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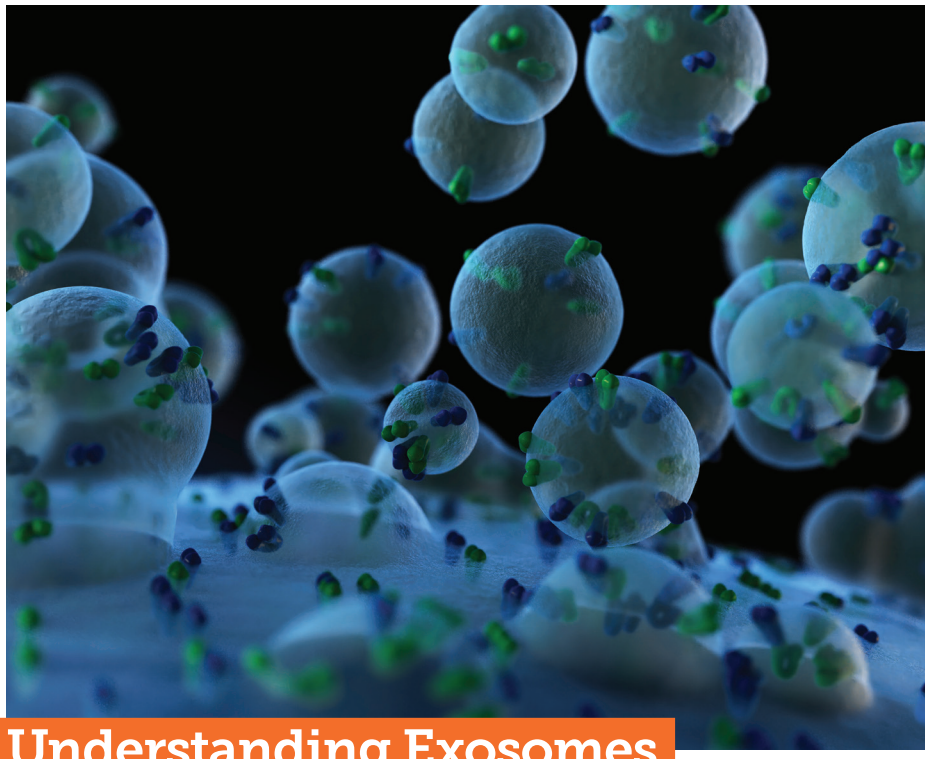

Understanding Exosomes

Mr Tunc Tiryaki and Serli Canikyan Turkay introduce exosomes in aesthetic regenerative medicine and present their latest clinical research

Unlike 10 years ago, there is a public interest from both patients and practitioners surrounding adipose-derived stem cells. These stem cells are suitable for the treatment of diseases such as autoimmune illnesses, soft tissue defects and even ageing.[1-3] Whilst we are still trying to understand this revolution in regenerative medicine, scientists and clinical researchers are already moving to the next stage, away from autologous stem cells to their messengers – exosomes. Therefore, it is time for practitioners to understand this revolution in progress, moving us from traditional medicine to stem cell treatments and further to exosome research.

Background

Back in 2001, the industry learnt that we could harvest stem cells from fat tissue.[4] Since then, many different methods have been created to isolate our own stem cells and use them for different medical problems and diseases. Today, autologous fat-derived stem cells are successfully used for soft tissue defects, cancer reconstruction, radiotherapy injury and other indications.[4-6] However, if we take a close look into the mode of action of these cell treatments, we discover that these stem cells do not only fill defects or reinforce the host area, but they also send messages or orders out to the local cells to regenerate. The messengers carrying orders of anti-inflammation and regeneration are called exosomes.[7]

Recently, studies focusing on reversing the ageing mechanism and extending the lifespan of human beings have attracted the great attention of scientists and physicians.[8-10] The article, *Ageing research: Blood to Blood* published in 2015, brought realism to ancient 'vampire stories' that have existed for centuries.[11] Parabiosis is a 150-year-old surgical technique which includes the vascular structures of two living animals (the word comes from the Greek *para*, meaning 'alongside', and *bios*, meaning 'life') mimicking the natural instances of shared blood supply, like conjoined twins.[12] Here, by joining the circulatory system of an old mouse to that of a young mouse, scientists have produced results with the heart, brain, muscles and almost every other tissue examined, the blood of young mice brought youth to older organs, making old mice healthier and stronger.[11,13] Furthermore, several clinical trials are taking place to test the benefits of young blood in older patients with diseases like Alzheimer's or Parkinson's.[14,15]

In the last few years, scientists have begun to identify the components of young blood that are responsible for these changes. This transfer of youth is carried by small messengers in the plasma component of the blood called exosomes. Exosomes are messenger particles that release naturally from a cell and are responsible for cell-to-cell communication. They carry genetic information and proteins to cells throughout the body and create paths for communication between cells. Also known as extracellular vesicles (particles that release naturally from a cell that cannot replicate) they are responsible for cell-to-cell communication. Exosomes are released naturally from cells upon the fusion of a discontinuous closed membrane system, also known as the intermediate endocytic compartment.[16-18] Exosomes have begun to emerge in the UK market with skincare products containing exosome ingredients. If used intradermally, intramuscularly or intravenously, all these treatments need approval from the Medicines & Healthcare products Regulatory Agency (MHRA) and US Food and Drug Administration (FDA) and require an Investigational New Drug Application (IND) submission.[19] This is because we cannot read and control what is written in those exosomes.[20-21]

> Our capacity to utilise exosomes will ensure these regenerative treatments are more reachable, effective and affordable

However, cultured human stem cell-derived exosomes have major drawbacks. As Good Manufacturing Practices (GMP) facility products, they are very sensitive to environment conditions and parameters such as pH and temperature affecting their function dramatically.[22] Another drawback is the ageing of stem cell cultures. After a few cell-division rounds, these cultures age, sending ageing exosomes out of the cell.[23] However, the most important drawback of GMP-derived exosomes is that as they are human-derived products, they cannot be sold as either medical devices or cosmetic products.[24]

While investigating potential alternatives to human-derived GMP based exosomes, the literature showed that we can harvest exosomes from different organisms, including plants and animals. The recent data suggests that the efficacy of exosomes is dependent on the genetic similarity of the donor and the host.[25] To have optimal treatments for human use, one solution is to use animal-derived exosomes, such as calf cord blood, due to biological recognition between the two species.[26] Furthermore, the study discussed below conducted by Mr Tunc Tiryaki and Recure Biotechnology suggests that the usage of calf cord blood-derived exosomes are easily stabilised and taken up by human cells, making them capable of increasing collagen production, increasing the speed of wound healing in cell cultures and preventing cell death.

Clinical research

The initial stage of exosome-based therapies is to develop a technique that can efficiently produce stable and concentrated exosome samples. Exosome concentration is significantly hampered by the operational complexity, time requirements, huge sample volumes and low purity of typical exosome concentration procedures.

Exosome isolation from various biofluids, such as blood, urine, milk and cell culture media, has been conducted with the help of different types of techniques.[27] Common separation methods mainly introduce ultracentrifugation, which is the gold standard in exosome isolation, size exclusion chromatography, ultrafiltration, polymer precipitation and immunoaffinity. All exosome isolation methods available in literature have both pros and cons. For example, while exosomes obtained from polymer precipitation and differential centrifugation isolation techniques have poor molecular purity, ultracentrifugation can just be applied in large amounts of samples due to its low efficiency. These obstacles are one of the reasons for the delay in the usage of exosomes in clinical applications.[28] Considering these disadvantages, new isolation methods continue to be developed to facilitate the active use of exosomes in clinical and cosmetology.

In the study, we combined different methods of ultrafiltration and flow filtration together. Post isolation, animal-based cord blood exosomes characterisation, cell proliferation assay, wound healing scratch assay, immunohistochemistry analysis, anti-inflammation assay, RT-PCRi ELISA and cellular uptake studies were performed. After in vitro studies, a third-party controlled study was conducted on 108 subjects. This study was also carried out with retinol, in which the effectiveness has been proven in cosmetic raw materials, as a control group.

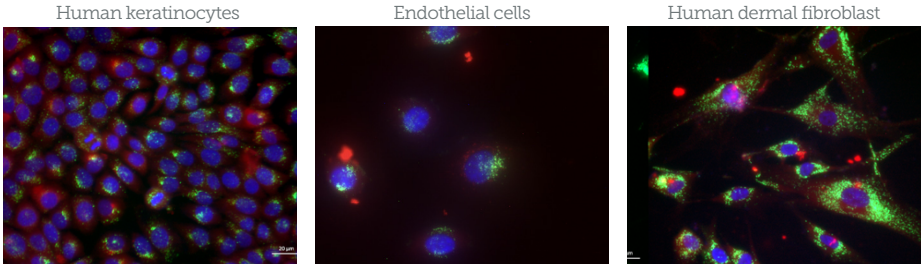

Figure 1: Human cells with uptake of animal-based cord blood exosomes. Green shows exosomes, red is cytoplasm and blue is the nucleus.

Cellular uptake

Results of the study displayed that uptake of animal-based cord blood plasma exosomes into human dermal fibroblasts, endothelial cells and keratinocyte cells began about four hours after application, and the uptake of animal-based exosomes was high in cell lines at six hours after the treatment.

In vitro wound healing capacity

Fibroblast cells are the primary effectors for soft tissue wound healing and their migration is necessary for wound contraction, collagen formation and tissue remodelling. Here, we assessed how animal-based cord blood exosomes affected the behaviour of fibroblasts in vitro. The scratch assay is widely considered the standard for determining wound closure rate. A scratch assay was performed to demonstrate the potential effect of animal-based cord blood exosomes on cell migration. In this assay, a gap formed by scratching the plate is tracked over time to determine the efficacy of gap closure in terms of cell migratory patterns, which is used to represent wound closure. The scratch assay revealed that, while control group cells modestly closed the scratched area after 48 hours, human

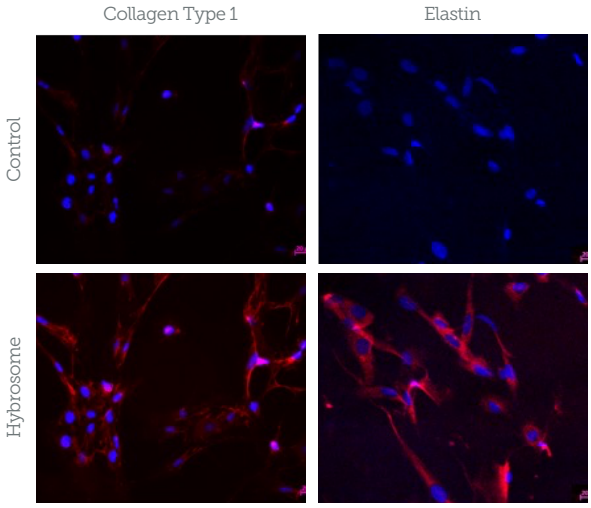

Figure 2: Immunocytochemistry analysis after treatment of animal-based cord blood exosome on dermal cells. Red shows increase of collagen and elastin.

Literature showed that we can harvest exosomes from different organisms, including plants, and animals

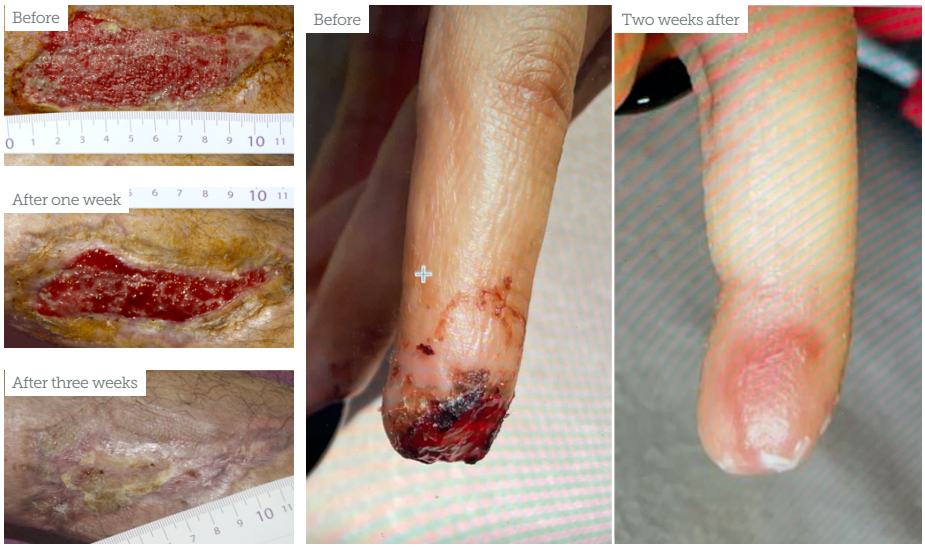

Figure 3: Diabetic unhealing wound progression after two years and a finger amputation. Both cases used an animal-based cord blood exosomes cream topically.

dermal fibroblast (HDF) cells treated with cord blood serum exosomes migrated faster, resulting in a 50% higher wound closure rate after 24 hours. The findings showed that these nanoparticles could be internalised by fibroblasts and dramatically increased their proliferation and migration potential, demonstrating that the activation of fibroblasts is a way through which cord blood exosomes facilitate wound healing.

Cell proliferation assay

In acute wounds, the proliferative phase starts after the inflammatory process. To bridge the gap, this stage involves both cell migration and proliferation steps together. This is a rate-limiting healing factor that must be presented for effective healing. The application of cord blood exosomes to the cell lines evaluated resulted in a considerable increase in cell proliferation (40-50%) in a dosage-dependent manner.

Collagen and elastin assay

Collagen and elastin are extracellular matrix components that play a vital role in healing. As a result, our findings support the hypothesis that cord blood exosomes increase collagen and elastin expression levels based on immunocytochemistry data. According to our findings, fibroblast transforming growth factor-β (TGFB) expression levels increased in response to cord blood exosomes administration. Furthermore, after cord blood exosomes treatment of HDF and human umbilical vein endothelial cells (HUVEC), vascular endothelial growth factor (VEGF) was upregulated in the vascularisation and wound healing (**Figure 2**).

Anti-inflammation effect of hydrosomes

Anti-inflammation is a major process in healing. Hydrogen peroxide (H_2O_2) tests are used during in vitro research to drive the onset and stimulate inflammation via the activation of nuclear factor kappa B (NFκB) transcription factors. H_2O_2 was utilised and shows that when it is only used, high cellular toxicity was detected, whereas when H_2O_2 and cord blood exosomes were administered at the same time onto healthy cell lines to initiate an inflammation reaction, results showed similarities with positive control, indicating that animal-based cord blood exosomes influenced pulling inflammation back to the control level. It can be concluded that cord blood exosomes can shield fibroblast cells against inflammation caused by reactive oxygen species.

Third party clinical trials

The aim of the test was to define the direct influence of the tested product on the reduction of the wrinkle area. The measurements have been performed at the site of application, before product application, after 28 days and after 56 days of regular use (**Figure 3**).
The animal-based cord blood exosome formulation was shown to reduce wrinkle length, depth, count, volume and area after 56 days from the product application.

The future of exosomes

Regenerative and cellular treatments are proven to be effective in problems such as hematopoietic disease, soft tissue defects and radiation injury. However, these methods still harness surgical intervention, limiting their availability. Our capacity to utilise exosomes will ensure these regenerative treatments are more reachable, effective and affordable. Treatments with exosomes or expanded mesenchymal stem cells are not yet authorised by the MHRA and FDA regulatory bodies. The invasive treatments, such as injections, require approval from the MHRA or similar organisations, but as we have shown in our pre-clinical studies, the topical applications seem to work successfully. While increasing our experience and understanding of this technology through topical use, it is vital to have reliable data from more invasive clinical studies in human subjects which can prove that exosome products are safe and effective.

Disclosure: Mr Tiryaki is currently working on a topical skincare product which contains exosomes.

Mr Tunc Tiryaki is a consultant plastic surgeon and works in facial regeneration. He was one of the first surgeons to use fat-derived stem cells for mini facelift procedures. Mr Tiryaki is the chair of the Humanitarian Programmes of ISAPS and founder of ISAPS-LEAP Surgical Relief Teams. He is a section editor for the *Aesthetic Plastic Surgery Journal* and has authored several internationally published publications on regenerative facelifts and micro-lifting.
Qual: MD

Serli Canikyan Turkay is a genetic bioengineer. After graduation she worked as a research assistant at Harvard Medical School on stem cells and microchip technology. She continued her career in a stem cell GMP lab as a coordinator of production and research and development. For the last 10 years, she has worked as the chief research officer at Mane Biotech.
Qual: Msc

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