A two-pronged strategy to increase Nicotinamide Mononucleotide for NAD+ recycling in the skin

NT-197

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Introduction:

Nicotinamide adenine dinucleotide (NAD^+) is an essential cofactor for the cellular energy metabolism in all living cells as well co-substrate for various enzymes involved in numerous cellular pathways, including DNA repair, epigenetic modification, inflammation, circadian rhythm and stress resistance[1,2]. NAD^+ levels are known to decline during aging throughout the body. In the skin in particular, sun-exposed areas show the most drastic decline in NAD^+ levels, which has been linked to increased UV sensitivity and visible signs of aging[3].

Interestingly, boosting NAD^+ levels have been shown to extend lifespan in various model organisms[4]. This has mostly been achieved by supplementation with precursors, as NAD^+ itself cannot easily be transported through cell membranes. Such a direct precursor to NAD^+ is nicotinamide mononucleotide (NMN), which thus serves as an important regulator for energetic processes within cells. NMN, however, is unstable in water.

With this study, we provide a solution to formulate NMN into a watery phase by incorporating NMN into Solid Lipid Nanoparticles (SLNs). Additionally, we investigated possibilities to boost the gene expression of NAMPT, the rate-limiting enzyme in the recycling pathway of NAD^+ recycling (Figure 1).

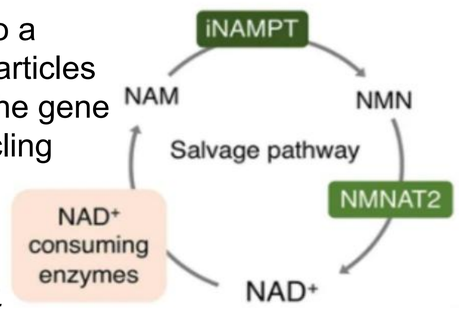

Figure 1: The NAD+ salvage pathway. Image adapted from [2].

Materials & Methods:

Encapsulation of NMN into SLNs
To encapsulate NMN into SLNs, a butter such as Shea Butter, was heated up to 55 °C under stirring. NMN (2% of final concentration) was added and immediately homogenized with a rod-homogenizer (IKA T25 digital Ultra-TURRAX). Afterwards, water (RT), containing soy lecithine (2% of final formulation) was added and homogenized with the lipid phase The suspension was then microfluidized to yield the nano suspension (Figure 2). To investigate the properties and stability of the nano suspension, the pH, particle size and Zeta potential were measured over time.

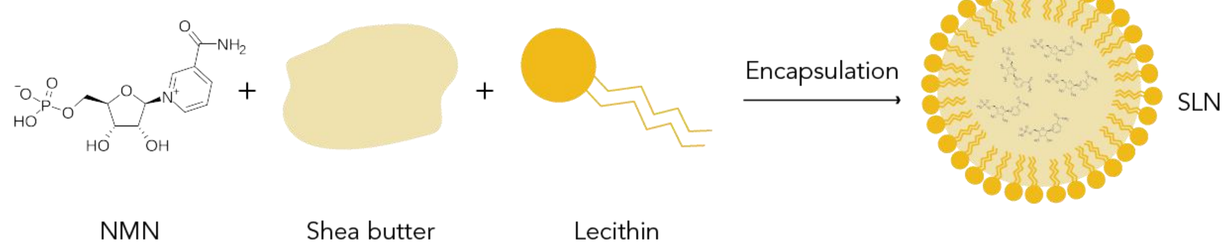

Figure 2: NMN encapsulation mechanism.

Cell cultivation and gene expression analysis
Normal human dermal fibroblasts (NHDF) were treated or not (control) with 1% *Helianthus Annuus (*sunflower) sprout extract for 48 hours. Gene expression of antioxidant and NAD+-recycling genes was analyzed by RT-qPCR using the LightCycler® system (Roche).

ATP analysis in reconstructed human epidermis
Reconstructed human epidermis (RHE) was cultivated for 4 weeks, with or without 2% sunflower sprout extract. Fresh RHE served as control. ATP concentration was determined using the ATP Colorimetric/Fluorometric Assay Kit (BioVision) and expressed in relation to the weight of RHE samples.

Induction and prevention of cellular senescence
Normal human dermal fibroblasts (NHDF) grown until the 10th passage were stressed for 2 hours with culture medium containing 600 µM H_2O_2. For recovery, NHDF were incubated for 144 hours with medium containing or not (control) 2% sunflower sprout extract. β-galactosidase staining was performed to quantify cellular senescence.

Results & Discussion:

NMN encapsulation into SLNs
Investigation of the stability of NMN and the encapsulation trials yielded the following results:

- NMN is unstable in water and decays into nicotinamide riboside as well as nicotinamide.
- The size of NMN powder particles was measured to be in the µm range. To eliminate interference of the particle size with encapsulation, experiments were performed with NMN dissolved in water or melted into the lipid phase.
- By testing various encapsulation methods and parameters, we were able to successfully and stably incorporate NMN into SLNs.

Improving the NAD^+ salvage pathway
Treatment with 1% sunflower sprout extract significantly stimulated gene expression of NAMPT by 111% after 48 hours. Further, treatment with 1% sunflower sprout extract induced the expression of SOD2 (superoxide dismutase 2), an enzyme that clears mitochondria of reactive oxygen species, by 1482%, thereby supporting the cellular oxidative stress response (Figure 3).

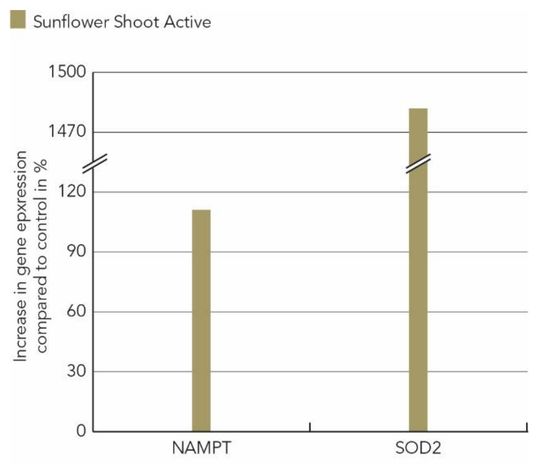

Figure 3: Increase in gene expression after treatment of NHDF with 1% sunflower sprout extract.

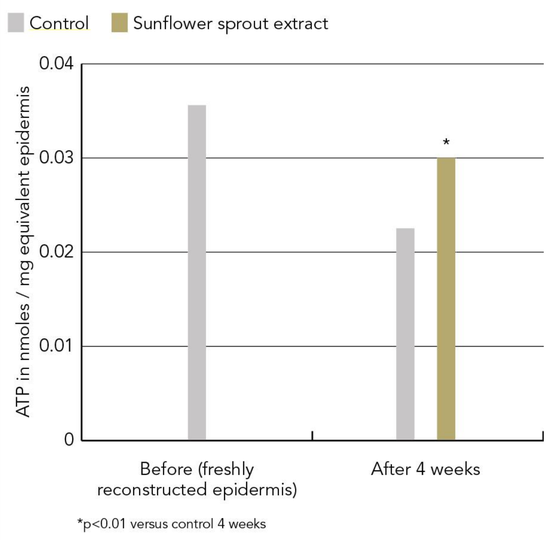

Figure 4: Measurement of ATP production in young and aged RHEs.

Compared to young reconstructed human epidermis (RHE), ATP production is reduced by about 1/3 in aged RHE. Treatment with 2% sunflower sprout extract preserved the ATP production in aged RHE 2.35-fold compared to untreated aged RHE (Figure 4).
This further supports the enzymatic activity in the salvage pathway, which is dependent on ATP.

Moreover, NHDF treated with the sunflower sprout extract were protected from oxidative stress-induced senescence, with 55% fewer senescent cells compared to untreated control cells, further contributing to the prevention of cellular aging.

Conclusions:

A two-pronged approach for effectively increasing cellular NAD^+ levels was successfully introduced:

1) Supply cells with the precursor NMN in a stable formulation through encapsulation into SLNs to ensure water solubility and prevent decay
2) Simultaneously activate the salvage pathway in skin cells to efficiently recycle NAD^+ for long-term rejuvenation effects.

Acknowledgements:

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References:

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