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The Role of NAD+ in Regenerative Medicine

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Summary: The understanding of the molecular and cellular basis of aging has grown exponentially over recent years, and it is now accepted within the scientific community that aging is a malleable process; just as it can be accelerated, it can also be slowed and even reversed. This has far-reaching implications for our attitude and approach toward aging, presenting the opportunity to enter a new era of *cellular* regenerative medicine to not only manage the external signs of aging but also to develop therapies that support the body to repair and restore itself back to a state of internal well-being. A wealth of evidence now demonstrates that a decline in cellular nicotinamide adenine dinucleotide (NAD+) is a feature of aging and may play a role in the process. NAD+ plays a pivotal role in cellular metabolism and is a co-substrate for enzymes that play key roles in pathways that modify aging. Thus, interventions that increase NAD+ may slow aspects of the aging trajectory, and there is great interest in methods for cellular NAD+ restoration. Given these recent advancements in understanding the cellular aging process, it is important that there is an integration between the basic scientists who are investigating the underlying mechanisms of cellular aging and the surgeons and aesthetic practitioners who are providing antiaging therapies. This will allow the effective translation of this vastly complex area of biology into clinical practice so that people can continue to not only stay looking younger for longer but also experience improved health and wellness. (*Plast. Reconstr. Surg.* 150: 41S, 2022.)

In general terms, aging is considered the organism-wide loss of homeostasis, innate repair, and regenerative capacity, resulting in an accumulation of damage and the development of multiple copathologies. Aging is a complex and multifactorial phenomenon that includes many effects at the systemic level which are ultimately driven by critical changes at the cellular level. It is recognized that there are nine key cellular changes that underpin the cascade of events that lead to systemic age-related decline. These cellular causes of aging have been well characterized and are collectively referred to within the aging research community as the "hallmarks of aging."[1]

The identification of the hallmarks of aging has marked a shift toward understanding aging not as a single process, but instead as a combination of multiple cellular changes. This has allowed the molecular and cellular root causes of many common aging phenotypes to be identified. For example, skin aging—arguably the most recognizable sign of aging—has traditionally been described at the histological level, but it is now understood that these changes result from more specific failures at a cellular level, revealing new therapeutic targets with the potential to address aging at its root cause (Fig. 1).

NAD+ AS A TARGET FOR CELLULAR AGING

One area of intense research within the field of cellular aging surrounds the molecule nicotinamide adenine dinucleotide (NAD+). Metabolomics-based studies of aging have identified NAD+ as a central metabolic intermediate linked to many of the hallmarks of aging.[2] NAD+ is a cellular coenzyme that plays an essential role in both metabolic and

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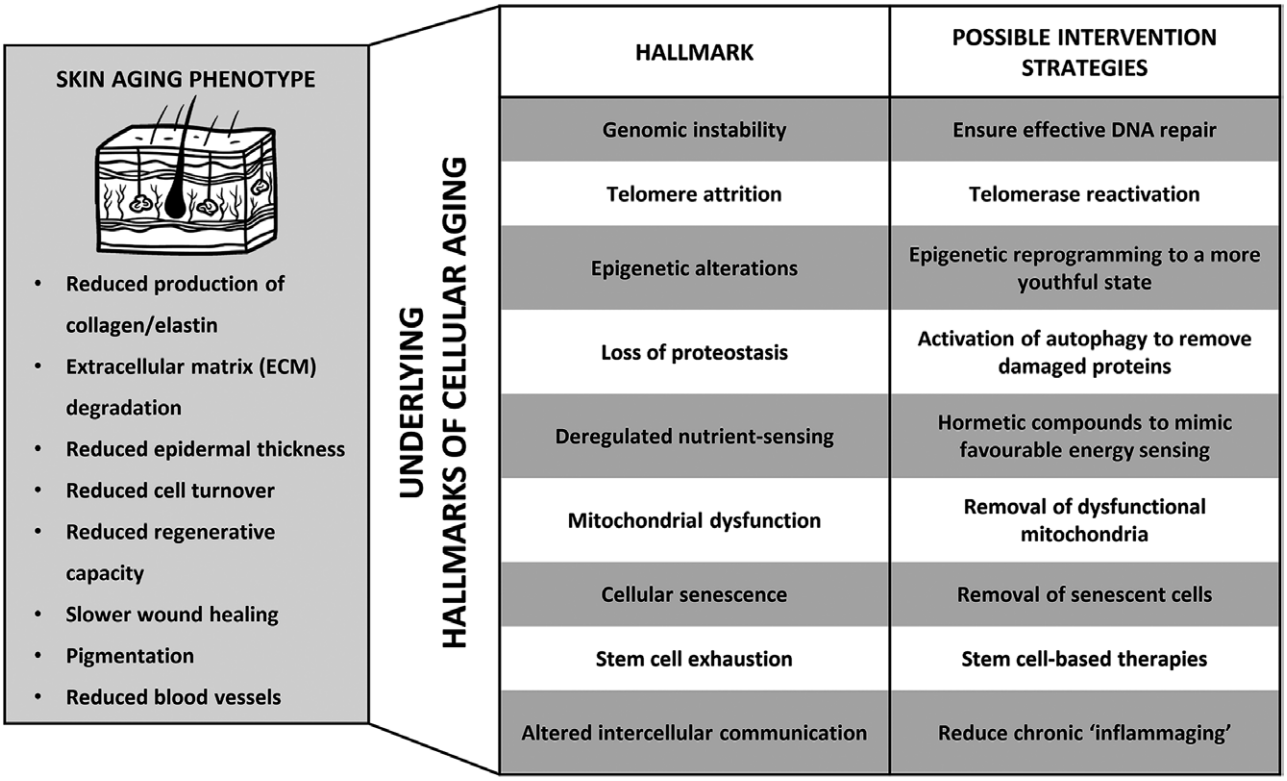

Fig. 1. Traditionally, age-related dysfunction has been described at the histological level, but it is now known that these changes result from more specific failures at the cellular level. These key cellular changes are collectively known as the "hallmarks of aging" and designing interventions that target these hallmarks is currently an area of intense research.

signaling reactions. During its role in metabolism, NAD+ participates in redox reactions leading to the formation of ATP. Aside from this key role, NAD+ is also a critical regulator of a wide array of enzymes involved in making posttranslational modifications to proteins that change their activity.[3] This combination of metabolic and cell-signaling functions means that NAD+ acts as a metabolic messenger providing an important link between the energy status of the cell and downstream signaling for appropriate cellular adaptation to bioenergetic stress. Therefore, a proper maintenance of NAD+ levels is required to maintain tissue homeostasis and stress response.[4]

Despite its critical role, an age-dependent decrease of cellular NAD+ is observed across species. In humans, age-related NAD+ decline has been observed in the liver,[5] skin,[6] brain,[7,8] plasma,[9] skeletal muscle,[10] and monocyte-derived macrophages.[11] Chronically low NAD+ has been observed in accelerated aging disorders[12] and age-related disease states,[13–15] and has been linked to multiple hallmarks of aging.

Low NAD+ contributes to aging because NAD+ serves as an exclusive co-substrate for two key families of enzymes that affect cellular repair and longevity—the sirtuins (SIRTs) and the poly(ADP-ribose) polymerases (PARPs). These enzyme families regulate many signaling processes associated with cellular health and longevity and are directly dependent on NAD+ availability to perform their functions.

PRECLINICAL BENEFITS OF NAD+ RESTORATION

Restoration of NAD+ in vivo has been investigated extensively and has demonstrated whole-body benefits. This has been reviewed in detail recently.[16] Briefly, in mice, NAD+ levels have been found to decrease twofold by mid-age, correlating with the onset of multiple age-related issues.[5] Successful restoration of NAD+ to youthful levels resulted in cardiovascular improvements[17] and the reversal of multiple metabolic conditions.[18–21] Improvements to muscle function and endurance[20] were also observed together with increased mitochondrial function, ATP production[14] and an increased number and quality of muscle stem cells.[22] An increased capacity for organ protection and regeneration after injury was found in the liver, heart, and kidneys,[23–25] and NAD+ restoration was also found to rescue vision by reversing retinal degeneration.[26] Significant neurological benefits have also been

demonstrated in Alzheimer disease animal models on NAD+ restoration, including improved cognition and nerve regeneration. NAD+ availability also appears to impact fertility, as strategies to boost NAD+ levels were found to improve oocyte quality and restore fertility in aged mice.[27]

CLINICAL BENEFITS OF NAD+ RESTORATION

These impressive preclinical results have now shifted the focus to human clinical trials with the hope of translating the benefits of NAD+ restoration to humans (**see Table, Supplemental Digital Content 1**, which shows human clinical trials that have measured NAD+ levels and clinical outcomes after NAD+ restoration, ***http://links.lww.com/PRS/F374***). Notable observations so far include a trend towards an improvement in indicators of cardiovascular function including lower systolic blood pressure and aortic stiffness,[28] a promising reduction in the levels of circulating inflammatory cytokines in older males after only 3 weeks of NAD+ restoration[29] and an NAD+-associated increase in mitochondrial function and decreased proinflammatory factors in heart failure patients.[30] The diverse protective and regenerative capacity of NAD+ has been attributed to its involvement in the prevention of multiple hallmarks of cellular aging (Table 1).

THE ROLE OF NAD+ IN SKIN AGING

There is also growing evidence that NAD+ decline plays a critical role in the biology of skin aging. DNA damage and genomic instability are key features of skin aging due to the continued exposure of the skin to UV radiation and sophisticated DNA repair mechanisms exist to quickly repair damage before it becomes harmful to the cell. It has emerged that several of these repair mechanisms are directly dependent on NAD+ to perform their function, so its decline with age is problematic. For example, the DNA repair enzyme PARP1, and SIRTs 1 and 6, which are integral elements of the DNA repair response, are all critically dependent on NAD+ to function.[31–33] Decreasing NAD+ levels therefore contribute to reduced DNA repair and an accumulation of DNA damage. Accordingly, an age-associated decrease in both NAD+ and SIRT1 is observed in skin, whilst DNA damage is found to accumulate,[6] ultimately triggering other hallmarks of aging such as cellular senescence.

Cellular senescence is characterized by the cell entering a state of irreversible cell cycle arrest.[34] Both fibroblasts and keratinocytes have been found to become senescent with age[35] and, while they persist in the skin and remain metabolically active, they do not perform their normal function in contributing to skin health. For example, senescent fibroblasts no longer produce collagen and elastin resulting in a dysfunctional support matrix and skin that is not capable of efficient damage repair.[36] Senescent cells also have a distinct inflammatory secretory profile termed the "senescence associated secretory phenotype" (SASP), which has a profound detrimental effect on surrounding cells, leading to altered intercellular communication.[37] Senescent fibroblasts promote degradation of the extracellular matrix (ECM) by secreting matrix metalloproteinase-1 (MMP1) and other proinflammatory factors,[38] ultimately leading to thinning of the epidermis and decreased barrier

Table 1. Hallmarks of Aging Are Key Cellular Changes That Underpin the Cascade of Events that Lead to Systemic Age-Related Decline*

Hallmark of Aging	Role of NAD+	References
Genomic instability	Adequate NAD+ availability is critical to drive DNA repair enzymes and pathways such as PARP1, SIRT1, and SIRT6	31–33
Cellular senescence	Low NAD+ promotes senescence in skin whilst restoration of NAD+ reduces the burden of senescent cells	40,41
Epigenetic alterations	NAD+-dependent sirtuins are critical for youthful epigenetic regulation. Reduced NAD+ means sirtuins cannot perform this critical role	43
Mitochondrial dysfunction	Adequate NAD+ is critical to healthy mitochondrial function and for the removal of damaged mitochondria	79
Telomere attrition	NAD+ restoration is found to alleviate telomere dysfunction	80
Altered intracellular communication	Low NAD+ promotes age-related inflammation	81
Loss of proteostasis	NAD+ is required for SIRT1-mediated activation of autophagy to clear damaged cellular proteins	60,61
Deregulated nutrient sensing	NAD+ levels are critical to sense the energetic status of the cell for adaptation to energy stress	4
Stem cell exhaustion	NAD+ restoration leads to stem cell rejuvenation	22

*NAD+ has been identified as a key metabolic intermediate linked to many of the hallmarks of aging.

function,[39] while the selective removal of senescent cells leads to normalization of the ECM and a reduction in inflammation.[37] Low NAD+ has also been found to promote senescence by reducing SIRT1 activity, which in turn reduces p63 expression, leading to a reduction in cell proliferation.[40] NAD+, SIRT1, and p63 are all found to decline in aged keratinocytes leading to senescence,[35] while the restoration of NAD+ reduces the senescent cell burden in dermal fibroblasts.[41]

As well as genomic instability, the aging process is characterized by changes to DNA methylation patterns known as "epigenetic drift," which ultimately alters gene expression.[42] This has led to the development of "DNA methylation clocks" that predict the biological age of cells based on these measurable epigenetic changes. The NAD+-dependent SIRTs play a key role in epigenetic regulation, highlighting a major role for NAD+ in the cross talk between the metabolic state of the cell and epigenetic regulation of gene expression.[43] Indeed, many of the beneficial antiaging effects of healthy lifestyle practices such as fasting and exercise are coordinated by increasing NAD+ levels, which in turn activates SIRT1 to change the expression of beneficial genes. In skin, the SIRTs are linked to the preservation of collagen in the dermis and their activation is important in wound healing and regeneration of skin by promoting keratinocyte proliferation.[44] SIRT6 promotes genes associated with collagen production,[45] and both SIRT1 and SIRT6 mediate the inhibition of MMP-1 gene transcription, which degrades collagen.[46] Both SIRT1 and SIRT6 are found to be downregulated in older skin, and this correlates with a reduction in available NAD+.[47,48]

Aged skin also demonstrates mitochondrial dysfunction,[49] an aging hallmark that is directly linked to oxidative stress, increased MMP-1 expression, dermal atrophy, and epidermal hyperplasia.[50] Adequate cellular NAD+ is critical for normal mitochondrial function both directly through its role in oxidative phosphorylation and indirectly through activation of SIRT1 and SIRT3, which are involved in the biogenesis and degradation of damaged mitochondria.[51,52] Increasing cellular NAD+ has been found to improve mitochondrial function, activate mitophagy (the recycling of dysfunctional mitochondria), and improve keratinocyte regenerative capacity.[53,54]

An age-dependent dermal accumulation of oxidatively modified and damaged proteins has also been found to cause skin dysfunction.[55] ECM proteins such as collagen become glycated to form Advanced Glycation End products (AGES) leading to dermal stiffness and decreased flexibility.[56] Autophagy mediates the recycling of AGES and other defective proteins and an age-related reduction in autophagic activity in dermal fibroblasts is found to reduce collagen, hyaluronan, and elastin, collectively leading to deterioration of dermal integrity and skin fragility.[57–59] Increasing data indicates that the maintenance of high NAD+ is critical to SIRT1-mediated activation of autophagy pathways and the clearance of damaged cellular proteins.[60,61]

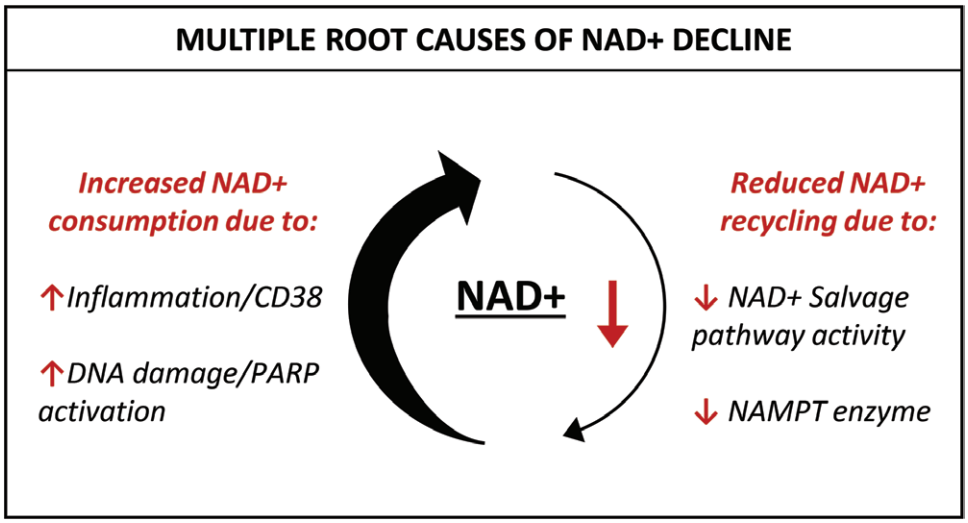

Fig. 2. There are multiple root causes of NAD+ decline. Older cells exhibit excessive NAD+ consumption due to chronic inflammation and DNA damage which increases the activity of the NAD+ consumers CD38 and the PARPs. At the same time, reduced expression of the NAMPT enzyme means the salvage pathway is less efficient at recycling NAD+, resulting in cells that struggle to meet the demand for NAD+.

CAUSES OF NAD+ DECLINE

The above discussion demonstrates clear evidence for the role of NAD+ decline in the development of the hallmarks of aging in the skin, and there has been great interest in understanding the root causes of NAD+ decline to determine strategies to successfully restore cellular NAD+ levels.

It is known that NAD+ metabolism comprises multiple precursors, production routes, recycling pathways, and a myriad of consuming enzymes. Evidence now suggests that a major cause of NAD+ decline is a disruption of this finely controlled network. Specifically, it has been found that NAD+ consumption starts to outpace NAD+ production and recycling with age[62] (Fig. 2).

In its role as a coenzyme, NAD+ acts as a substrate that is irreversibly degraded by NAD+-consuming enzymes, including the SIRTs, PARPs, and CD38. The expression and activity of these NAD+-consuming enzymes have been found to increase with age meaning that the demand for NAD+ also increases. For example, age-associated increases in DNA damage activates NAD+-dependent PARP1,[63,64] and although PARP1 is a critical DNA repair enzyme, its persistent activation is harmful due to this contribution to NAD+ depletion. Indeed, overactivation of PARPs with resulting severe depletion of NAD+ has been highlighted recently by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), which is found to severely deplete cellular NAD+ due to overactivation of PARPs.[65]

The NAD+ glycohydrolase CD38 has also been recognized to consume large quantities of NAD+.[66] CD38 is found throughout the body and plays an important role in multiple aspects of the inflammatory response.[67] It is now clear that CD38 becomes overexpressed during aging due to chronic activation from persistent low-level "inflammaging," which in turn results in NAD+ depletion.[68,69]

This overactivation of NAD+-consuming pathways with age and disease can severely compromise NAD+ availability in cells, subsequently limiting utilization of NAD+ by other critical NAD+-dependent enzymes that promote good health, such as the SIRTs. Despite this increased demand for NAD+ throughout life, NAD+ levels should, in theory, be self-sustaining as cells have the ability to rapidly recycle the breakdown products of NAD+ consumption to replenish NAD+. This occurs via the salvage pathway, which plays a major role in restoring NAD+. Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in this recycling process,[70] and it is now known that NAMPT levels decline with age in parallel with the decline of NAD+ in aged tissues.[5,71–77] This reduction in NAD+ biosynthesis via the salvage pathway is a significant factor in older cells because, as NAD+ consumption increases concurrently with age and demands for NAD+ replenishment and recycling increase, the resulting degraded NAD+ is no longer efficiently recycled, exacerbating a situation of declining NAD+ levels.[5]

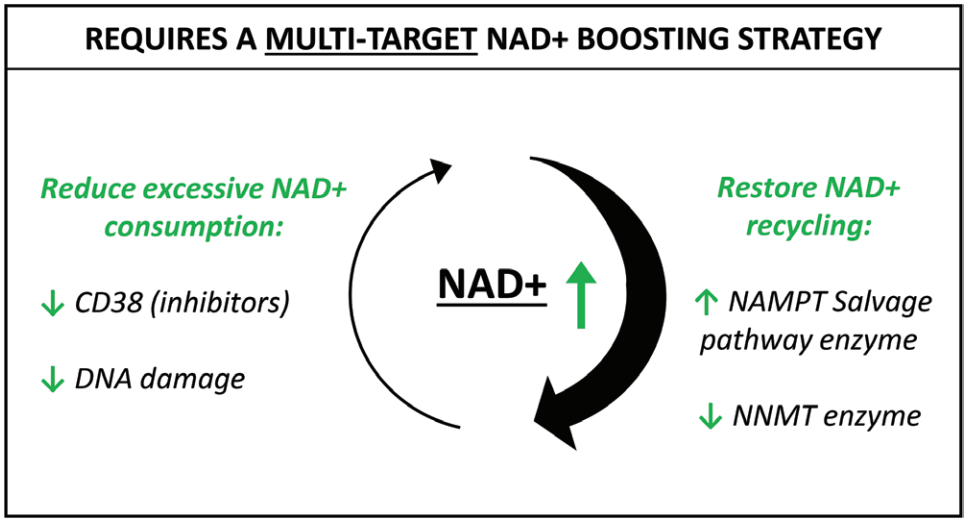

Fig. 3. Successful NAD+ restoration requires a multitargeted strategy that simultaneously addresses the root causes of NAD+ decline. Therapies must reduce the excessive consumption of NAD+ with approaches such as CD38 inhibition and reduction of DNA damage, while improving the efficiency of NAD+ recycling by promoting upregulation of the rate-limiting salvage pathway enzyme NAMPT and inhibition of NNMT, an enzyme that promotes the removal of NAD+ breakdown products from the cell rather than recycling.

Table 2. Potential Clinical Applications, Routes of Administration and Benefits of NAD+-Restoration Therapies

	Method of Administration	Potential Benefits
Systemic applications for NAD+-restoration	Oral supplementation	Whole-body improvements in cellular health contributing to improved healthspan Improved energy, cognitive function, and sense of well-being Improved sleep quality
	Intravenous	Pre-procedure administration to prime cells for optimal response to aesthetic treatments Improve healing/regenerative capacity pre/postsurgery
Localized applications for NAD+-restoration	Topical	Concentrated treatment for problematic areas Concentrated application to improve healing/regeneration postsurgery
	Injectable	Use in combination with aesthetic procedures such as microneedling

METHODS TO RESTORE NAD+

The evidence demonstrates that the biology behind NAD+ decline is complex. NAD+ restoration using pure exogenous NAD+ is often not practical due to its unstable nature and poor bioavailability to most cell types, so efforts have focused on oral supplementation with the NAD+ precursor compounds nicotinamide (NAM), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN). These precursors are utilized by NAD+ biosynthesis pathways and converted to NAD+ within the cell. However, it is now clear that this popular approach ignores the root causes of NAD+ decline meaning supplementation with precursors alone such as NR or NMN do not offer a long-term efficacious solution for NAD+ restoration. Instead, strategies that simultaneously address the multiple root causes of NAD+ decline such as the combined administration of a NAD+ precursor, a CD38 inhibitor, and an NAMPT activator, hold potential for NAD+ restoration with greater measurable benefits (Fig. 3). Furthermore, there is already a wealth of evidence to support these targets as interventions to successfully restore cellular NAD+ levels and multiple safe and well-tolerated active ingredients against these targets already exist.[62] This presents the opportunity for the development of oral, topical, and injectable formulations, allowing for both whole-body NAD+-restoring benefits alongside more targeted administration, resulting in the ultimate inside-outside approach to aging (Table 2).

THE FUTURE OF NAD+ IN CLINICAL PRACTICE

Until recently, antiaging therapies were limited to repairing the consequences of aging, but now there is clear evidence that aging can be targeted from its root cellular cause giving the opportunity to slow and even reverse aspects of aging.[78]

NAD+ restoration has been identified as a key therapeutic target that can positively impact many of the hallmarks of cellular aging. Not only does it play a key role in skin aging but also demonstrates a great potential to improve multiple aspects of age-related decline across the whole body. This offers an unprecedented opportunity for practitioners to introduce NAD+-restoring therapies that not only impact the appearance of their patients but also their health and well-being (Table 2).

Given the rapidly aging population, addressing aging at the cellular level is now critically important. Many surgical procedures rely on the healing and regenerative capacity of the skin which is known to decline with age, leading to disappointing results or negative postsurgery outcomes. Improving health and resilience at the cellular level with NAD+ restoration could be harnessed as a way to ensure consistent results and recovery irrespective of patient age. It should also be noted that the efficacy of many nonsurgical aesthetic procedures such as microneedling and laser technologies ultimately rely on the activation of cellular stress pathways to trigger the clearance of damaged cells and stimulate the production of new collagen. Many of these pathways require adequate NAD+ levels for optimal function, meaning NAD+ restoration before treatment could be a strategy to ensure the underlying cells are in an optimal condition to respond to the treatment.

With this greater understanding of the benefits of NAD+ and how to design targeted strategies to maintain its availability, collaboration with clinical practitioners, who have firsthand experience of the clinical manifestations of aging and access to patient groups for clinical trials, is now crucial to translate this exciting science into the clinic.

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