Senolytics: eliminating "zombie cells" in the skin

A novel anti-aging mechanism to combat senescent cells

FRANZISKA WANDREY*, DANIEL SCHMID, FRED ZÜLLI
*Corresponding author
Mibelle Biochemistry, Buchs, Switzerland

Abstract

Cellular senescence is one of the hallmarks of aging. Senescent cells, also called "zombie cells", a result of the aging process and oxidative stress, secrete pro-inflammatory factors that further contribute to aging. Therefore, eliminating senescent cells has emerged as a promising anti-aging therapy in the medical field in the past few years. This novel concept known as "senolytics" helps to clear tissues of senescent cells without affecting healthy cells in order to reduce inflammation and rejuvenate the tissue. For the first time, this concept has been adapted for cosmetics. An extract from organic alpine rose leaves demonstrated a clear senolytic activity on senescent fibroblasts. In a placebo-controlled clinical study, treatment with alpine rose extract significantly reduced skin redness and increased elasticity.

Keywords:

- Senescent cells
- Senolytics
- Fibroblasts
- Rejuvenation
- Skin care
- Peer Reviewed

INTRODUCTION

One of the main contributors to the aging process in our body is cellular senescence (1). The word senescence, its definition being "becoming or being old", is derived from the Latin word *senex* (=old). While senescence can describe the aging of a whole organism, cellular senescence describes the aging process of cells within an organism. It was first shown in the 1960s by Hayflick & Moorhead that normal human fibroblasts grown in a petri dish had a finite number of cell divisions before they stopped dividing and became senescent (2).
Since then, a myriad of research groups has investigated senescent cells, also called "zombie cells", their contribution to the aging process and how their detrimental effects could be averted.

In the field of cosmetics, senescent cells in the skin have been a target for active ingredients for quite some time, mainly by mitigating the cellular damage that leads to senescence. However, a novel mechanism to eliminate senescent cells, called senolytics, is a promising new way to fight the aging process, in the medical as well as in the cosmetic field.
This article will explain in depth how senescent cells are formed, what their role is in our skin and how they can be efficiently targeted for tissue rejuvenation.

Why do healthy cells become "zombie cells"?

Too many cell divisions, as mentioned above, trigger cellular senescence because of the shortening of telomeres, the repetitive DNA sequences at the end of each chromosome. As the function of telomeres is to protect the DNA during replication, once they have reached a critically short size, the cell will stop dividing to prevent DNA damage and become senescent. However, there are other triggers of senescence: too much oxidative stress, such as through exposure to UV light and pollution, causes damage to the DNA. When the DNA is damaged beyond repair, these damaged cells face a choice. They can either continue to divide despite the accumulated damage that would be propagated to their daughter cells, which potentiates the harmfulness. A second option is to undergo apoptosis, programmed cell death, to stop themselves from spreading the damage. Senescence is a third option, which permanently blocks the cells from undergoing further cell divisions.

Senescent cells are also called "zombie cells" (3) for the following reason: whilst they no longer divide, they are also far from being dead. Instead, senescent cells block an intracellular pathway that promotes their apoptosis in order to prevent their own elimination. Moreover, they continue to secrete signaling molecules such as cytokines that promote inflammation. This complex secretory program has even been shown to promote senescence in previously normal surrounding cells (Figure 1) (4).

Why are "zombie cells" harmful?

Senescent cells play an important role in some processes, for example during embryogenesis and tissue regeneration.
They also prevent cells with significant damage from proliferating (5). However, the senescent cells formed during these processes are only temporary and are usually quickly cleared by the immune system. If many senescent cells form simultaneously and for a longer time period through oxidative stress, they often cannot be cleared by the immune system, and accumulate in the tissue (6). These zombie cells are characterized by a senescence-associated secretory phenotype (SASP), which includes chemokines, inflammatory cytokines (such as IL-1 and IL-8) and matrix remodeling proteases (such as MMP-1 and MMP-2) (7).
The SASP thus leads to latent tissue inflammation and remodeling which accelerates and exacerbates the aging process. Further, the stress of the SASP on the surrounding tissue can turn healthy cells into zombie cells as well, leading to a vicious cycle of aging (8):
More and more cells become senescent, inflammation is increased due to higher SASP output, which further destroys the extracellular matrix leading to tissue degeneration and aging.

One of the best studied cell types that undergo senescence are fibroblasts in the dermis. Fibroblasts are exposed to intrinsic aging as well as external stresses such as UV radiation and toxic compounds from particulate matter that can enter the skin. Whereas most keratinocytes in the epidermis can avoid damage accumulation by the constant shedding of terminally differentiated keratinocytes, fibroblasts are long-lived and thus more prone to damage accumulation (9).
The SASP of senescent fibroblasts in the dermis leads to constant inflammatory reactions that cause skin redness and dilated blood vessels.

In addition, extracellular matrix degradation is promoted, which leads to a lack of skin elasticity and sagging. It is therefore important to keep the senescent cell population under control.

How to combat "zombie cells"?

There are several options to deal with senescent cells. The first is prevention: when the damage that leads to senescence is repaired, the cells would not turn senescent. However, this is not possible for all damage occurring in the cells, as our body, and especially the skin, is constantly exposed to internal and external stressors. Another option would be to prevent senescence from happening despite the damage. This would prove harmful for the tissue as cells dividing with extensive DNA damage and passing these errors onto their daughter cells should be avoided at all costs as it could lead to uncontrolled cell growth. The third option, which has become a highly promising anti-aging therapy in the past few years, is to eliminate senescent cells in a targeted way so that the surrounding healthy cells are not affected. This novel concept known as "senolytics" helps to clear tissues of senescent cells in order to reduce inflammation and rejuvenate the tissue. The term senolytics was coined in 2015 by researchers from the Mayo Clinic and the Scripps research institute in the USA, who described a mechanism through which senescent cells are selectively eliminated without harming healthy dividing cells (10).

The targeted senescent cells are going into apoptosis and are subsequently cleared from the tissue. Clearing senescent cells both reduces negative effects of aging pathologies and extends median lifespan (11). Despite being a brand-new life science topic, there have already been more than 300 scientific publications that investigate the senolytic activity of numerous compounds.

So far, the concept of senolytics has not yet been applied in the cosmetic field despite its great potential for skin rejuvenation. Here we describe an extract of organic *rhododendron ferrugineum*, also called alpine rose, leaves and its senolytic effect on human fibroblasts. Moreover, an improvement of skin redness and elasticity was observed in a placebo-controlled, randomized clinical study.

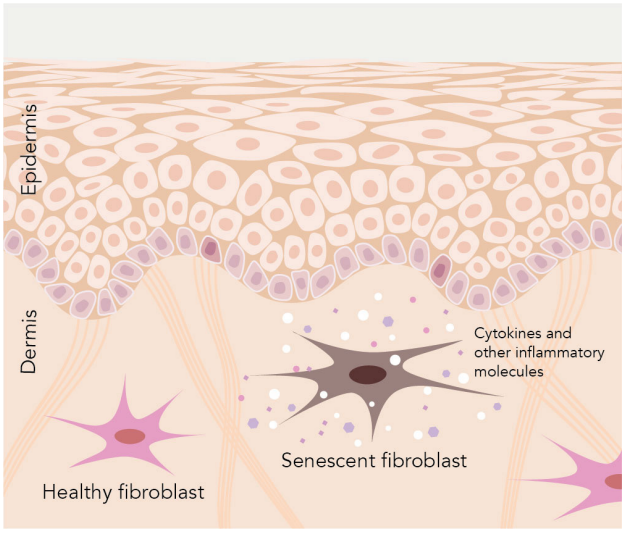

Figure 1. Schematic representation of a senescent fibroblast in the dermis. Secretion of cytokines by these senescent cells leads to constant inflammation in the skin, which results in collagen degradation and accelerated skin aging.

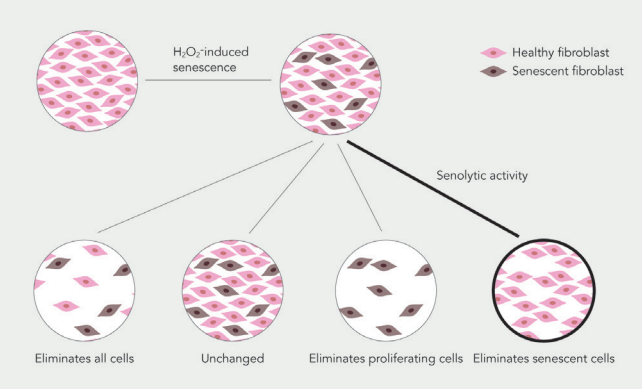

Figure 2. Schematic representation of the senolytic study concept. The desired senolytic activity depicted on the right results in the depletion of senescent cells while not affecting the healthy cell number.

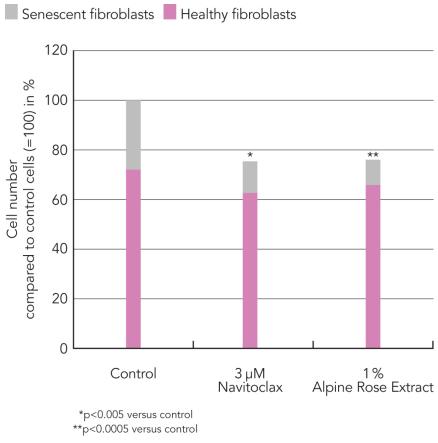

Figure 3. The alpine rose extract exhibits senolytic activity. Cell numbers of senescent and non-senescent cells are shown normalized to control cells in which senescence was induced with hydrogen peroxide.

MATERIALS AND METHODS

Senolytic assay

In order to distinguish between the prevention of senescence and true senolytic activity, Normal Human Dermal Fibroblasts were first stressed with 500 µM H_2O_2 for 2 hours to induce premature senescence through oxidative stress.

The medium was then exchanged, and the cells were grown for 3 days to fully establish the senescent phenotype in a subpopulation of the cells. This mixture of senescent and healthy fibroblasts was then treated for 48 hours with either 1% alpine rose extract or Navitoclax (Cayman Chemical, Ann Arbor, USA), a known senolytic drug, or not treated (control). Following fixation with 2% formaldehyde and 0.2% glutaraldehyde, cells were stained with DAPI and the relative total cell number was determined by fluorescence measurement. Senescence-associated β-galactosidase activity assay was performed according to (12) and a total of 400 cells were counted. Counting the β-gal-positive cells as a marker for senescence and calculating the percentage compared to the total cell number revealed the treatment efficacy.

Clinical anti-aging study

In a double-blind, placebo-controlled clinical study, forty-four Caucasian women aged between 40 and 65 years (mean age: 55 years) with redness on the cheeks were split into two groups. One group applied a cream with 2% alpine rose extract and the other group applied the corresponding placebo cream on the entire face and neck twice daily for 28 days. Skin color was measured using a Spectrocolorimeter CM700-d (Konica Minolta, Japan) and skin elasticity was determined with a Cutometer MPA 580 (Courage + Khazaka, Germany).

In addition, macrophotographs were taken before and after treatment with the Visia skin analysis system (Canfield Scientific, Germany).

RESULTS AND DISCUSSION

Alpine rose extract has senolytic activity

A challenge when screening for a senolytic effect is to distinguish between senescence delaying actives, such as antioxidants that minimize the damage that could lead to senescence, and true senolytic activity. To assess the latter, senescence was induced in fibroblasts by treatment with H_2O_2 for 2 h first and cells were then cultured for three more days. Only afterwards, when the senescent phenotype was established, incubation with the potential senolytic active took place. The number of senescent cells was quantified and compared to total cell number. Several different outcomes of the experiment are shown in Figure 2. Only actives that eliminate senescent cells while not affecting healthy fibroblasts are considered to possess senolytic activity (right option in Figure 2).

Treatment with 1% alpine rose extract significantly reduced the number of senescent cells while not affecting the number of healthy fibroblasts. Compared to control cells, which had 28.1% senescent cells in comparison to total cell number, treatment with alpine rose extract reduced that number to 10.1% senescent cells compared to the total cell number. The effect was similar to a treatment with the known senolytic drug Navitoclax, which reduced the percentage of senescent cells to 12.3% (Figure 3). The alpine rose extract therefore exhibits senolytic activity.

Increase in skin elasticity and decrease in redness

In a double-blind, placebo-controlled clinical study, twice daily application of 2% alpine rose extract for 14 days resulted in a reduction of the redness parameter a* by 8.4 %, which was significant compared to initial conditions as well as the placebo (Figure 4). The effect was also visible in macrophotographs taken of the volunteers (Figure 5). Furthermore, an increase in skin lightness by 2.1% was measured in volunteers who applied 2% alpine rose extract, which was significant compared to initial conditions and the placebo (data not shown). After 28 days of treatment, skin elasticity increased by 16.1%, which was significant compared to initial conditions and the placebo (Figure 6).

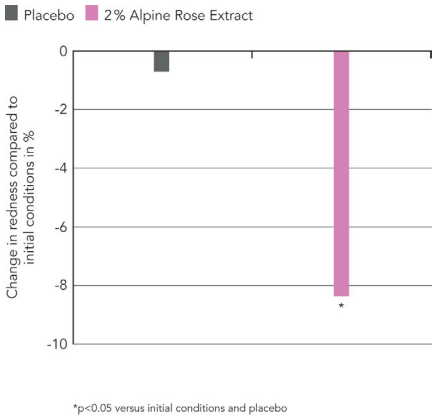

Figure 4. Decrease in skin redness after 14 days treatment with 2% alpine rose extract.

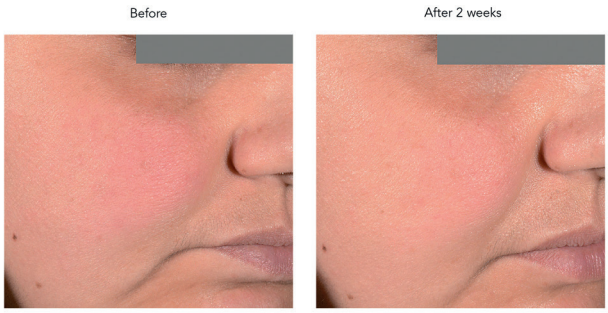

Figure 5. Before and after picture taken of a volunteer who applied 2% alpine rose extract twice daily for 14 days.

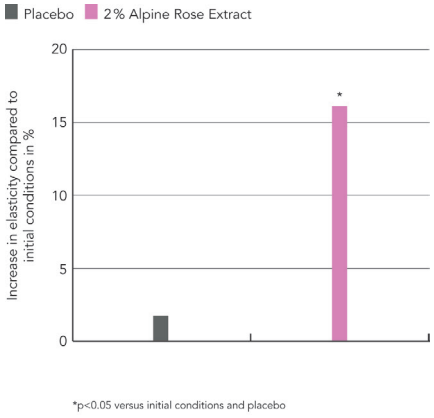

Figure 6. Increase in skin elasticity after 28 days treatment with 2% alpine rose extract.

CONCLUSION

Senescent cells greatly contribute to the aging process in the skin by inducing and accelerating inflammatory reactions and the degradation of extracellular matrix.

For the first time, a senolytic activity was shown *in vitro* for a cosmetic active ingredient, an extract of organic alpine rose leaves. Furthermore, the alpine rose extract reduced redness and increased elasticity in a placebo-controlled clinical study.

REFERENCES

1. López-Otín C, Blasco MA, et al., Cell, 153(6), 1194-217 (2013).
2. Hayflick L, Moorhead PS., Exp Cell Res.;25:585-621 (1961).
3. Scudellari M., Nature; 550, 448–450 (2017).
4. Acosta, J., Banito, A., Wuestefeld, T. et al., Nat Cell Biol 15, 978–990 (2013).
5. Ritschka, B. et al., Genes Dev. 31, 172–183 (2017).
6. Muñoz-Espín D, Serrano M., Nat Rev Mol Cell Biol., 15(7):482-496 (2014).
7. Toutfaire M, Bauwens E, Debacq-Chainiaux F., Biochem Pharmacol., 142:1-12 (2017).
8. Coppé JP, Desprez PY, Krtolica A, Campisi J., Annu Rev Pathol., 5: 99–118 (2010).
9. Tigges J, Krutmann J, Fritsche E, et al. Mech Ageing Dev., 138:26-44 (2014).
10. Zhu Y, Tchkonia T, et al., Aging Cell, 14 (4), 644-58 (2015).
11. Xu M, Pirtskhalava T, et al., Nat Med, 24(8): 1246–1256 (2018).
12. Zhao J, Fuhrmann-Stroissnigg H, et al., Curr Protoc Cytom., 79: 9.51.1-9.51.25 (2017).

ABOUT THE AUTHOR

Franziska Wandrey received her Ph.D. in biochemistry in 2014 for investigating ribosome biogenesis in human cells at ETH Zurich, Switzerland. Since 2015 she is working as a Research and Study Manager at Mibelle Biochemistry, where she contributes to the scientific active ingredient concepts and oversees the in vitro and clinical efficacy studies.