure exosomes are efficacious, but the effects could be further improved. From our studies, exosomes are found to be significant "seeds" or triggers for regenerative or anti-inflammatory effects, because exosomes contain a huge number of growth factors, cytokines, short-chain RNAs, peptides, and lipids. However, for example, when we added specific amino acids or other small peptides, efficacy was better for regeneration as well immune modulation in our experiments. Furthermore, due to the high cost of pure exosomes, market acceptance has been limited. ExoCoBio has decided to change its product strategy and become the first company to develop a combination of formulation processes in order to stimulate the potency of stem cell-derived exosomes. In this way, we could provide quality products based on ASCE. ExoCoBio is very proud to help people suffering from a variety of problematic skin conditions in addition to regular cosmetic uses around the world.

5. A similar question for extraction. Did you try ultracentrifugation and then discard that method? Why?

Since the beginning of 2019, ExoCoBio has conducted various studies on the method of isolating exosomes. There are about 9 different methods to extract

exosomes from conditioned media of stem cell culture. The most frequent way is ultracentrifugation, well known by many publications at that time [21].

We saw the advantages of ultracentrifugation for exosome isolation as follows:

- Easy to Use: Ultracentrifugation is one of the most effective methods for isolating exosomes from biological fluids and can provide high yields of pure exosomes.
- Versatility: Ultracentrifugation can be used to isolate exosomes from a wide range of biological fluids, including blood, urine, and saliva.
- Established protocol: Ultracentrifugation is a well-established technique for exosome isolation, with many published protocols and established best practices.
- Compatibility with downstream analysis: Exosomes isolated by ultracentrifugation are compatible with a wide range of downstream analyses, including Western blotting, ELISA, and electron microscopy.

However, the long-term goal of ExoCoBio was to provide high quality exosomes at an affordable price. From that point of view, there were these disadvantages of ultracentrifugation for mass manufacturing of exosomes as follows:

- Time-consuming: Ultracentrifugation can be a time-consuming process, especially when processing large volumes of the sample or separating exosomes from other vesicles with similar sedimentation rates.
- Expensive: Ultracentrifuges are expensive instruments that can require specialized training and maintenance, making them inaccessible to some researchers.
- Potentially damaging: Ultracentrifugation can potentially damage exosomes, especially if the conditions are not optimized for the specific sample being processed.
- Limited specificity: Ultracentrifugation can isolate a range of vesicles, including exosomes, but may also co-isolate other vesicles or non-vesicular components, reducing the specificity of the isolation method.

Therefore, ExoCoBio decided to utilize another technology and process, namely tangential flow filtration (TFF). This is a membrane-based separation technique used to separate and concentrate biological molecules and particles from a liquid sample. It involves passing the sample across a semipermeable membrane under pressure, allowing smaller molecules or particles to pass through the membrane while retaining larger molecules or particles on the membrane surface.

TFF could be used for a variety of applications that can process the large volume of stem cell conditioned media, including:

- Concentrating and purifying proteins, viruses, and other macromolecules.
- Removing impurities, such as salts, sugars, or detergents, from a sample.
- Clarifying cell culture media or biological fluids.

- Harvesting and concentrating cells or cell debris from a culture or fermentation broth.

Actually, at that time, ExoCoBio found that TFF was very similar to the serial filtration method used in the first publication of Dr. SK Lim about 15 years ago [2]. To find exosomes, Dr. Lim performed a series of filtration with different pore sizes to track down the paracrine effect of stem cell-derived exosomes.

TFF is a versatile and scalable technique, which can be easily adapted to process large or small volumes of a sample. It can be combined with other separation techniques, such as chromatography, to achieve a higher degree of separation or purification. TFF is widely used in bioprocessing, biopharmaceutical production, and research applications.

6. Now that you have your new facility, tell us what your company is currently doing

After 3 years and still ongoing about $20 million investment, ExoCoBio built the world's largest GMP mfg. facility of ASCE production, named ExoGMP™ in Osong, South Korea. The purpose of ExoGMP is to produce intravenously injectable grade exosomes that are fully GMP-compliant, for regenerative medicine and regenerative esthetics as well. Though neither KFDA nor FDA approval for this use has been

Figure 3.
ExoGMP™ in Osong, South Korea.

achieved, the company plans to be ready with this type of product once regulatory approval has been achieved (**Figure 3**).

ExoCoBio has installed more than 200 instruments and equipment, trying to establish about 300 standard operating procedures (SOP). Our team is committed to the production of quality exosomes and finalizing all the qualifications to hopefully produce the first batch of clinical-grade exosomes in the second quarter of 2023. ExoCoBio plans to initiate a Phase 1 clinical study in 2025.

The future of exosomes in esthetic medicine looks strong. However, regulatory issues are still a hurdle. Currently, there are no approved uses for exosomes, either in the medical or esthetic field. While the FDA does not regulate topical cosmetic use, a provider cannot claim to be using the product "off label" if injecting into patients. To stay safe, all exosome use should remain as a topical cosmetic product until full regulatory approval has been obtained.

Many postulations regarding future uses for exosomes have been made. From curing cancer to the reversal of genetic mutations and epigenetic cellular change, the potential for exosome therapies appears to be broad and strong. A current challenge is reading the "message" or contents of each exosome. Targeted or programmed exosomes would be able to direct recipient cells to behave in a certain way. Because of the popularity of the term, many products claiming to have exosomes either have none or have a minimal amount. While exosomes are not living cells, proteins in the contents will degrade over time, so without proper storage formulation, long-term shelf stability is not possible. Exosomes are merely a vehicle for the message they contain. Once we can safely and cost-effectively tailor the directions for cellular change that these tiny particles carry, we can potentially direct recipient cells to repair, reverse such processes as methylation or senescence, and reacquire lost metabolic functions.

7. Plant-derived extracellular vesicles

Plant exosomes, also known as extracellular vesicles, are small membrane-bound vesicles that are released by plant cells into the extracellular space. They are similar in structure and function to exosomes found in animals and other organisms. Plant exosomes contain various molecules such as proteins, lipids, and nucleic acids, which can be delivered to target cells and tissues to regulate various biological processes [22].

Research on plant exosomes is still relatively new. However, plant exosomes are involved in various physiological and developmental processes, such as cell-to-cell communication, stress response, and defense mechanisms. They have also been shown to play a role in inter-kingdom communication, where they can be taken up by other organisms such as fungi and bacteria [23]. In terms of anti-inflammation or immune modulation, edible *P. lobata*-derived exosomes promoted M2 macrophage polarization [24].

On Pubmed (pubmed.ncbi.nlm.nih.gov), we can find about 450 publications. One of the earliest publications is about multivesicular bodies (MVBs)-derived exosomes [23]. Further, one of the most recent publications is about the drug-delivery approach based on plant-derived exosomes for the treatment of inflammatory bowel disease and colitis-associated cancer [25]. In this publication, the isolation of plant-derived exosomes was done by ultracentrifugation mostly and it was found that the intake of plant miRNA may have a variety of effects on our bodies.

One of the R&D projects of ExoCoBio was to expand and apply ExoSCRT™ technology into plant- or microbial-derives extracellular vesicles or exosomes, to find new

material. In that way, I was very interested in rose stem cell (Callus) – derived EVs (RSCE), because (1) roses have been the most popular plant and a cosmetic ingredient for humans, (2) there has still no scientific discovery on the contents of the rose stem cell-derived exosomes. ExoCoBio has been researching to isolate and characterize RSCE for the last 3 years and found a few biological functions as follows (As of now, all the data on RSCE are unpublished, to be submitted for publication soon.):

- Rose stem cell exosomes (RSCE) were obtained by separating and refining the RSC culture supernatant. The physical characteristics of the lipid membrane and the sizes of 30–200 nanometers were confirmed through Nano Tracking Analysis (NTA) and Transmission Electron Microscope (TEM) (**Figures 4** and **5**).

- Preliminary miRNA analysis revealed that RSCE has more than 1000 kinds of miRNA which are mostly de novo sequences. Only about 30 kinds of them have been matched to human-derived miRNA sequences. Most of them are related to housekeeping functions.

- RSCE could increase the collagen production of human dermal fibroblasts by 40–120% in a dose-dependent manner and promote cellular migration by more than 20%.

- RSCE was found to have an anti-inflammatory function that the IL-6 production of macrophages was also reduced to 50–60%, depending on its concentration (**Figure 6**).

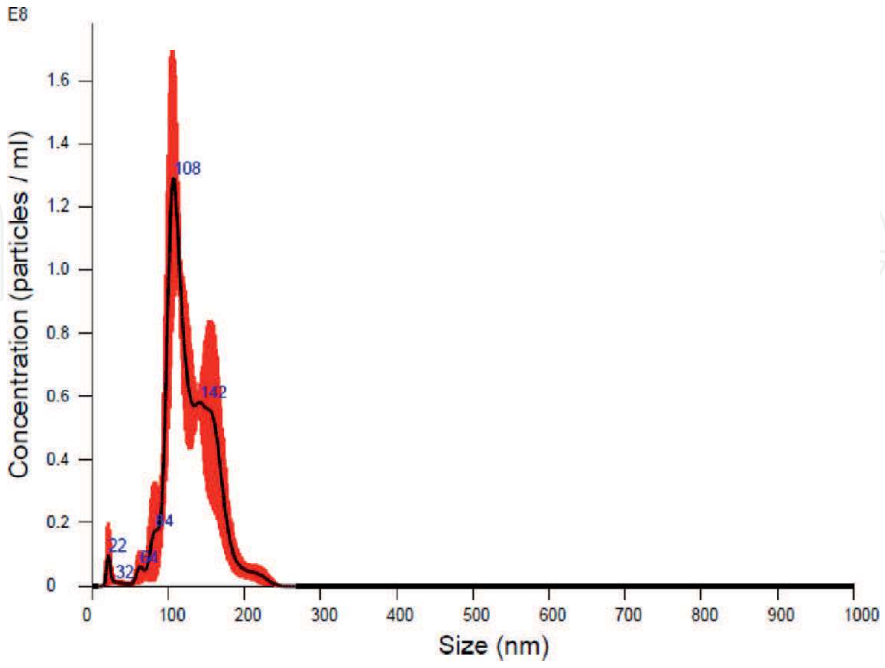

Figure 4.
RSCE NTA analysis. Source: ExoCoBio Inc. (unpublished data).

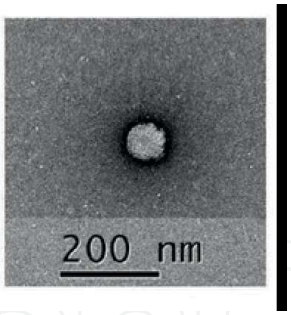

Figure 5.
RSCE TEM image. Source: ExoCoBio Inc. (unpublished data).

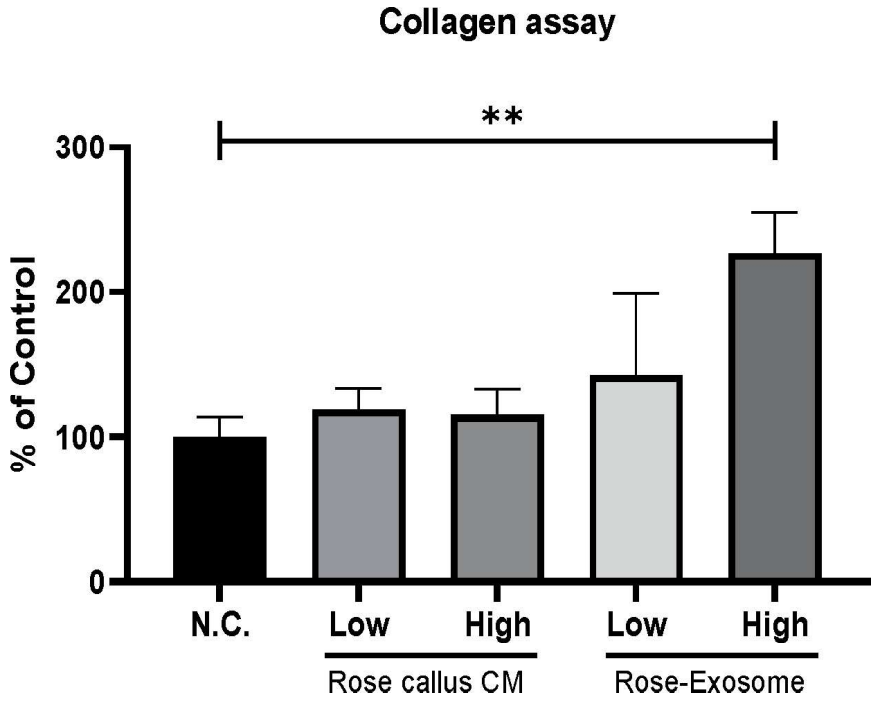

Figure 6.
*Collagen synthesis of RSCE in human dermal fibroblasts (HDF). *note: Rose callus CM is the supernatant of the stem cell (callus) culture of rose. Source: ExoCoBio Inc. (unpublished data).*

One disadvantage of plant-derived exosomes is that there is no universal quality standard yet. Many of the previous studies were done before the announcement of the minimal requirements established by the International Society of Extracellular Vesicles (ISEV). So, we need to be cautious in evaluating the data and results and make sure how to isolate and characterize plant-derived EVs. Moreover, the biogenesis pathways of plant-derived exosomes are also not well defined yet. Classifying plant-derived exosomes with the current terms used for animal EVs is still difficult due to a lack of current level of scientific discovery.

I believe that when we consider that microbes or bacteria are releasing extracellular vesicles and that kind of biology is universal across the kingdoms and species, it is very worth researching plant-derived EVs indeed for next-generation plant biology. ExoCoBio and other companies are trying to commercialize them as cosmetics or skincare products on the market.

8. Previous Writing

8.1 What is skin?

Our skin is a vital and complex organ that plays a crucial role in maintaining our overall health and well-being. Skin is the largest organ of the human body, and it is the outermost layer that covers and protects our entire body. It is a complex and multifunctional organ that serves many purposes. Some of the primary functions of the skin include:

- Protection: The skin acts as a barrier against harmful environmental factors such as UV radiation, pollution, and bacteria, which can cause damage or infection.
- Sensation: The skin contains a vast network of nerve endings that detect sensations such as touch, pressure, temperature, and pain.
- Thermoregulation: The skin helps to regulate body temperature by releasing sweat, which cools the body down, and constricting or dilating blood vessels to conserve or release heat.
- Hair: The skin plays an essential role in the growth and maintenance of hair. Hair is produced by hair follicles, which are small structures located in the dermis (the middle layer of the skin). The hair follicles contain specialized cells called keratinocytes, which produce the hair shafts that grow out of the follicle.
- Vitamin D synthesis: The skin produces vitamin D when exposed to sunlight, which is essential for maintaining healthy bones and teeth.

8.2 The structure of skin

The skin is the largest organ of the human body and has three main layers:

- Epidermis: This is the outermost layer of the skin and is made up of several layers of cells. The top layer of the epidermis is called the stratum corneum, which is composed of dead skin cells that have been shed from the skin surface. Further, we have a great skin barrier generated by a lipid layer of key lipid molecules like ceramides or dihydroceramides, or others. This skin barrier is critical for our skin health and aging. The epidermis also contains melanocytes, which are responsible for producing the pigment melanin that gives the skin its color.
- Dermis: This is the middle layer of the skin and contains various types of connective tissue, including collagen, elastin fibers, and hyaluronic acids. It also houses hair follicles, sweat glands, and sebaceous glands. The dermis is responsible for providing the skin with its strength and elasticity.
- Subcutaneous tissue: This is the innermost layer of the skin and is made up of adipose tissue (fat) and connective tissue. This layer contains the biggest number of stem cells in our body, which means that adipose stem cells and their exosomes are critical for skin health and aging, even though this is not well known previously. It helps to regulate body temperature and provides cushioning for the body's organs and bones.

8.3 The skin aging

Skin aging is a natural process that occurs as we age, and it is characterized by various changes in the skin's appearance, texture, and function. Some of the most common signs of skin aging include:

- Wrinkles and fine lines: As we age, the skin loses its elasticity, and wrinkles and fine lines begin to appear.
- Age spots: These are brown or black spots that appear on the skin, often on the face, hands, or arms, as a result of prolonged sun exposure.
- Dryness: As we age, the skin becomes drier and less able to retain moisture, leading to a rough, scaly texture.
- Thinning of the skin: The skin becomes thinner and more fragile as we age, making it more susceptible to injury.
- Loss of collagen and elastin: Collagen is a protein that gives the skin its strength and elasticity, and as we age, the production of collagen decreases, leading to sagging and loss of firmness.

9. Current trend in skin rejuvenation & exosomes

The trend in skin rejuvenation currently is towards non-invasive, minimally invasive, and more importantly, regenerative procedures that provide natural-looking results with little to no downtime. Patients are increasingly seeking out treatments that can address a variety of skin concerns, including wrinkles, fine lines, sun damage, and loss of volume, without the need for surgery or extensive recovery time. Some of the most popular non-invasive and minimally invasive treatments for skin rejuvenation include:

- Neurotoxins: Botulinum toxin injections, such as Botox® and Dysport®, can be used to temporarily reduce the appearance of wrinkles and fine lines caused by facial expressions.
- Dermal fillers: Injectable dermal fillers can be used to restore volume to the face, reduce the appearance of wrinkles and fine lines, and enhance facial features.
- Energy-based devices: Energy-based devices, such as lasers, radiofrequency, ultrasound, and light therapy, can be used to stimulate collagen production, tighten the skin, and reduce the appearance of wrinkles and fine lines.
- Chemical peels: Chemical peels use a solution to remove the outer layer of skin, revealing smoother, more youthful-looking skin.
- Microneedling: Microneedling involves using a device to create small punctures in the skin, stimulating collagen production and improving the texture and appearance of the skin.

- Platelet-rich plasma (PRP) therapy: PRP therapy involves injecting a concentrated solution of the patient's blood platelets into the skin, stimulating collagen production, and promoting tissue repair.

- Stem Cells: Stem cells have shown great promise in skin rejuvenation due to their ability to differentiate into various types of cells, including skin cells. In skin rejuvenation, stem cells can be used in various ways. Stem cells can be injected or applied topically to the skin, where they can release growth factors and cytokines that promote the growth of new cells, stimulate collagen production, and reduce inflammation. Another approach is to use stem cells to enhance the effects of other skin rejuvenation treatments, such as dermal fillers or energy-based devices. Stem cells can be mixed with dermal fillers or injected into the skin before or after energy-based treatments to improve their efficacy and enhance tissue repair.

- Exosomes: Exosomes are small vesicles that are released by cells, including stem cells. They contain various molecules, including proteins, lipids, and nucleic acids, that can regulate cellular functions and promote tissue repair and regeneration. Exosomes have emerged as a promising new approach to skin rejuvenation due to their ability to stimulate collagen production, promote tissue repair, and reduce inflammation. Exosomes can be obtained from various sources, including mesenchymal stem cells, and can be applied topically or injected into the skin. When applied to the skin, exosomes can penetrate the epidermis and dermis, where they can stimulate fibroblasts and other cells to produce collagen, elastin, and other extracellular matrix proteins that improve skin texture and reduce the appearance of fine lines and wrinkles. Exosomes can also promote tissue repair and reduce inflammation by modulating immune responses and promoting the growth of new blood vessels. This can help to improve skin health and reduce the risk of skin damage caused by environmental factors such as sun exposure and pollution. Clinical studies have shown promising results for the use of exosomes in skin rejuvenation.

10. Adipose stem cells and aging

Adipose stem cells, also known as adipose-derived stem cells (ASCs), are a type of stem cell found in adipose tissue (fat tissue). These cells have the ability to differentiate into various cell types, including adipocytes (fat cells), chondrocytes (cartilage cells), and osteocytes (bone cells). Adipose stem cells also have the strongest anti-inflammatory and regenerative properties, which make them valuable in the field of regenerative medicine.

During aging, there is a gradual loss of adipose stem cells in the body including facial skin and scalp, which can contribute to various age-related health problems. This loss of stem cells is thought to be due to a combination of factors, including decreased stem cell proliferation and increased cell death.

As the number of adipose stem cells decreases with age, the body's ability to regenerate and repair damaged tissues also declines. This can lead to a range of skin and health problems, including slower wound healing, more inflammation in the skin and other organs, decreased muscle mass, and decreased bone density.

Scientists have been actively researching ways to preserve and replenish adipose stem cells in the body, in order to promote better health and slow down the aging

process. One promising approach involves the use of stem cell therapy, which involves the transplantation of stem cells into the body to replace damaged or depleted cells.

Recently, exosomes derived from adipose stem cells are being applied to treat a variety of diseases including dermatological and esthetic uses. Exosome esthetics refers to the use of exosomes in cosmetic procedures and treatments to improve the appearance of the skin, hair, and other parts of the body. In the field of esthetics, exosomes are used to stimulate the growth and regeneration of skin cells, reduce inflammation, and improve the overall appearance of the skin. Exosome-based treatments can be used to address a variety of cosmetic concerns, including fine lines and wrinkles, age spots, uneven skin tone, and acne scars. These treatments may involve the injection or topical application of exosomes directly to the skin or hair follicles. The exosomes can be derived from various sources, including mesenchymal stem cells, which are known to produce particularly potent exosomes with regenerative properties.

10.1 Exosomes for clinical applications

Exosomes are nano-sized vesicles of 30–200 nanometers that are released by cells and contain a variety of biomolecules, including proteins, lipids, and nucleic acids such as cytokines, growth factors, & microRNAs. Exosomes play important roles in cell-to-cell communication and have been found to have a wide range of potential clinical applications.

Here are some examples of the clinical applications of exosomes:

- Regenerative medicine: Exosomes have regenerative properties and can be used to promote tissue repair and regeneration. They have been studied for their potential use in treating various conditions, such as heart disease, liver disease, and neurodegenerative diseases.

- Anti-aging treatments: Exosomes have been studied for their potential use in anti-aging treatments. They contain growth factors and other molecules that can promote tissue repair and regeneration, which may help to slow down the aging process.

- Anti-inflammatory function: Stem cell exosomes, especially adipose stem cell-derived exosomes, have been found to have anti-inflammatory properties, which is one of the reasons they have potential therapeutic applications in various inflammatory diseases. For example, adipose stem cell exosomes have been found to inhibit the production of pro-inflammatory cytokines, such as TNF-alpha and IL-6, and promote the production of anti-inflammatory cytokines, such as IL-10. This can help to suppress the inflammatory response and promote tissue healing and regeneration like dermatitis or inflammatory bowel diseases.

- Drug delivery: Exosomes can be used as a vehicle for drug delivery, as they are capable of crossing biological barriers such as the blood-brain barrier. This makes them a promising tool for the targeted delivery of drugs to specific cells or tissues.

- Cancer treatment: Exosomes have been studied for their potential use in cancer treatment. They can be engineered to carry therapeutic agents, such as drugs or

small interfering RNAs (siRNAs), to cancer cells. Additionally, exosomes can be used to stimulate the immune system to attack cancer cells.

- Diagnosis and monitoring of diseases: Exosomes contain biomolecules that can serve as biomarkers for various diseases. This makes them a potential tool for the diagnosis and monitoring of diseases, such as cancer and neurodegenerative diseases.

10.2 The differences & combination between exosomes and current technologies

Botulinum toxin is a neurotoxic protein produced by the bacterium *Clostridium botulinum*. This toxin is known to cause a severe form of food poisoning called botulism, which can be fatal in some cases. However, botulinum toxin has also been found to have therapeutic uses, particularly in the field of cosmetic and medical dermatology. In dermatology, botulinum toxin is used as a muscle relaxant to temporarily paralyze facial muscles and reduce the appearance of wrinkles and fine lines. The injection of botulinum toxin blocks the release of acetylcholine, a neurotransmitter that signals muscle contraction. This causes the targeted muscles to relax and reduces the appearance of wrinkles and fine lines.

Exosomes and botulinum toxins are two very different substances with distinct mechanisms of action and therapeutic applications. While both exosomes and botulinum toxins have potential applications in dermatology, they work through different mechanisms and have different therapeutic effects. Exosomes promote tissue repair and regeneration and have anti-inflammatory effects, while botulinum toxins are primarily used to reduce muscle activity and smooth wrinkles.

Dermal fillers are injectable substances used to restore volume and fullness to the face, reduce the appearance of wrinkles and fine lines, and enhance facial features. They are typically composed of a variety of materials, including hyaluronic acid, calcium hydroxylapatite, poly-L-lactic acid, and polymethylmethacrylate beads. Hyaluronic acid fillers are the most commonly used type of dermal filler. Hyaluronic acid is a naturally occurring substance found in the body that helps to hydrate and plump the skin. When injected into the skin, hyaluronic acid fillers can restore volume to the face, smooth out wrinkles and fine lines, and enhance facial features, such as the lips and cheeks. Mostly, dermal fillers are primarily used to restore volume to the face and reduce the appearance of wrinkles and fine lines. Only a few fillers have collagen-boosting effects, while exosomes can promote tissue repair and regeneration and have anti-inflammatory effects synergistically.

Energy-based devices for skin are non-invasive or minimally invasive devices that use various types of energy, such as light, radiofrequency, ultrasound, or laser, to improve the appearance of the skin. These devices can be used to address a range of skin concerns, including wrinkles, fine lines, sagging skin, hyperpigmentation, and acne scars. Some common types of energy-based devices for skin include:

- Laser: Lasers emit concentrated beams of light that are absorbed by the skin to stimulate collagen production and reduce the appearance of wrinkles, fine lines, and hyperpigmentation.

- Radiofrequency: Radiofrequency devices use electric energy waves to heat the skin and stimulate collagen production, resulting in tighter, firmer skin.

- Ultrasound: Ultrasound devices use sound waves to heat the skin and stimulate collagen production, resulting in tighter, firmer skin.

- Light therapy: Light therapy devices use different wavelengths of light to improve the appearance of the skin. For example, blue light therapy can help to kill acne-causing bacteria, while red light therapy can stimulate collagen production and reduce the appearance of fine lines and wrinkles.

Exosomes have been proven to work synergistically with these energy-based devices in dermatology. While exosomes give bio-stimulating signals to cells in the skin, energy-based devices provide manageable damage. Both mechanisms of action showed significant improvement to treat acne scars in combination with fractional CO2 laser and adipose stem cell exosomes recently.

11. Stromal vascular fraction (SVF), nanofat, and exosomes

SVF, Nanofat, and exosomes have shown promise in skin rejuvenation and other regenerative medicine applications. Stromal vascular fraction (SVF) and exosomes are both derived from stem cells and have been investigated for their potential applications in regenerative medicine and skin rejuvenation. However, there are some key differences between the three:

- SVF is a mixture of cells, including adipose-derived stem cells, as well as other cell types such as endothelial cells, smooth muscle cells, and immune cells. SVF also contains extracellular matrix proteins and growth factors that can promote tissue repair and regeneration.

- Nanofat is a minimally invasive cosmetic treatment that uses a small amount of the patient's fat to promote tissue regeneration and rejuvenation. The term "nanofat" refers to the small size of the fat particles that are injected, which are typically less than 500 microns in diameter. During the procedure, a small amount of fat is harvested from the patient using liposuction. The fat is then processed and emulsified to create a solution of small fat particles. The nanofat solution is then injected into the desired area of the body, such as the face or hands, using a fine-gauge needle.

- Exosomes, on the other hand, are tiny vesicles that are secreted by cells, including stem cells. Exosomes contain various molecules, including proteins, lipids, and nucleic acids, that can regulate cellular functions and promote tissue repair and regeneration. Stem cell exosomes are not or minimally immunogenic, which means that they are generally recognized as safe.

One key difference between SVF/Nanofat and exosomes is that SVF/Nanofat contains a mixture of cell types, while exosomes contain only the molecules that are secreted by stem cells. This means that exosomes are a more focused approach to stem cell-based therapies, as they target the specific molecules that are responsible for promoting tissue repair and regeneration. Another difference between SVF/Nanofat and exosomes is that SVF/Nanofat is typically obtained autologously by processing

adipose tissue, while exosomes can be obtained from a variety of cell sources, including adipose-derived stem cells, bone marrow-derived stem cells, and mesenchymal stem cells. SVF/Nanofat is an autologous treatment while exosomes can be allogenic as "off-the-shelf products."

12. Key exosomes science & patents for dermatological use

Exosome research is a rapidly evolving field with many scientists and researchers contributing to its development. There are two famous scientists in exosome research:

- Dr. Jan Lötvall: He is a Swedish immunologist who is widely regarded as one of the pioneers of exosome research. He discovered the RNA transfer medicated by exosomes for the first time in the world in 2007 and his publication has the biggest citation number of 12,000 times in exosome science. He has made important contributions to the understanding of exosome biology and function, and his research has paved the way for the development of exosome-based therapies. He founded the International Society of Extracellular Vesicles (ISEV) and is the Chief Editor of the Journal of Extracellular Vesicles (JEV) at present. He has been doing a lot of jobs to set the standards of exosomes for years.

- Dr. Sai Kiang Lim: She is the first discoverer of stem cell-derived exosomes, which defined the "paracrine effect" of stem cells. Now she is a Senior Principal Investigator and Deputy Director at the Institute of Medical Biology at A*STAR in Singapore. Dr. Lim's research has focused on the use of stem cell-derived exosomes for tissue engineering and regenerative medicine applications. She has been developing stem cell exosomes as drugs for various inflammatory diseases.

In last years, lots of scientists and companies have created key patents for esthetic and dermatological uses based on exosomes. They are stem cell, plant, & microbe-derived, as follows:

- US Patent No. 10,071,050: This is the first patent on the skin rejuvenation effect of adipose stem cell exosomes in the world. It relates to a cosmetic composition for skin whitening, wrinkle improvement, or skin regeneration and includes, as an active ingredient, exosomes derived from stem cells comprising proliferating stem cells.

- US Patent No. 11,529,306: The lyophilized formulation of stem cell-derived exosomes and the anti-inflammatory composition including the same as an active ingredient is able to stabilize stem cell-derived exosomes and exhibit excellent anti-inflammatory effects, and particularly, exhibit remarkable anti-inflammatory effects as compared with not-lyophilized stem cell-derived exosomes isolated and purified from conditioned media of stem cells. Therefore, the lyophilized formulation of stem cell-derived exosomes and the anti-inflammatory composition including the same as an active ingredient is able to effectively prevent, suppress, alleviate, ameliorate, or treat inflammatory response or inflammatory diseases.

- US Patent No. 11,529,370: This patent is about composition for strengthening the skin barrier or improving skin barrier function that is able to improve objective indicators related to the protection of the skin barrier, the strengthening of the

skin barrier, and/or the improvement of skin barrier function. The composition exhibits the effects of increasing the number of ceramides, dihydroceramides, and sphingoid bases, increasing the activities of enzymes that are involved in the synthesis thereof, and decreasing the activities of enzymes that are involved in the degradation thereof. In addition, the composition is able to restore skin barrier function by reducing TSLP, IL-4, and IL-13 which are closely associated with skin barrier damage, thus interrupting a vicious circle in which the lipids and proteins contributing to skin barrier decrease.

- US Patent No. 11,446,333: The present invention provides a composition for preventing, suppressing, alleviating, ameliorating, or treating pruritus comprising stem cell-derived exosomes as an active ingredient. The composition of the present invention is able to act against pruritus-inducing multiple cytokine targets, for example, IL-4, IL-31, and TSLP, and thus is able to be widely applied against pruritus caused by various factors and is able to effectively suppress and alleviate pruritus. In addition, when the composition of the present invention is applied directly to human skin, it is able to remarkably ameliorate pruritus-associated clinical scores, erythema, and the like. Thus, the composition of the present invention is able to be used as a pharmaceutical composition, a skin external preparation, and a cosmetic composition for preventing, suppressing, alleviating, ameliorating, or treating pruritus.

- US Patent No. 11,337,419: This patent relates to a method of lyophilizing exosomes using a cryoprotectant comprising methionine, mannitol, and trehalose is disclosed. The lyophilized exosome product shows a good appearance which maintains a porous sponge shape without forming ice crystals. In addition, the lyophilized exosome product can be applied to a pharmaceutical composition, a skin external preparation, and a cosmetic composition. For example, the lyophilized exosome product can be used as a solution obtained by simply mixing it with a diluent.

- WO 2020022731: This patent is the first plant stem cell-based in the world. The present invention provides a cosmetic composition comprising rose stem cell-derived exosomes as an effective ingredient for skin regeneration, skin elasticity improvement, or skin wrinkle reduction. The cosmetic composition of the present invention has excellent effects on skin regeneration, skin elasticity improvement, and/or skin wrinkle reduction.

- US Patent No. 11,534,392: This is a cosmetic composition including exosomes derived from Galactomyces as an active ingredient that is provided for skin regeneration, skin elasticity improvement, or skin wrinkle reduction. Cosmetic composition has excellent effects on skin regeneration, skin elasticity improvement, and/or skin wrinkle reduction.

13. Exosomes manufacturing & quality standards

Almost every cell is releasing exosomes so there are a huge number of types and sources of exosomes. So, it is very important to set up quality standards to produce exosomes for commercialization and clinical applications. There is currently no universally accepted quality standard for exosomes, as the field of exosome research is

still evolving and the properties and characteristics of exosomes can vary depending on the source, isolation method, and intended use.

However, the ISEV has been trying to give minimal requirements on exosome standards from time to time. And there are generally accepted guidelines and best practices that have been proposed for the characterization and quality control of exosomes. These include:

- Size and morphology: Exosomes should be characterized by their size and morphology using techniques such as electron microscopy, dynamic light scattering, or nanoparticle tracking analysis. The size range of exosomes is typically between 30 and 200 nm, and they should exhibit a characteristic cup-shaped morphology.

- Protein markers: Exosomes should be analyzed for the presence of specific protein markers that are typically associated with exosomes, such as CD63, CD81, and CD9. The absence of contaminating proteins or markers from other cellular components should also be confirmed. Unfortunately, there are no generally accepted protein markers existing on or inside bacterial or plant exosomes, which needs vigorous research in the future.

- Nucleic acid content: Exosomes should be analyzed for their nucleic acid content, including DNA, RNA, and miRNA. The quantity and quality of nucleic acids can provide valuable information on the biological activity and potential therapeutic applications of exosomes.

- Purity and concentration: Exosomes should be purified to remove contaminants and characterized for their concentration using appropriate techniques such as Bradford assay, BCA assay, or nanodrop. Especially, blood-derived exosomes contain lots of lipoproteins or protein aggregates which are 100 times more than actual exosomes. A simple manufacturing process can not discriminate those impurities from exosomes due to their very similar size.

- Functional assays: Finally, exosomes should be tested for their biological activity using appropriate functional assays. For example, exosomes derived from stem cells should be evaluated for their ability to promote tissue regeneration or reduce inflammation, while exosomes intended for drug delivery should be evaluated for their efficacy and safety.

In particular, to get the best quality of exosomes, the manufacturing process must be Good Manufacturing Practices (GMP)-compliant. The ISEV and the International Society of Cellular Therapy (ISCT) gave a guideline for GMP manufacturing, for example, which requires the creation of master cell banks (MCB) and working cell banks (WCB) to get consistent stem cell quality. Those MCB or WCB are required to initiate stem cell therapy worldwide, while blood-derived exosomes are limited in terms of consistent quality due to a lack of cell bank establishment.

While these guidelines can help ensure the quality and consistency of exosome preparations, it is important to note that the field of exosome research is still evolving and there is ongoing debate and discussion around the best practices for exosome characterization and quality control.

13.1 Exosome stability & long-term storage

The stability of exosomes is an important factor that can affect their safety and efficacy. Exosomes are sensitive to environmental conditions such as temperature, pH, and oxidation, which can affect their structural integrity and functional activity. Therefore, proper storage and handling of exosomes are critical to maintaining their stability and potency.

Some factors that can affect exosome stability include:

- Lyophilization: The best storage condition for exosomes is considered lyophilization as published by 3 groups so far. Lyophilization removes water molecules from the exosome raw material and prevents protein or RNA degradation. Liquid- or frozen-exosomes are found to be very short-term stable around 3 ~ 6 months, depending on the storage temperature like −20 ~ −80°C.

- Temperature: Exosomes are sensitive to changes in temperature, and high temperature can damage their structure and function. Exosomes can be stored at a temperature between 2 and 8°C for long-term storage of up to 3 years after lyophilization, and, −20 ~ 80°C for short-term storage.

- pH: Changes in pH can also affect exosome stability. Exosomes should be stored in a buffer solution with a pH between 7 and 8 to maintain their stability.

- Oxidation: Exposure to oxygen can cause oxidative stress and damage to exosomes. Therefore, exosomes should be stored in an environment free from oxygen or under reduced oxygen conditions.

- Freezing and thawing: Repeated freezing and thawing can damage the structural integrity of exosomes. Therefore, it is important to avoid repeated freeze-thaw cycles and to store exosomes in small aliquots.

- Contamination: Exosomes can be sensitive to contamination from bacteria, viruses, and other contaminants. Therefore, it is important to maintain strict aseptic techniques when handling and storing exosomes.

Overall, proper storage and handling of exosomes are critical to maintaining their stability and potency.

13.2 Exosome-based aesthetic products

A huge number of exosome-based cosmetic products are available on the market worldwide. The regulatory requirements for exosome-based cosmetic products vary depending on the country or region where the products are intended to be marketed. In general, exosome-based cosmetic products may need to comply with the following regulatory requirements:

- Ingredient safety: Exosome-based cosmetic products must use ingredients that are safe for use in cosmetics. The safety of the ingredients must be supported by

data from safety assessments, including toxicology studies, clinical studies, and other relevant data.

- Labeling: The labeling of exosome-based cosmetic products must provide accurate information about the product's ingredients, claims, and directions for use. The labeling must comply with the applicable regulations in the country or region where the product is intended to be marketed.

- Manufacturing: Exosome-based cosmetic products must be manufactured in compliance with Good Manufacturing Practices (GMP) to ensure product quality, safety, and consistency.

- Claim substantiation: The claims made about the product must be substantiated by scientific evidence. The evidence must be adequate to support the claims made in the labeling and advertising of the product.

- Registration/notification: Depending on the country or region where the product is intended to be marketed, exosome-based cosmetic products may need to be registered or notified to the regulatory authorities. The registration/notification requirements may vary depending on the product's intended use, claims, and ingredients.

In the United States, exosome-based cosmetic products are regulated by the Food and Drug Administration (FDA) or Personal Care Products Council (PCPC) as cosmetic products. The FDA does not require pre-market approval of cosmetic products.

To be a successful exosome product, it should be affordable with acceptable efficacy proved by non-clinical and clinical studies. The cost of exosome-based cosmetic products can vary depending on several factors, including the source of the exosomes, the manufacturing process, and the quality of the product. As exosome-based cosmetic products are still a relatively new technology, they may be more expensive than traditional cosmetic products. However, as the technology becomes more widely adopted, the cost of exosome-based cosmetic products may become more affordable.

It is important to note that affordability should not compromise safety and efficacy. Consumers should always choose exosome-based cosmetic products from reputable manufacturers, fully GMP-compliant and with lots of scientific publications, that have undergone rigorous safety and efficacy testing. The proven quality by a series of scientific publications and patents, rather than the simple number of exosomes, is the most important factor to choose products.

IN CONCLUSION, EXSOMES ARE NEW TECHNOLOGIES FOR ESTHETIC USE AND WE MUST CHOOSE SCIENTIFIC EVIDENCE-BASED EXSOME PRODUCTS.

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