Stem cell activation for a V-shaped face

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Abstract

As we age, the production of collagen and elastin is reduced, which results in sagging skin that can most notably be observed at the face contours of the jawline. Mesenchymal stem cells have been shown to improve collagen production and regenerate the skin, for example during wound healing. These processes are mediated by vesicles known as exosomes which are produced and secreted by these stem cells. A novel active ingredient based on goji plant stem cells was shown to improve the stemness of aged mesenchymal stem cells as well as increase exosome signalling by mesenchymal stem cells, which in turn improves extracellular matrix production in fibroblasts. The improved extracellular matrix rejuvenates the skin by improving skin density, reducing wrinkles and reshaping the face for an improved V-shape of the face..

Mesenchymal stem cells (MSCs) reside in many different tissues, including the subdermal adipose tissue. They are multipotent precursors of many different cell types, although they are morphologically and characteristically highly similar to fibroblasts.[1] Interestingly, MSCs were shown to improve and accelerate various regeneration processes such as cutaneous wound healing. Even more notably, it has been shown that mainly the MSCs themselves are not needed for this process but rather a cell-to-cell signalling mechanism deriving from MSCs that promotes proliferation, migration and collagen production of dermal fibroblasts. This beneficial signalling from MSCs to fibroblasts has recently been shown to occur through vesicles called exosomes.[2-4]

Cell-to-cell communication via exosomes

Exosomes are small, approximately 100 nm vesicles that are surrounded by a membrane, and which contain messenger molecules such as RNA, DNA proteins and lipids.[5] They can be as durable as viruses and can maintain their vesicle integrity even under deformation stress.[6] Exosomes are formed in so-called multivesicular bodies (MVBs) derived from endosomes of the donor cell. The MVBs fuse with the plasma membrane of the donor cell to release the exosomes into the extracellular space. The exosomes are then taken up by the recipient cell through a variety of mechanisms, including direct membrane fusion, release their cargo into the recipient cell, which induces various cellular pathways (Fig 1). The topic of exosomes is quite new; while in 2006 there were less than 100 scientific articles on this subject, there were more than 9,000 scientific articles in 2018.[7] This underlines the unquestionable relevance of this relatively new field. Not surprisingly, exosomes are currently being heavily investigated for their diagnostic and therapeutic purposes.[8]

In the skin, exosomes have been shown to be involved in cutaneous immunity, pigmentation and the aforementioned skin repair and regeneration through MSC signalling.[6] By rejuvenating MSCs, which can produce new adipocytes to fill the skin from inside, and by boosting exosome-mediated collagen production in fibroblasts, facial sagging of the skin could be reduced and a defined jawline, also called V-shape of the face, could be achieved.

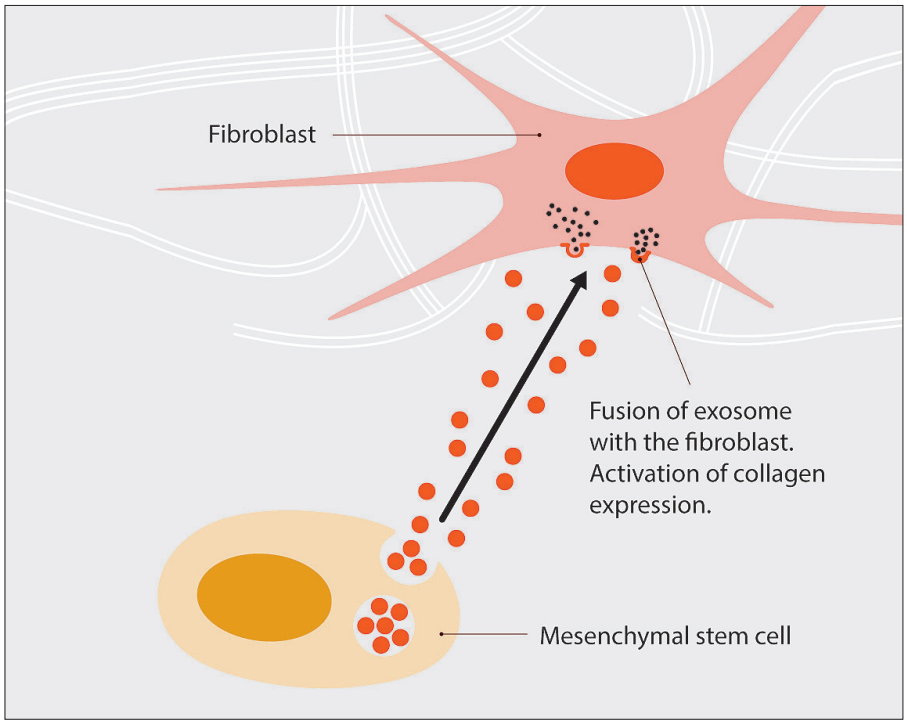

Figure 1: Schematic illustration of exosome signalling between mesenchymal stem cells and fibroblasts.

Goji – the superfruit

Goji (*Lycium barbarum*) plants are native to south east Europe and Asia. They belong to the nightshade family just like tomatoes and potatoes and grow up to 3 metres high. The red-orange goji berries harvested from this plant are one of the most famous super fruits.

According to a legend, goji berries were first discovered by a Buddhist monk and the monks who incorporated them into their diets lived longer than those who did not. For hundreds of years, goji berries have been

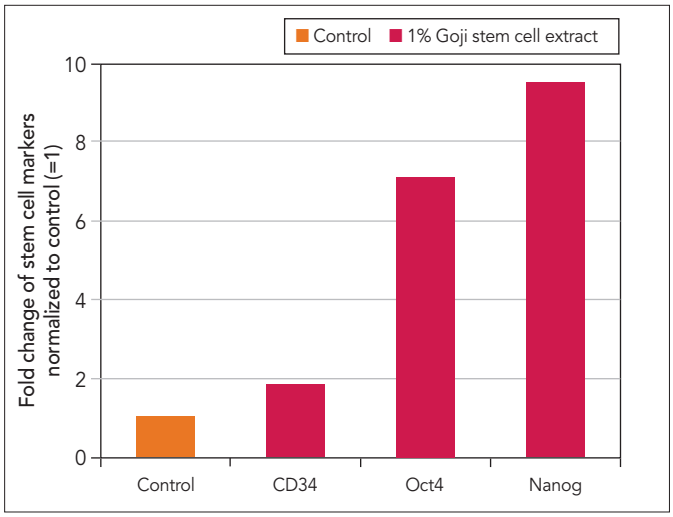

Figure 2: Increase in stem cell marker expression in aged mesenchymal stem cells after treatment with goji stem cell extract.

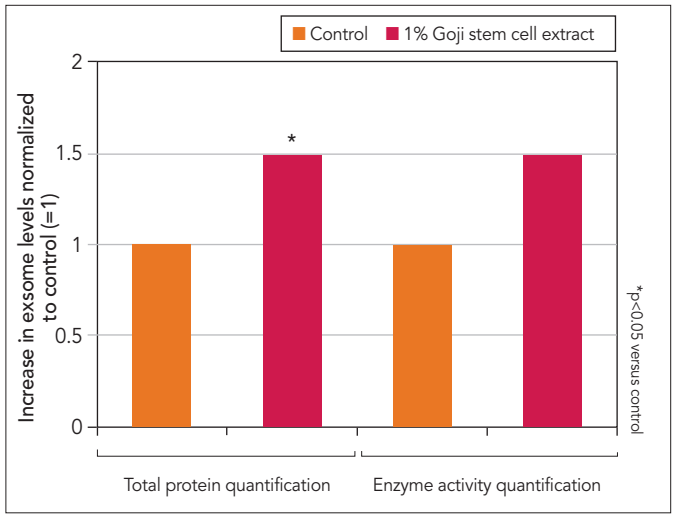

Figure 3: Increase in exosome production by mesenchymal stem cells after treatment with goji stem cell extract.

eaten in Asia for their medicinal and nutritive properties. Today, goji berries are eaten all over the world, mostly in dried form, and there are various goji berry supplements on the market with the reported effects ranging from anti-ageing, immune boosting and energising to improved memory.

Producing plant stem cells

To enable the large scale cultivation of plant stem cells the technology PhytoCellTec™ was used. This technology relies on the wound healing mechanism of a plant: part of a plant is wounded to induce the formation of callus cells. This healing tissue consists of dedifferentiated cells which are stem cells. Callus cells are harvested and cultivated in a suspension in a unique bioreactor. The goji stem cell active [PhytoCellTec™ Goji, INCI: Lycium Barbarum Callus Culture Extract (and) Isomalt (and) Lecithin (and) Aqua/Water] is based on a plant stem cell culture of a goji seedling and was tested for its effect on mesenchymal stem cells and exosome production.

Materials and methods

Expression of stem cell markers in aged MSCs

Adipose-derived human MSCs were grown for 14 passages to mimic the ageing process. These aged MSCs were then either treated or not (control) with 1% goji stem cell extract for 72 hours. At the end of incubation, the culture supernatants were discarded and cells were washed in phosphate buffered saline (PBS) solution and immediately frozen at -80°C. Total RNA was extracted from the cells and the expression of the stem cell markers CD34, Oct4 and Nanog was then assessed with RT-qPCR using the LightCycler System (Roche).

Quantification of exosome production by MSCs

Dermal derived human MSCs were either treated or not (control) with 0.1% goji stem cell extract for a period of 24 hours. The exosomes that were released from the cells were isolated using EXOPrep kit (Hansabiomed) and quantified in two different ways: quantification of total protein amount and quantification of the activity of Acetylcholinesterase, which is a known exosomal protein using the Pierce BCA Protein Assay kit (Thermo Fischer Scientific) and the Fluorocet exosome quantitation assay kit (System Biosciences), respectively.

Gene expression in fibroblasts after treatment with MSC-conditioned medium

Human MSCs were seeded in 6-well plates and cultured in culture medium for 24 hours. The medium was then removed and replaced with culture medium containing or not (control conditioned medium) 1% goji stem cell extract and cells were incubated for 72 hours. All experimental conditions were performed in n=2. At the end of incubation, the supernatants were collected, and the replicates of each condition were pooled to be used for the treatment of fibroblasts (conditioned medium). Fibroblasts were previously seeded in 12-well plates and cultured in culture medium for 72 hours and then in assay medium for further 4 hours. Afterwards, for one series, medium was removed and

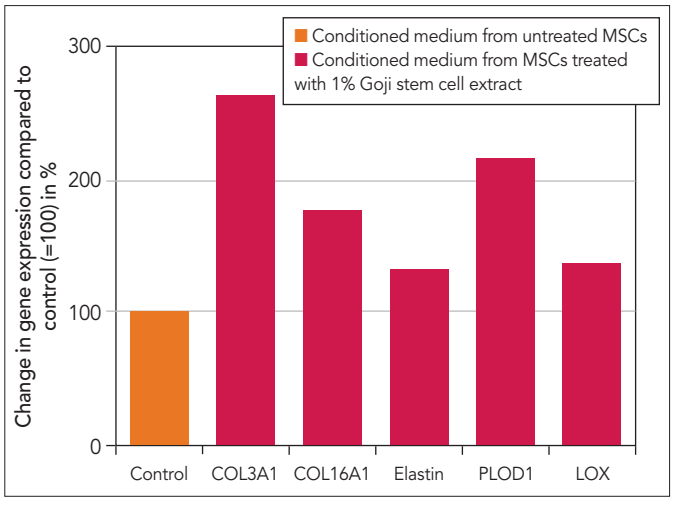

Figure 4: Gene expression of extracellular matrix genes by fibroblasts treated by conditioned medium from either untreated or goji stem cell extract treated mesenchymal stem cells.

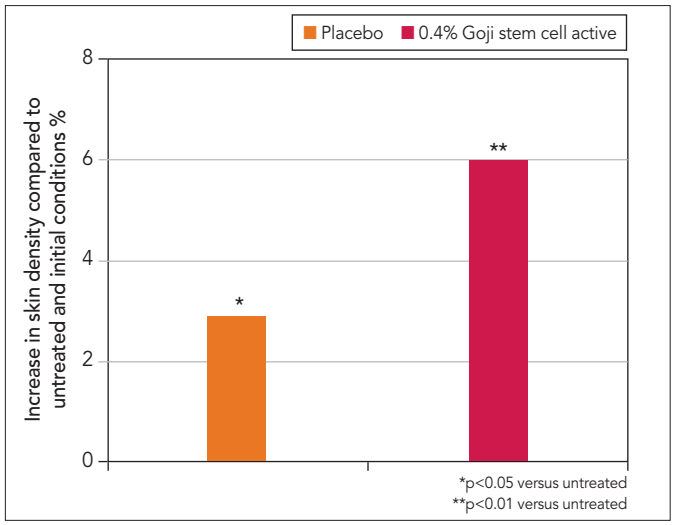

Figure 5: Increase in epidermis + dermis density after 28 days treatment with 0.4% goji stem cell active.

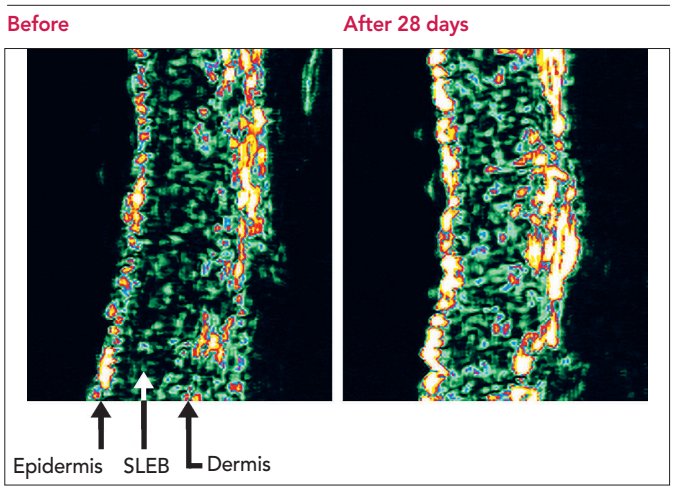

Figure 6: Representative ultrasound image of the increase in epidermis + dermis density after 28 days treatment with 0.4% goji stem cell active. SLEB = subepidermal low-echogenic band.

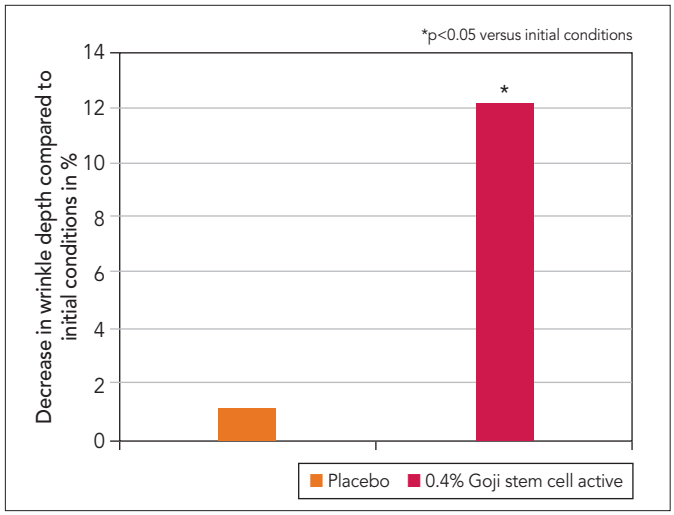

Figure 7: Decrease in wrinkle depth on crow's feet after 56 days treatment with 0.4% goji stem cell active.

replaced with assay medium containing or not (control) goji stem cell extract. For another series, medium was removed and replaced with the conditioned medium from MSCs (control or goji stem cell extract treated MSCs). Cells were then incubated for 24 hours. All experimental conditions were performed in n=3. At the end of incubation, cells were washed in phosphate buffered saline (PBS) solution and immediately frozen at -80°C. Total RNA was extracted from the cells and the expression of extracellular matrix genes was then assessed with RT-qPCR using the LightCycler System (Roche).

Clinical study on photoaged skin

In a randomised, placebo-controlled clinical study, twenty-three Caucasian women aged between 41 and 69 years (mean age: 56 years) who displayed signs of photoageing applied either a cream with 0.4% goji stem cell active on one half of the face and one forearm and the corresponding placebo on the other half of the face and the other forearm, twice daily for a period of 56 days. The wrinkle depth was measured using PRIMOS lite (Canfield, USA). The density of the skin (epidermis + dermis) was determined by ultrasonic measurements in triplicates using DermaScan C (Cortex, Denmark) which counts the number of low-echogenic pixels corresponding to low-density areas on a 2-D portion of epidermis and dermis. The lower the number of dark pixels the higher the skin density.

Clinical study on oval face shape

In a randomised, placebo-controlled clinical study, sixty-seven Caucasian women aged between 39 and 70 years (average age 57 years) with sagging facial skin were split into two groups. One group applied a cream with 0.4 % goji stem cell active and the other group applied the corresponding placebo cream on the entire face and neck twice daily for 28 days. The facial sagging and oval face shape was determined by measuring the size of the neck/submandibular triangle area by image analysis of the pictures of the volunteers' faces that were taken with Visioface 1000 D (Courage + Khazaka). For the image analysis, a straight line was drawn vertically through the middle of the chin. On either side of the face, vertical parallel lines were drawn to intersect at the start of the jawline/sagging skin. The points of the jawline intersection were then connected with the middle of the chin. The area below the drawn V lines was determined. A reduction of this area would suggest a reduction in facial sagging and an improved V-shape of the face.

Figure 8: Representative before/after pictures of two volunteers to illustrate the decrease in wrinkle depth on crow's feet after 56 days treatment with 0.4% goji stem cell active.

Results and discussion

Maintenance of stemness in aged MSCs

Although much slower than differentiated cells, MSCs also undergo ageing processes. This is characterised by the reduced production of stem cell markers and eventually a halt in proliferation.

To investigate whether the goji stem cell active influences the vitality of MSCs, aged MSCs were treated with 1% goji stem cell extract for 72 hours. Results showed that treatment with goji stem cell extract increased the expression of the stem cell markers CD34, Oct4 and Nanog in aged MSCs (Fig 2). This means the goji stem cell active is able to help mesenchymal stem cells to maintain their stemness.

Increase in MSC exosome production

In order to assess the effect of the goji stem cell active on the exosome production capability, human MSCs were either treated or not (control) with 0.1% goji stem cell extract for a period of 24 hours. The exosomes that were released from the cells were quantified in two ways, either measuring the total protein or enzymatic activity of a known exosomal protein. Both quantification methods revealed that treatment with goji stem cell extract leads to an increase in exosome production by MSCs (Fig 3).

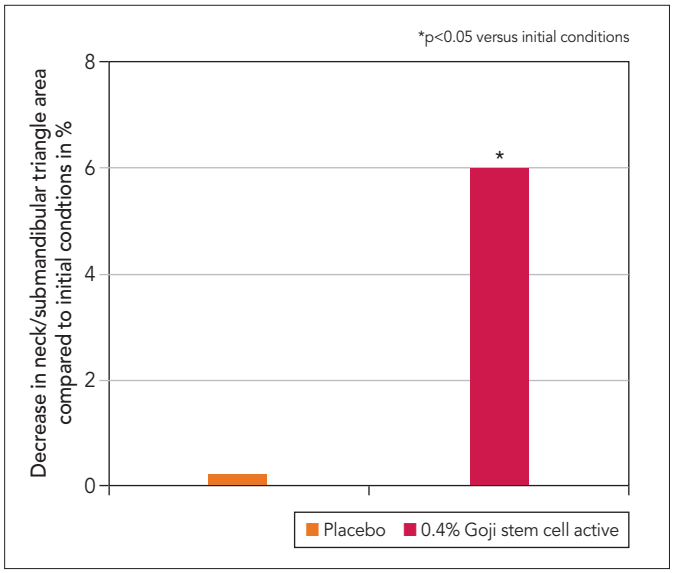

Figure 9: Improvement of oval face shape after 28 days treatment with 0.4% goji stem cell active.

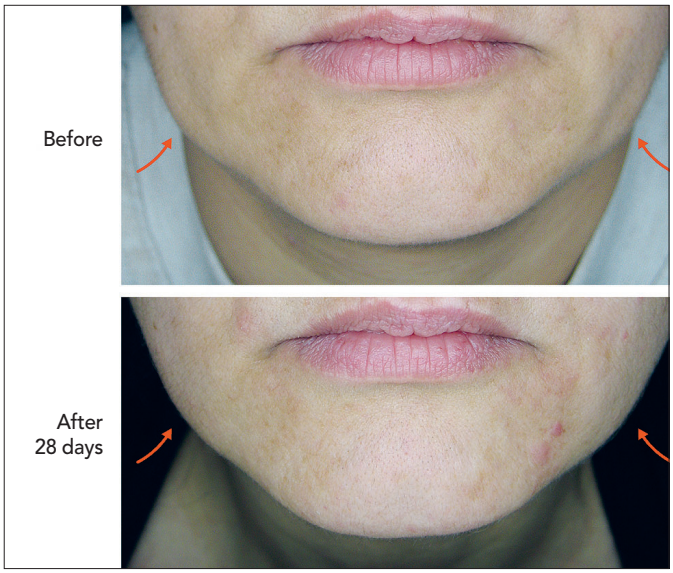

Figure 10: Visibly improved V-shape of the face after 28 days treatment with 0.4% goji stem cell active.

Stimulation of extracellular matrix genes through cell-to-cell communication

After demonstrating the effect on exosome production in MSCs, it was investigated as to whether MSCs treated with goji stem cell extract are able to communicate with fibroblasts in order to produce more extracellular matrix (ECM) proteins. For this purpose, human MSCs were treated with 1% goji stem cell extract. The supernatant, which includes soluble factors as well as exosomes ("conditioned medium"), was added to fibroblasts for a period of 24 hours. Fibroblasts treated with medium from untreated MSCs served as the control. Results showed that the conditioned medium from goji stem cell extract-treated MSCs increased the gene expression of several ECM factors such as collagen 3 and 16 and elastin (Fig 4). PLOD1 is crucial for collagen production, while LOX (lysyl oxidase) connects collagen and elastin to ensure more stability and elasticity. The direct treatment of the fibroblasts did not have the same effect. Therefore, the goji stem cell active improves cell-to-cell communication between MSCs and fibroblasts.

Improvement of wrinkle depth and skin density in photoaged skin

In a clinical study with twenty-three Caucasian women aged between 41 and 69 years (mean age: 56 years) who displayed signs of photoageing, applied a cream containing 0.4% goji stem cell active and the corresponding placebo cream for two months. After 28 days, a significant improvement of skin density could be observed via ultrasonic measurements for the treatment with the goji stem cell active (Fig 5). The collagen and elastic fibre structure of an intact dermis yields many reflections from the ultrasonic wave that are visible as bright colours in the ultrasonographic image. However, disruption to this regular architecture leads to weaker reflections and dark patches, as can be seen in the ultrasonographic image at Day 0. These so-called subepidermal low-echogenic bands (SLEB) are commonly found in aged and photodamaged skin. The SLEB could be reduced after 28 days treatment with 0.4% goji stem cell active (Fig 6). Furthermore, after 56 days, a significant decrease in wrinkle depth in the crow's feet area could be measured (Fig 7). Notably, not only fine wrinkles below the eye appeared smoother (see upper panel in Fig 8), but deeper lines were also visibly improved (see lower panel in Fig 8).

Improvement of the oval face shape

Sixty-seven Caucasian women aged between 39 and 70 years (average age 57 years) with sagging facial skin were split into two groups. One group applied a cream with 0.4 % goji stem cell active and the other group applied the corresponding placebo cream on the entire face and neck twice daily for 28 days. The facial sagging and oval face shape was determined by image analysis of the pictures of the volunteers' faces. Results showed that treatment for four weeks with 0.4 % goji stem cell active significantly improved the oval face shape compared to initial conditions (Fig 9). The improved V-shape of the face was also visible in the pictures of the jawline (Fig 10). This demonstrates that the goji stem cell active is indeed able to reduce facial sagging and improve the facial V-shape.

Conclusion

The novel active ingredient based on goji plant stem cells was shown to maintain the stemness of aged mesenchymal stem cells, to increase their exosome production and consequentially the production of collagen and elastin by fibroblasts. In placebo controlled clinical studies, treatment with the goji stem cell active significantly improved skin density and wrinkle depth. Furthermore, a significant improvement of oval face shape through reduced sagging of facial contours was observed. Therefore, the goji stem cell active rejuvenates the skin from inside out for an improved V-shaped face. PC

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