

8 April 2024

ETERWELL™ YOUTH – FAQ

What are senolytics?

Senolytics are compounds that selectively induce cellular apoptosis in senescent cells. It comes from the Latin words senex (old) and lytic (destroying), roughly translated as “destroying the old.”

What are SASP?

The abbreviation stands for senescence-associated secretory phenotype, which is a collective term for all that is secreted by senescent cells. This can be proteins, molecules, peptides, DNA, RNA, etc., and the majority of these are not favorable long-term for the surrounding (happy cells).

Is senescence cell and zombie cell the same?

Yes, these two terms are used to describe the same when in the proper context. A “senescent cell” is the technical term, while the “zombie cell” is an analogue and a more slang-like term. The zombie term fits as senescent cells are not really living and not really dead, and they are harmful to their surroundings, which results in additional senescent cells (i.e., zombie aspects).

What is the difference between (senolytics), autophagy and apoptosis?

Senolytics are compounds that selectively induce apoptosis in senescent cells.

Autophagy is the process of the cell’s internal recycling system. The cells collect damaged proteins or waste materials and reprocess them back into useable building blocks. There is research that shows that boosting this process can help against ageing and prevent senescence to a degree.

Apoptosis is the technical term for when cells are self-sacrificing themselves. This occurs to about 60–80 billion cell in your body every day and help to keep the whole organism healthy.

What is the difference between senolytic and senomorphics?



Senolytic is the process of selectively inducing apoptosis of senescent cells. Senomorphics do not induce apoptosis in senescent cells but mitigate the harmful effects of senescent cells (i.e., SASP). We currently advocate against this as there are many types of SASPs (+100), which might also differ per location or tissue or age. Furthermore, the SASPs provide a tumorigenic environment, meaning that cancer can occur in the long-term.

If senescence occurs in any cell, why the efficiency of an active is not the same on hair follicle and on fibroblast?

While senescence can occur in any cell (type), each cell type has its unique factory and mechanistic system to fulfill its duty/role. This means that there can be different ways for a cell to become damaged to a certain degree that leads to senescence.

Additionally, there are multiple so-called senescence anti-apoptotic pathways (SCAPs) that allow senescent cells to persist. Senolytic compounds aim to stop these pathways. There could be differences per cell type or tissue or area that requires a different approach to induce apoptosis of them.

What happens with ETERWELL™ YOUTH on keratinocyte?

Based on our ex vivo data (epidermis) that showed reductions in p16 and p21 (both well-known senescent markers), senescent keratinocytes should be reduced.

Is ETERWELL™ YOUTH killing the senescent fibroblasts and how does it do that selectively (not harming surrounding healthy cells)?

Technically ETERWELL™ YOUTH helps senescent cells to fulfill their intended program.

Senescent cells have two programs running: 1) self-sacrifice by apoptosis and 2) survival. In normal cases, when a cell becomes too damaged beyond repair, it will only have program 1 running to self-sacrifice. However, for unknown reasons, senescent cells also have the survival program running. Hence, ETERWELL™ YOUTH stops the running survival program in senescent cells. As a result, senescent cells go themselves into apoptosis. Normal cells do not have the self-sacrifice program running and are therefore unaffected.

How are we sure that ETERWELL™ YOUTH will not clear/remove also healthy cells?

In vitro we have shown, using our holotomography microscope technology, that cell death of normal cells is not increased but only with senescent cells.

Ex vivo we also show that overall tissue viability is not negatively impacted by ETERWELL YOUTH.

Can we really say that 52% zombie cells are reduced if this test was conducted with a 70/30 proportion of senescent cells and normal cells? And why?

Yes, we can. The percentual change focuses on the senescent cells. We go from 31% to 15% senescence, which is a percentage difference of 52% (15 is 52% of 31). This also means that we went from 69% to 85% normal cells.

The rationale behind the 30% senescent cells is based on the notion that we found in literature that skin has been recorded up to 60% senescence cells. We wanted to be in the middle of



this as we don't want to test at the end spectrum of age. It is also not that important. Whether we use 70/30 or 50/50, often we still see the 50% improvement.

Are we able to link percentage of senescent cells to age?

Currently this is not possible. There is a very huge variability from a person to another one as lifestyle plays too much of a role. There might be a statistical average for the population, but it will depend where in the world you look at as cultures differ.

How works the apoptosis (or the self-sacrifice mechanism)?

1. Initiation: Apoptosis can be initiated by a variety of signals, including DNA damage, oxidative stress, and activation of cell surface receptors. These signals activate a family of proteins called caspases, which are proteases that cleave other proteins and ultimately lead to cell death.
2. Execution: Once caspases are activated, they cleave a variety of target proteins that are involved in maintaining the integrity of the cell. These include cytoskeletal proteins, nuclear proteins, and proteins involved in DNA repair. The cleavage of these proteins leads to the characteristic morphological changes that are observed during apoptosis, such as cell shrinkage and fragmentation.
3. Clearance: Once the cell has undergone apoptosis, it needs to be cleared by neighboring cells or by phagocytic cells such as macrophages. The cellular debris is then broken down and recycled.

Is ETERWELL™ YOUTH active on immune system to remove senescent cell?

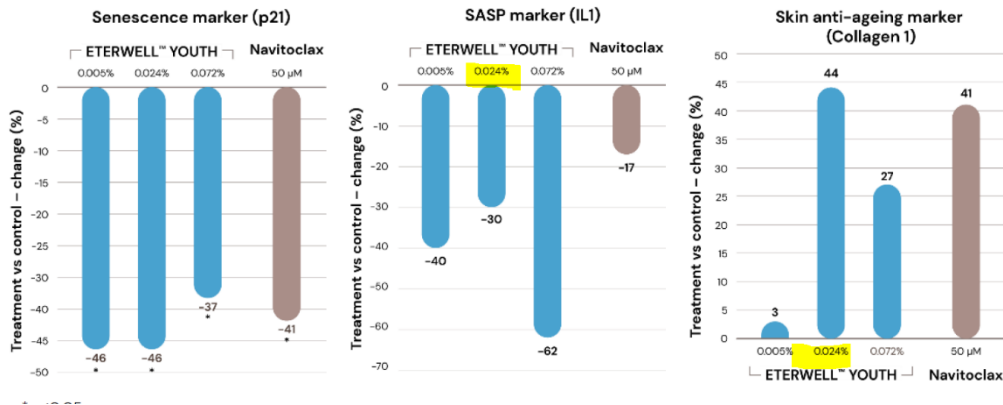
We do not have data that investigates this topic. However, it is not expected that ETERWELL™ YOUTH enhances the immune system that in turn removes senescent cells.

However, we can state that we see limited benefits in this approach for products that do so. For instance, these ingredients assume that there is a healthy and vital immune system to be activated for the removal of senescent cells. The issue is that with age the immune system also weakens, making these ingredients assumingly less effective in people that need it the most. Often, these conditions are also not considered when testing for their effect. So, the results shown are from very healthy immune cells.

Overall, our ETERWELL™ YOUTH product is powerful on its own and does not rely on the immune system.



How do we explain that middle part of dosage seems to have 'strange' results, i.e. higher or lower than highest dosage?



This is quite complicated to answer but will do my best. 😊

1. The results here show transcriptomics, meaning gene expression. In basic biology, when there is a need for something (e.g., collagen 1), genes (DNA) are expressed. Simply said, this "expression" is the making of many molds (messenger RNA) from one blueprint (the gene) for the cellular factories (ribosomes) to use to make for example collagen 1. What should not be forgotten is that just because you see lots of molds, it does not mean you have lots of collagen 1. The factories still need to do their jobs (filling and reusing the molds to make collagen 1). However, usually when you have more mold copies to make collagen 1, you will have more collagen 1. Finally, it depends per mold (p21, IL1, or Collagen 1), but they disappear at some point. This can be minutes to hours to days.
2. So, what you see in the graph is the percentage change in the number of molds for that specific protein at day 10.
3. So, the theory is that we have these results because we are looking at a moment in time to see mold number, which is likely not the optimal one for all concentrations/proteins. With higher concentrations, the stimulation to make molds is probably higher/faster, the molds are used faster, and then they disappear faster. Again, this can depend on various things.
4. To make it more complex, we are counting mold numbers of the epidermis and dermis together. Histology results showed that the dermis had no change, likely it being too deep for only 10 days treatment in this model. Also, a reason why we don't reach statistical significance everywhere.

And to make it even more complex, sometimes too high mold numbers (too much gene stimulation to express) can actually lead to the opposite effect.

Do we offer TGF-B in any commercial form?

TGF-B is a hormonal growth factor and can be only used for in vitro purposes. Therefore, we do not offer a commercial form.

For the synergetic assay with retinol, what is the concentration of Retinol?

We have used 10uM retinol or about 0.0003%.



What is the difference between ETERWELL™ YOUTH and ALPAFLOR® ALP-SEBUM CB
ETERWELL™ YOUTH is optimized to work on senescent cell removal as a senolytic. This means that only the leaves and stem are collected for harvest. Furthermore, the harvest itself is optimized for highest myricitrin flavonoid content, which means early harvest in comparison. ALPAFLOR® ALP-SEBUM CB is optimized to work on sebum reduction via alpha-reductase. The harvesting focuses on the whole plant and on Oenothrin active compounds.

What are the key active compounds of ETERWELL™ YOUTH

Currently, the main known active compound is myricitrin, which is a flavonoid high in concentration in *Epilobium fleischeri*. However, it is not the only active compound, other flavonoids (Myricetin 3-(6''galloyl)galactoside), Myricetin-3-O-galactoside,...) show also activity and we have tested additional fractions (even non-flavonoid fraction) that also showed some activities. As such, the complex natural mixture contributes to ETERWELL™ YOUTH the overall powerful senolytic effect.

What is the kinetic of Myricitrin during the growth of the plant, one of the key active compounds of ETERWELL™ YOUTH

The content of flavonoids drops during the growth of the plant, from about 800 mg myricitrin/100g dry plant at the start of the season to 500mg myricitrin/100g dry plant when the plant blooms. But at the start of the season, the yield of dry plant is pretty low, and we have to find the best compromise between yield and content of actives. This is why we have decided to harvest the plant just before blooming.

Is the tested concentration of Myricitrin for the senolytic in vitro assay closed to the concentration in the plant extract?

Yes, we have in our extract about 350mg myricitrin/kg ETERWELL™ YOUTH. The concentration tested of pure myricitrin is between 25 uM and 1 uM, which respectively correspond to 0.4 mg myricitrin/kg and 10 mg myricitrin /kg. Therefore, the concentration in the plant extract is 35 times higher than the highest concentration tested, therefore we are confident that myricitrin contributes to the senolytic activity of our ETERWELL™ YOUTH.

What exactly do we mean when we say that ETERWELL™ YOUTH is a patented application?

We have filed the IP about *Epilobium fleischeri* extract as senolytic bioactive with anti-ageing skin benefits, and also about myricitrin as senolytic compound. This patent is still pending.

What happens if one uses more than recommended 3% maximum?

Most likely you will have a stronger effect on senescence reduction.