CAUSES OF SKIN AGING

Prepared by: Charlene DeHaven, MD, FACEP, Clinical Director

There are between 25 and 30 different theories of aging recognized by the NIH. Although some of these have much credible scientific support, they are still classed as “theories” because none have been irrevocably proven. There is no unified theory of aging. If there were, one ingredient in one skincare product would serve to treat aging skin. As it is, aging must be approached from the standpoint of multi-factorial causation with multiple ingredients and products. We are still learning about aging and how it affects the individual.

This article will discuss 4 key processes of aging. These processes apply to the entire organism and every cell within it. Of course, they also apply to skin. However, skin is unique in that is the organ that shields the interior of our body from the environment and all types of environmental assaults including solar damage, injury, pollution, and others.

Theory 1—Oxidative Stress

This theory is more commonly known as the free radical theory of aging. All cells need energy to perform their particular function. This energy is a very “hot” process and uses free radical generation to burn fuel. In this process, extra free radicals are created. These extra free radicals bounce around inside the cell, damaging all cellular structures they contact. Over a lifetime, these free radical “hits” gradually accumulate leading to a physiologic decline in structure and function. We label this decline “aging.”

Skin cells being metabolically active are subject to the same free radical damage as other cells of the body. In addition, skin cells are damaged by energy packets of solar rays termed photons. Photons are themselves very high-energy particles that are free radicals. Depending on whether the sunscreen chosen is physical or chemical, these solar free radicals can be blocked or neutralized.

Antioxidants are helpful because they combine with free radicals and prevent the ongoing cascade of free radical damage. Only about one percent of oral antioxidants reach the skin so topical antioxidants are also critical.

Suboptimal health states such as severe burns, diabetes, critical illnesses, infections, vascular diseases, endocrine diseases and others are associated with excess free radical generation. Smokers have huge amounts of free radicals floating about in their bodies.

All life forms must deal with free radical damage from internal metabolism and from the sun. It is not surprising that plant substances usually have some amount of antioxidant activity. Some plant substances such as Centella asiatica are especially potent antioxidants. The 3 most active components of C. asiatica are madecassic acid, asiatic acid and asiaticoside.

Theory 2—Inflammation

A certain amount of inflammation is required for health. Through its inflammatory response the body combats infections, clears away damaged tissue and heals sunburn and other oxidative processes. Excess inflammation results in accelerated rates of aging, scarring and destruction of normal tissue architecture.

Free radical damage is well-known to trigger excess inflammation. The inflammatory response is elevated

in those having higher levels of oxidative stress byproducts.

Oleuropein is an anti-inflammatory substance found in the olive tree, Olea europaea. More anti-inflammatory activity is contained within the olive leaf than in other parts such as olive oil.

Theory 3—Glycation

The process of attaching a sugar to a protein is called glycation. Oxidative damage is an intracellular process, i.e. occurs inside the cell whereas glycation is an extracellular process and occurs outside the cell. Glycation occurs in protein-rich tissues that contain large amounts of the protein collagen. Collagen-rich tissues include the skin, blood vessels, joints and lens of the eye. Glycated collagen is damaged collagen and less able to respond to physical stress by stretching. Glycated collagen has much less deformability and resilience.

Persons most subject to glycation have excess amounts of extracellular sugar (glucose). In this group are diabetics, the obese and the elderly. These 3 groups are all relatively "insulin resistant"; that is, they do not respond to insulin in a normal way by moving glucose inside the cell. The excess extracellular glycose will glycate collagen and damage tissues containing large amounts of it. This illustrates why "tight glycemic control" or keeping glucose at a normal level is so important in preventing the complications of diabetes. Excluding diabetics, before the age of 40, the level of obesity is most important in determining the rate of glycation. After age 40, chronological age is more important than the amount of obesity.

As we age, our ability to synthesize cellular products such as collagen decreases. This makes it more difficult for us to repair collagen damaged by glycation and other types of injury.

Resveratrol is an anti-aging substance found within red wine that prevents vascular disease. Resveratrol is found in the outer covering of the grape seed. Grape seed extract also contains resveratrol.

Theory 4—DNA Damage

DNA is contained in the helical structure inside chromosomes in the nucleus of the cell. This DNA contains our genetic material and also directs the function of the cell in which it resides. A cell with damaged DNA cannot properly function and may even become cancerous. Increased DNA damage in skin occurs with photoaging and high oxidative stress. Not as much DNA damage is found with glycation because DNA is protected from glycation within the cell. Most glycation occurs extracellularly.

Genetic defects such as Werner's Syndrome illustrate the importance of DNA repair. Werner's Syndrome is one of the progerias, the diseases manifesting greatly accelerated aging. Persons with Werner's Syndrome lack the helicase enzyme required for DNA repair. In the teen years, these individuals look much like everyone else. By the age of 30, they look many, many decades older and have an accompanying high rate of disease and decreased lifespan.

DNA is subject to free radical damage so antioxidants improve rates of DNA damage. Some growth factors such as EGF (Epidermal Growth Factor) or HGF (Hepatocyte Growth Factor) can direct the DNA of fibroblasts to synthesize more collagen.

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Improving Aging Rate and Surgical Outcome. Improving any of these 4 processes decreases the rate of aging and also improves surgical outcome. Individuals with excess free radical damage, glycation, inflammation or DNA damage have decreased healing capacity.

Certainly, attacking the processes of aging in younger years is preferable. But even though “younger is better”, any time is better than never. This is illustrated by experiments on rodents the equivalent of 60 in human years. These rodents, prior to their rodent age of 60, lived a life full of stress, free radical damage and high carbohydrate diets. At 60, they were put on high doses of antioxidants. Their lifespan significantly increased even at this late age. Similarly, when anticipating a procedure, “sooner is better” for instituting good skincare practices. If begun just prior to surgery, then 2-4 weeks is a good estimate of the minimum time required to begin a skincare regime.

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OXIDATIVE STRESS AND FREE RADICAL DAMAGE TO SKIN

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FREE RADICAL THEORY OF AGING

The National Institutes of Health (NIH) recognizes 20–30 different theories of aging, the most widely accepted of which is the Free Radical Theory. This theory was first developed by Denham Harman and has been discussed for more than 50 years. The Free Radical Theory states that accumulated free radical damage and oxidative stress alter biochemical and cellular processes as aging damage accumulates. Most free radical damage occurs during times of active metabolic turnover. In humans, this occurs in early puberty for males and in pre-puberty and early puberty for females. Also during this time, humans possess the most physiologic reserve. However, as damage accumulates, our physiologic reserve becomes depleted. A 20-year-old faced with trauma or biochemical assault can accommodate and recover faster than an 80-year-old, whose physiologic reserve has been depleted.

A great deal of scientific evidence supports the Free Radical Theory. When more free radical damage occurs than can be neutralized by internal defenses, a state of oxidative stress exists. Living in stressful environments overwhelms the body's natural defenses against free radical damage. Ongoing oxidative stress directly relates to an increased rate of aging and eventual illness. Other biologic mechanisms such as inflammation, glycation, and DNA damage also play roles in the process of aging. But even these additional causes of aging are arguably related to or subsets of oxidative stress.

OXIDATIVE STRESS AND DISEASE

When a critical amount of oxidative stress occurs, "diseases of aging" can manifest as diabetes, atherosclerosis, strokes, or cancer. Many common diseases, such as infection, injury, or trauma, are the result of simple insult to the organism. Infection and trauma are not the results of aging but simply the risks of living in one's environment. As the average lifespan of humans has lengthened, there has been an increased frequency of age-related diseases associated with oxidative stress. These particular diseases occur in the older members of the population and are rarely seen in younger persons.

A recently discovered strain of yeast, found to age faster with better resistance to oxidative stress, has shed light on the enormous complexities of aging mechanisms. The yeast's ability to control oxidative stress may be related to a process called hormesis. Hormesis is a complicated internal process designed to help neutralize low levels of free radical formation by increasing other oxidative processes. All forms of oxidative stress may not be equal in their effects on aging, hence the yeast's successful employment of specific oxidative reactions to combat aging.

INFLAMMATION

Inflammation accompanies and potentiates free radical damage. As free radical damage occurs, cells and tissues are damaged. The body attempts to clear away damaged cells by activating various inflammatory pathways. Activated cells release assorted chemicals, triggering inflammation. Inflammation destroys and liquefies damaged tissue so it can be removed. However, inflammation is never restricted only to damaged cells, and it spills over into surrounding healthy tissue. Thus, inflammation, although designed to heal, might collaterally damage normal tissue.

SOURCES OF FREE RADICAL DAMAGE

Skin receives 80% of its free radical damage from exposure to the sun's rays. Solar rays are composed of packets of energy called photons, which are very high-energy particles—i.e. free radicals.

For tissues other than skin, more than 85% of all free radical damage comes from the cell's own metabolism. Our cells take oxygen inhaled by the lungs and use it in enzymatic reactions to burn fuel—glucose, fat, or even protein—and create energy. Each cell uses its energy to perform its own individual function. However, each cell makes extra energy. As energy is created, radicals are created. The extra radicals "spin off" as free radicals. These extra packets of energy are called free radicals because they are not committed to any particular ongoing biochemical reaction. Free radicals penetrate into the interior of the cell, combining with whatever structure they strike, damaging that structure.

The skin, being the body's first environmental defense, is exposed to other sources of free radical damage in addition to sun and internal cellular metabolism. Other sources of free radical damage to the skin include ozone, pollutants, applied substances (some sunscreens), alcohol, severe physical and emotional stress, poor nutrition, obesity, and toxins. Smoking is also critically damaging to cells and tissues, by delivering massive amounts of free radicals with every puff.

FREE RADICALS AND ANTIOXIDANTS

There are mechanisms for neutralizing free radicals, such as antioxidants. Antioxidants absorb free radicals and stop the negative cascade of molecular damage. There are lipid-soluble antioxidants, such as vitamin E, which target the lipid-rich or fat-containing parts of cells. There are aqueous antioxidants, such as vitamin C, which protect the water-containing fluid portions of cells. Extrinsic antioxidants are ingested or applied topically, whereas intrinsic antioxidants are present inside cells and are synthesized by the body. Intrinsic antioxidants include enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes are manufactured within the interior of the cell, as an inherent defense against free radical damage.

A condition of oxidative stress occurs when more free radicals exist than can be neutralized by all antioxidants combined. Humans are always in states of oxidative stress. There are always excess free radicals causing damage and the slow decline of the body, and this translates to a natural process of aging. For skin, the most effective strategy to combat oxidative stress is to avoid sun exposure, use sunscreen, and apply a combination of topical antioxidants.

FREE RADICAL DAMAGE CASCADE

The abbreviated chemical reactions below illustrate the accumulation of free radical damage and oxidative stress. The free radical O* contains very high energy and combines with the first molecule it touches. In combining with this structure, O* damages it, and in the process, another free radical is created. The second free radical then combines with the first structure it touches. The negative cascade continues until the free radicals are neutralized. For this reason, antioxidants are crucial to maintaining cellular function as we age.

- O* + cell membrane → damaged cell membrane + A* (Cell membrane protects integrity of cell)
- A* + mitochondria → damaged mitochondria + B* (Mitochondria produce energy for the cell)
- B* + DNA → damaged DNA + D* (DNA is the genetic mechanism of the cell that directs all cellular function and reproduces itself to create another cell. Damaged DNA leads to a cancerous or malignant cell)
- D* + cellular protein/collagen/elastin → → damaged elastic tissue (wrinkles) + E*

The free radical damage cascade shown above depends upon the unpaired electron in the free radical's outer orbit, which captures an electron from other molecules, creating more free radicals in this process and causing ongoing collateral damage. The beauty of antioxidants is that they serve as electron donors to free radicals, thus neutralizing the free radicals but remaining stable

themselves. Antioxidants stop the ongoing cascade of perpetual free radical creation and minimize collateral damage.

Skin cells, being near the surface of the body, are bombarded with additional stressors from the environment that fail to reach other cells deep within the body's interior. This increases the volume of oxidative stress present in the skin. Once a free radical touches the cell, the cascade of free radical damage begins, as illustrated in the above reactions.

Antioxidants can neutralize free radicals before they touch the cell, as well as stop ongoing interior cellular damage. Antioxidants stop the cascade of free radical damage by donating an electron, thus ending the cycle of further free radical creation. It is important to have antioxidant protection at all cellular layers, because it is impossible to stop all free radicals at the surface. Many of them get through the initial skin barrier or come from inside the cell itself via cellular metabolism. Antioxidants combining with free radicals "upstream" can help to prevent a large cascade of damage. In this way, antioxidants can prevent the multiplication of damage seen "downstream."

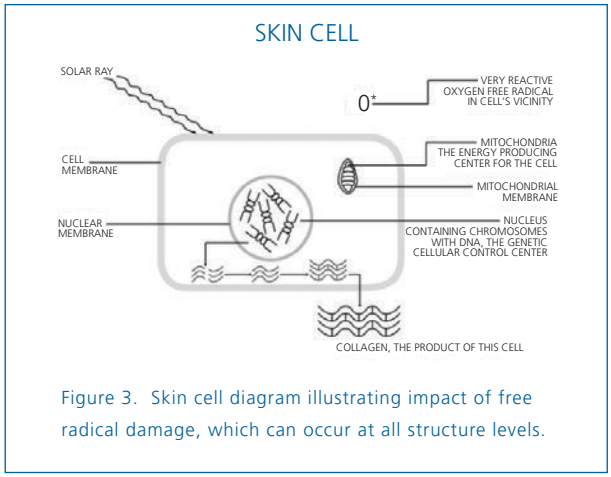

Figure 3. Skin cell diagram illustrating impact of free radical damage, which can occur at all structure levels.

CELL STRUCTURE AND OXIDATIVE STRESS

Membranes are lipid-soluble barriers designed to enclose cells and also interior organelles such as the mitochondria and nucleolus. If they become damaged, they have difficulty protecting their interior structures, as well as letting the right substances in while keeping other substances out. Lipid-soluble antioxidants like vitamin E protect cell membranes and other lipid structures.

Interior cellular structures and interstitial fluid between cells contain mostly water. Therefore, aqueous or water-soluble antioxidants like vitamin C protect these areas from free radical damage.

DNA directs cell function and regulates cellular reproduction. In the case of skin, the function of one cell type might be to make collagen. If the DNA is damaged, it may direct the formation of collagen containing mistakes. Biochemically inaccurate collagen would be unable to function properly. Impaired collagen might have poor elasticity, causing wrinkles, or be unable to bind with other collagen chains, causing wrinkles, loss of resilience, and improper scarring. If enough DNA damage occurs, the cell can become malignant—i.e. cancerous.

Damaged mitochondria are unable to produce adequate energy for the cell. A specific amount of energy is required for the cell to function. Damaged mitochondria may produce too little or too much energy, contributing to an increase in free radical damage.

TYPES OF ANTIOXIDANTS

Antioxidants can be ingested (taken orally) or applied topically to the skin. As little as 1% of antioxidants taken orally reach the surface of the skin. To maximize antioxidant protection, it is necessary to apply effective topical antioxidants to the skin.

Effective topical antioxidants must be high-quality, stable, and in the purest form. Since skin's primary function is to keep some substances in and other environmental agents out, the design of topical antioxidants becomes particularly challenging. Successfully formulated topical antioxidants penetrate the outermost barriers and reach the interior of the cell.

Figure 2. Illustration of a free radical damage cascade with ongoing electron theft and perpetual free radical creation. This results in an ever-widening circle of structural damage and further free radical creation.

Suggested Products—All iS CLINICAL® and iS products are helpful for oxidative stress and free radical damage.

ANTIOXIDANTS

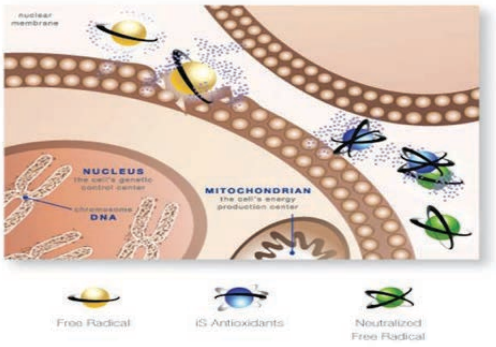

Figure 3. Antioxidants neutralize free radicals by donating an electron, thus stopping the ongoing cascade of damage and further creation of free radicals.

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TRIPEPTIDE-1

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The tripeptide copper complex, Glycyl-L-Hidtidyl-L-Lysine-Copper, is a biomimetic oligomeric peptide. This means that it possesses the same activity as a protein found naturally within the body and it is composed of a small-peptide chain (a chain containing fewer than ten amino acid subunits.) It is a type of growth factor, belonging to the general group of cytokines or proteins that direct cellular activity. Growth factors specifically encourage and direct cell maturation and development. The use of growth factors in medicine is one of the most interesting and valuable areas of research.

Tripeptide-1 mimics the effects of Hepatocyte Growth Factor or HGF. Although the term “hepatocyte” refers to liver cells, Hepatocyte Growth Factor was so named because it was first discovered and studied in liver cells. However, HGF effects all epithelial cell types. Skin cells are types of epithelial cells and, as such, are subject to the direction of HGF, which signals molecules between cells and promotes skin differentiation and development. When cells and organs of epithelial origin are injured, they express HGF to modulate the healing process. The HGF encourages repair, growth and differentiation of new, healthy epithelial cells.

In the case of skin, damage may occur through an actual wound or, more commonly, by the free radical damage of intrinsic aging and photodamage. The consequences of oxidative stress in skin lead to destruction of the normal support matrix of the skin, sagging, pigmentary irregularities and wrinkle development, all of which increase over time. New research into growth factors has demonstrated that skin aging can actually be improved by modulating normal cellular regenerative processes within skin, thus restoring structural integrity of age-damaged tissues.

Tripeptide-1 encourages tissue repair by activating normal proteins of the extra-cellular matrix. These include collagen (responsible for resilience and strength against skin physical stress), elastin (responsible for elastic recoil and elasticity) and laminin (responsible for normal cellular adhesion, particularly at the dermal-epidermal junction). Healthy collagen, elastin and laminin are all required for beautiful skin, which appears healthy and is able to perform its normal functions of protecting the deeper layers of the body, guarding against environmental stress, healing wounds and supporting the structure and function of skin organelles.

Tripeptide-1 is able to penetrate skin and reach fibroblasts in the connective tissue. Is effect on fibroblasts is to encourage the synthesis of healthy collagen. Induction of collagen I and collagen III is particularly encouraged as well as other components of fibronectin, laminin and elastin.

Topical application of this substance has no side effects. This most likely relates to its structure which is very similar to a naturally occurring substance and which has an identical function.

In animal studies, Tripeptide-1 was shown to improve healing in sutured wounds. Strength of the wound was tested by traction upon the wound. Speed of healing was much faster than in control wounds where Tripeptide-1 was not used. Tensile strength was improved 60% using Tripeptide-1.

Wrinkle development proceeds in a fashion quite similar to scarring from surgical wounds. Oxidative damage encourages the inflammatory process with resultant efforts at healing, leading to deep wrinkle development that becomes more severe as aging continues. Tripeptide-1 encourages healing of the pathological effects of aging, encouraging healthy dermal restructuring and healing of wrinkles and stretch marks.

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WHITE PAPER

COPPER TRIPEPTIDE-1

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DEFINITION

Copper tripeptide-1 is a small protein composed of the three amino acids (protein building blocks) glycine, histidine, and lysine combined in a specific geometric configuration with the physiologically beneficial mineral copper. It is sometimes abbreviated GHK-Cu, indicating the chemical symbols for the four molecules composing it. These three amino acids have a very high affinity (attraction) for copper and are often found in association with this mineral in biologic systems. Within the body, Copper tripeptide-1 may be found both complexed with or without copper, although the form including copper is more beneficial. Since 1973 when Copper tripeptide-1 was discovered to cause aged liver cells to behave like young liver cells, a large body of scientific evidence has accumulated regarding the safety and beneficial effects of this fascinating compound.

LOCATION IN THE BODY

Injured tissues of all types contain Copper tripeptide-1, which acts as a signaling agent for processes of repair and regeneration, assisting damaged tissue to return to normal non-injured function. Copper tripeptide-1 was first found in human plasma, the liquid portion of blood minus the blood cells, and found to have helpful effects for liver cells. Soon, it was also found in saliva, urine, and collagen and then discovered to have important effects in repairing and maintaining all tissue types. Copper tripeptide-1 is bio-identical in that it has the exact chemical structure of molecules found naturally in the human body. Since it is a small molecule, it is able to move easily within tissue and around cells. Much of its benefit relates to its ability to efficiently bind and transfer copper ions. Copper tripeptide-1 is a member of a large family of copper-containing enzymes helpful in tissue repair, inflammation, metabolism, and synthesis of vital molecular structures.

ACTIONS

Copper tripeptide-1 belongs to a group of emergency response molecules which are released during injury and come to the body's aid when the processes below are activated.

Wound Healing

A huge body of scientific evidence supports the essential role of Copper tripeptide-1 in acceleration of wound healing. This compound is released during any tissue injury to signal repair processes to begin. Research has documented its benefit when used in various types of wounds, including surgical, post-laser, ischemic, burns, skin transplants, hair transplants, and diabetic ulcers. Diabetic wounds healed three times faster in the presence of Copper tripeptide-1. Time to re-epitheliazation is shortened.

Tissue Remodeling

Furthermore, Copper tripeptide-1 is active not only for primary healing but also for tissue remodeling, which is the return of injured tissue to normal architecture and function. It increases keratinocyte proliferation and normal collagen synthesis, improves skin thickness, skin elasticity and firmness, improves wrinkles, photodamage and uneven pigmentation, improves skin clarity, and tightens protective barrier proteins. Through effects on decorin, new collagen made in injured tissue assumes the correct anatomical configuration and structure rather than a disorganized scar. Matrix support structures of the dermis, including Collagen I, Collagen III, and glycosaminoglycans, are increased in the presence of Copper tripeptide-1 as normal tissue configuration is restored after injury. Scar-forming processes are minimoized and protein synthesis increases through direct effects on fibroblasts. Copper tripeptide-1 blocks the effects of toxins on liver cells. It improves surgical outcome of joint replacements by increasing bonding strength and new bone formation between hardware and native bone. It also encourages healing of all types of gastrointestinal ulcers, including

Anti-Tumor Effect and DNA Repair

Copper tripeptide-1 has a strong anti-tumor effect, partly mediated via decorin. It protects DNA from damaging effects of radiation, including radiation for cancer treatments and photoaging from sun exposure. Tumor cell lines died in the presence of Copper tripeptide-1 while healthy cell lines were encouraged. This compound is therefore not only biologically safe, but actually protective.

Stem Cell Anti-Senescence

By modulating integrins and p63, Copper tripeptide-1 promotes the survival of stem cells in the basal epidermis. This suggests it could have an important anti-aging role and, in fact, the anti-senescent effects of Copper tripeptide-1 have been demonstrated on a number of tissue types. Radiation severely damages DNA and induces aging but fibroblasts exposed to radiation were able to restore their function to that of intact cells in the presence of Copper tripeptide-1.

Anti-Inflammatory and Antioxidant Effects

Many of the body's intrinsic antioxidant systems, including superoxide dismutase, are induced by Copper tripeptide-1. Vitamin C and other antioxidant levels are increased in healing wounds. Its strong anti-inflammatory effect has been demonstrated during wound healing, photodamage, and wrinkle improvement.

Anti Microbial Effects

Copper tripeptide-1 lessens the risk of infection developing in a wound or in remodeled tissue. This partly relates to inhibition of ferritin ion release, a pro-oxidant.

Nerve Cell Maintenance

Copper tripeptide-1 has been suggested to have a potential therapeutic role in age-related neurodegeneration and cognitive decline. It improves axon survival and maintenance of nerves.

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Introducing iS CLINICAL® ADVANCE+

Stabilized L Ascorbic Acid Formulas

INNOVATIVE SKINCARE® has raised the bar once again in vitamin C technology with the iS CLINICAL® ADVANCE+ line. Featuring a cutting-edge vitamin C (L ascorbic acid) technology along with Copper Tripeptide Growth Factor (in select products) the ADVANCE+ line produces more rapid, pronounced improvements in the skin than ever before.

Superior Vitamin C technology:

The superior vitamin C technology employed in our remarkable ADVANCE+ formulas has been developed to increase stability and to offer time-released, continuous and steady delivery of L ascorbic acid to the cell, providing a more efficient and effective form of vitamin C. This powerhouse ingredient provides improved collagen production, exceptional antioxidant and UV-photo protection, as well as safe, natural lightening of the skin.

ADVANCE+ L Ascorbic Acid vs. Traditional L Ascorbic Acid

- **Improved Antioxidant Protection:** ADVANCE+ L ascorbic acid is better than traditional L ascorbic acid in its ability to squelch free radicals. On a per-molecule comparison, the number of free radicals neutralized is higher with ADVANCE+ L ascorbic acid. When both ADVANCE+ L ascorbic acid and traditional L ascorbic acid are combined with a specific number of free radicals, the traditional L ascorbic acid exhausted itself after 10 minutes. After 2 hours, ADVANCE+ L ascorbic acid was still active and neutralizing free radicals.

- **Ability to be Combined With Copper:** Previously incompatible, iS CLINICAL's ADVANCE+ L ascorbic acid successfully combines with copper, bringing together some of the most important elements in collagen synthesis.

  Until now, the addition of copper-containing substances to vitamin C products has been quite problematic because copper oxidizes the L Ascorbic Acid. The addition of another moiety to its chemical structure allows ADVANCE+ L ascorbic acid to become much more stable and facilitates easier formulation. This is particularly important when combined with substances that might react with it. This exact issue was investigated by using copper ions as a pro-oxidant and adding it to the ADVANCE+ L ascorbic acid. The graphs below demonstrate that no negative interaction occurred between the ADVANCE+ L ascorbic acid and the copper. At the experiment's conclusion, the amount of copper and the amount of ADVANCE+ L ascorbic acid remained unchanged.

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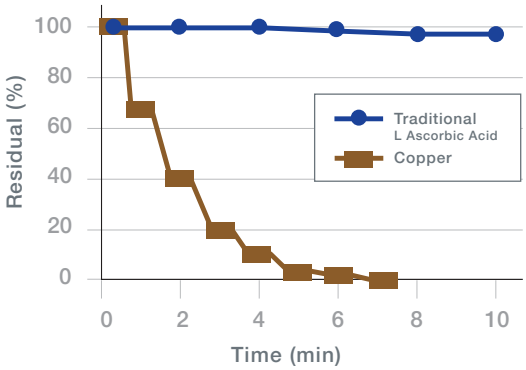
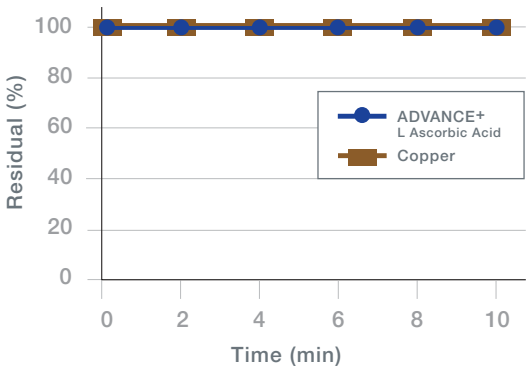
L Ascorbic Acid and Copper Can Be Combined



Combining traditional L ascorbic acid and copper causes a chemical reaction between the 2 substances nullifying the copper and making the L ascorbic acid much less effective for the skin. This means that neither substance remains fully available for beneficial activity in the skin.



Advance+ L ascorbic acid and copper, placed together in a formulation, work synergistically and do not react with each other, leaving both substances completely available to the skin.

Vitamin C is necessary for effective collagen synthesis. Copper is required as a cofactor in collagen synthesis. The unique ability of ADVANCE+ L ascorbic acid to combine with copper allows it to induce collagen synthesis much more effectively than traditional L Ascorbic Acid. Here is another set of chemical reactions (i.e. synthesis of collagen) that will potentiate each other due to their unique combination in iS CLINICAL® products.

- **Improved Collagen Production:** ADVANCE+ L ascorbic acid is more effective than L ascorbic acid for inducing collagen formation. Improvement is observed in more sustained collagen production when compared with ascorbic acid. This is thought to be related to the higher cellular levels of ascorbic acid produced by ADVANCE+ L ascorbic acid. L Ascorbic acid levels at the site of action are elevated for a more extended period with ADVANCE+ L ascorbic acid than with L ascorbic acid itself.

  In studies, fibroblasts from human skin were stimulated to produce collagen and then incubated in the presence of ADVANCE+ L ascorbic acid. The amount of collagen synthesized was compared to L ascorbic acid. Both types of L ascorbic acid were provided to the cells only at initiation of the experiment. With both types of L ascorbic acid, there was an immediate increase in collagen synthesis to approximately the same level. However, by the eighth day, the units of collagen production had dropped from 14 to 6 in the cells provided L ascorbic acid. In the cells provided ADVANCE+ L ascorbic acid, there was no decrease in collagen production at the eighth day.

- **Improved Lightening Effects:** ADVANCE+ L ascorbic acid reduces the accumulation of lipofuscin induced by UVB irradiation. This has not been shown to occur with ascorbic acid. Lipofuscin is a complex of oxidation products, including oxidized lipids that are found in “age spots”, the hyperpigmented areas seen with photoaging. This data suggests that ADVANCE+ L ascorbic acid is superior in ameliorating these “age spots”. Furthermore, since these lesions are comprised of oxidized lipids, the research suggests that ADVANCE+ L ascorbic acid can be used to treat lipid peroxidation in general. Lipid peroxidation is involved in numerous processes associated with aging, such as vascular disease, dementia, diabetes, and others. Because all cellular membranes are comprised of lipids, this protection against lipid peroxidation provides vast positive implications for the skin.

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When ADVANCE+ L ascorbic acid and traditional L ascorbic acid were each added to B16V melanoma cells to ascertain their ability to inhibit melanin production, ADVANCE+ L ascorbic acid tested superior to traditional L ascorbic acid after 1day. After 2 days, traditional L ascorbic acid had lost its effectiveness completely, while ADVANCE+ L ascorbic acid continued to decrease melanin content on day 2.

- **Activity of ADVANCE+ L ascorbic acid:** ADVANCE+ L ascorbic acid has pro-vitamin C activity. It is converted to L ascorbic acid within the cell. Its biologic effect, however, lasts longer than L ascorbic acid due to sustained cellular release of L ascorbic acid. This sustained activity is responsible for many of the advantages of ADVANCE+ L ascorbic acid over traditional ascorbic acid.

- **Maintains Higher Ascorbic Acid Levels In Cells:** Within the cell, ADVANCE+ L ascorbic acid converts to L ascorbic acid. Sustained cellular release of ascorbic acid occurs when ADVANCE+ L ascorbic acid is applied topically. ADVANCE+ L ascorbic acid is superior to L ascorbic acid in maintaining ascorbic acid levels in the cell.

  Compared to traditional L ascorbic acid, efficacy is prolonged as the conversion of ADVANCE+ L ascorbic acid to L ascorbic acid proceeds within the cell at a constant rate, providing a more sustained and reliable intracellular level of L ascorbic acid.

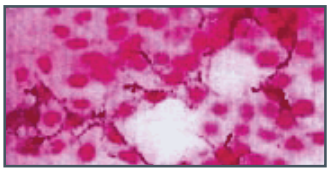
- **Improved Intracellular Antioxidant Protection:** ADVANCE+ L ascorbic acid is superior to L ascorbic acid in resisting oxidative degradation and higher intracellular levels of ascorbic acid are maintained for a longer period. The improved resilience of ADVANCE+ L ascorbic acid against oxidative stress is thought to be caused by the proprietary moiety to the chemical structure of the ascorbic acid.

  When a colorimetric assay was used to evaluate the free radical scavenging ability of L ascorbic acid compared to ADVANCE+ L ascorbic acid, the L ascorbic acid exhausted its free radical scavenging ability after 10 minutes. The ADVANCE+ L ascorbic acid was still fully active in scavenging free radicals after 2 hours. This indicates the far superior oxidative protection provided by the ADVANCE+ L ascorbic acid.

- **Inhibits Melanin Production:** ADVANCE+ L ascorbic acid inhibits the production of melanin.

Effects on Melanin Synthesis

Without ADVANCE+ L ascorbic acid



With ADVANCE+ L ascorbic acid

The efficacy of ADVANCE+ L ascorbic acid was tested using B16 melanoma cells treated for 12 hr with 2-O-alpha-D-glucopyranosyl-L-ascorbic acid (2.5mM) or a placebo. Following treatment theophylline (0.5mM) was added to stimulate melanin synthesis, and the cells were tested after 48 hr for the presence of DOPA quinone (a precursor to melanin) using a histochemical stain. In conclusion, ADVANCE+ L ascorbic acid acid demonstrated the ability to effectively prevent skin pigmentation.

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- **Helps Prevent UVB Damage:** ADVANCE+ L ascorbic acid helps prevent UVB damage in human skin keratinocytes and fibroblasts. Sunburn cells are a measure of skin damage from UVB rays. They are cells that have been severely damaged by the sun and are dying, releasing toxins and inflammatory mediators. When ADVANCE+ L ascorbic acid is applied to skin before UVB exposure, the number of sunburn cells is decreased by 400% compared to the control. Furthermore, traditional L ascorbic acid allows for the production of a larger number of sunburn cells caused by UVB exposure than does ADVANCE+ L ascorbic acid.

- **Promotes Production Of HGF:** ADVANCE+ L ascorbic acid promotes the production of HGF. HGF (Hepatocyte Growth Factor) is the growth factor that induces growth and development of all epithelial, cell types including skin. Measured basal HGF production by human skin fibroblasts increases with the addition of ADVANCE+ L ascorbic acid.

- **Protects Against Lipid Peroxidation:** ADVANCE+ L ascorbic acid protects against lipid peroxidation in the presence of vitamin E. When human keratinocytes were irradiated with UVB, the intracellular amount of peroxidized lipids doubled. If, prior to UVB irradiation, ADVANCE+ L ascorbic acid was added to the human keratinocytes, much less lipid peroxidation occurred.

- **Potentiates Vitamin E:** ADVANCE+ L ascorbic acid potentiates the function of vitamin E and vitamin E's antioxidant ability. When human keratinocytes were irradiated with UVB, adding ADVANCE+ L ascorbic acid and vitamin E completely protected the cells, i.e. the amount of damage was the same as without any radiation. This highly significant finding illustrates the power of the ADVANCE+ line in potentiating effects of other antioxidants. ADVANCE+ L ascorbic acid and vitamin E are a particularly good combination as together they give synergistic antioxidant protection to aqueous-soluble elements (within the cell and between cells) and to all lipid (fat) soluble elements (cell membranes enclosing the cell and its organelles to maintain their integrity).

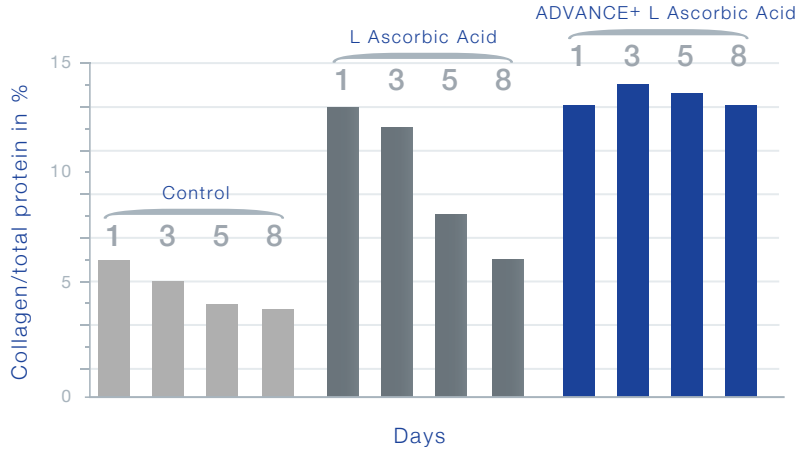
- **Increases Antibody Production and Stimulates Immune Function:** ADVANCE+ L ascorbic acid is a potent stimulator of immune function and antibody production in humans.

  Both ADVANCE+ L ascorbic acid and traditional L ascorbic acid are stimulators of immune function. Antibody production by peripheral lymphocytes was examined when the lymphocytes were stimulated with pokeweed mitogen or Staph aureus. ADVANCE+ L ascorbic acid and the L Ascorbic Acid induced equivalent amounts of antibody production.

- **Encourages Proliferation of Fibroblasts and Increases Collagen Production:** ADVANCE+ L ascorbic acid assists proliferation of human skin fibroblasts and collagen production.

Effects on Collagen Synthesis

Human skin fibroblasts were incubated in the presence of a control, ascorbic acid (AsA; 0.25mM) or ADVANCE+ L ascorbic acid (0.25mM) for 1- 8 days. The percentage of collagen synthesis to total protein synthesis was determined. ADVANCE+ L ascorbic acid was far superior to traditional L ascorbic acid at promoting collagen synthesis. Stimulatory effect of ADVANCE+ L ascorbic acid continued for the full 8 day period.



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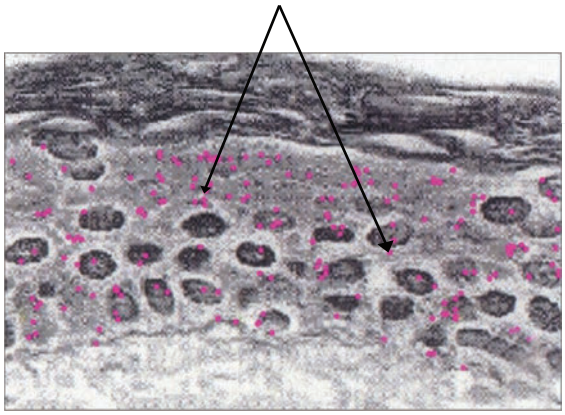
Absorption of ADVANCE+ L Ascorbic Acid

ADVANCE+ L ascorbic acid is well absorbed through intact skin. When applied topically, granules of ascorbic acid are shown microscopically within the skin. This illustrates that the ADVANCE+ L ascorbic acid is absorbed.

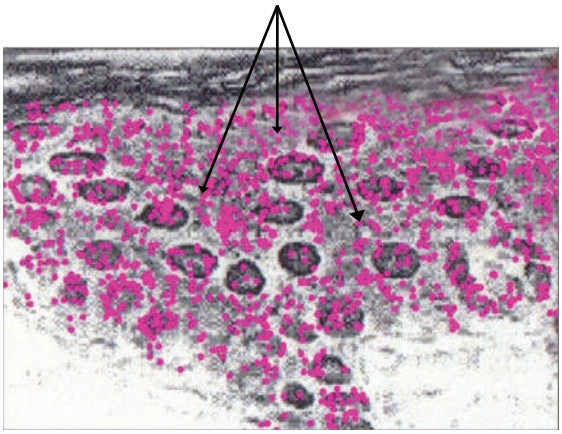
L-Ascorbic Acid



Placebo (containing NO source of L-Ascorbic Acid and NO ADVANCE+ L-Ascorbic Acid) was applied to a human forearm, covered with occlusive film for 12 hours, the film removed and the skin biopsied after 24 hrs.

L-Ascorbic Acid granules found normally in skin are stained by the special silver stain.

L-Ascorbic Acid



ADVANCE+ L-Ascorbic Acid was applied to a human forearm and the same experiment was completed. Numerous granules of L-Ascorbic Acid was absorbed quite well. Furthermore, this experiment illustrates that ADVANCE+ L-Ascorbic Acid was converted to L-Ascorbic Acid within the skin cells.

Natural Occurrence of ADVANCE+ L Ascorbic Acid ADVANCE+ L ascorbic acid is found in vivo in mammals, within all cells of their bodies, throughout their lives. In plants, it is found in the fruit of Lycium barbarum.

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iS CLINICAL® ADVANCE+ Formulas:

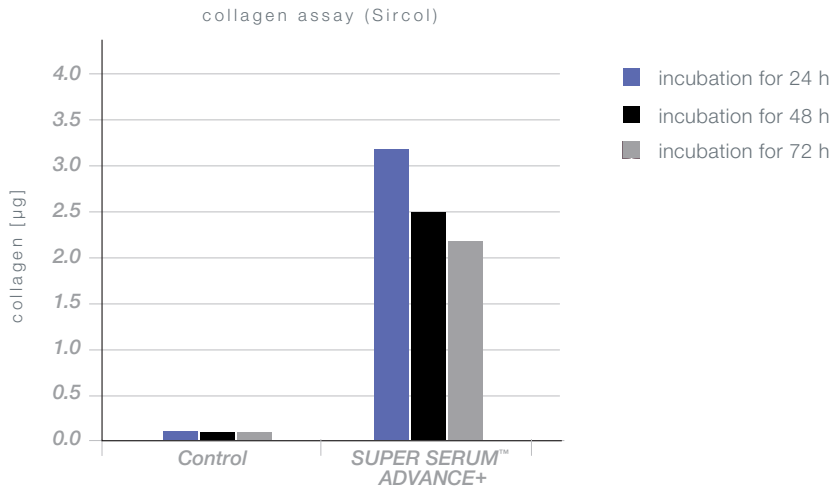
SUPER SERUM™ ADVANCE+ | PRO-HEAL® SERUM ADVANCE+ | C EYE ADVANCE+
C & E SERUM ADVANCE+ | C-15 SERUM ADVANCE+

SUPER SERUM™ ADVANCE+

SUPER SERUM™ ADVANCE+ features our stabilized L ascorbic acid (ADVANCE+ L ascorbic acid), which for the first time has been successfully combined with our featured copper tripeptide-1 growth factor (HGF, or Hepatocyte Growth Factor). These advanced ingredients are formulated with Centella asiatica, vitamin B5 (Calcium Pentothenate), Arbutin, mushroom extract (Polyporus umbellatus), and pure Hyaluronic acid. SUPER SERUM™ ADVANCE+ exhibits properties superior to other products on the market, including:

- **Anti-Aging Benefits:** For the first time vitamin C can be effectively combined with a copper substance without compromising efficacy. The Copper Tripeptide (HGF) Growth Factor formulated in SUPER SERUM™ ADVANCE+ is clinically proven to promote healing of the pathological effects of aging, encouraging healthy dermal restructuring and healing of wrinkles and stretch marks. ADVANCE+ L ascorbic acid and Copper Tripeptide (HGF) Growth Factor work synergistically to produce more rapid, pronounced improvements in the skin than ever before.

Effects on Collagen Synthesis using SUPER SERUM™ ADVANCE+



Collagen synthesis: Collagen serves as the elastic framework of the skin, providing tensile strength and resilience against mechanical stress. Fibroblasts are the cells within the dermis responsible for collagen synthesis. As the skin ages, collagen synthesis is impaired and the amount of functional collagen decreases. When human fibroblasts are exposed to SUPER SERUM™ ADVANCE+, collagen synthesis increases dramatically, as has been clinically validated.

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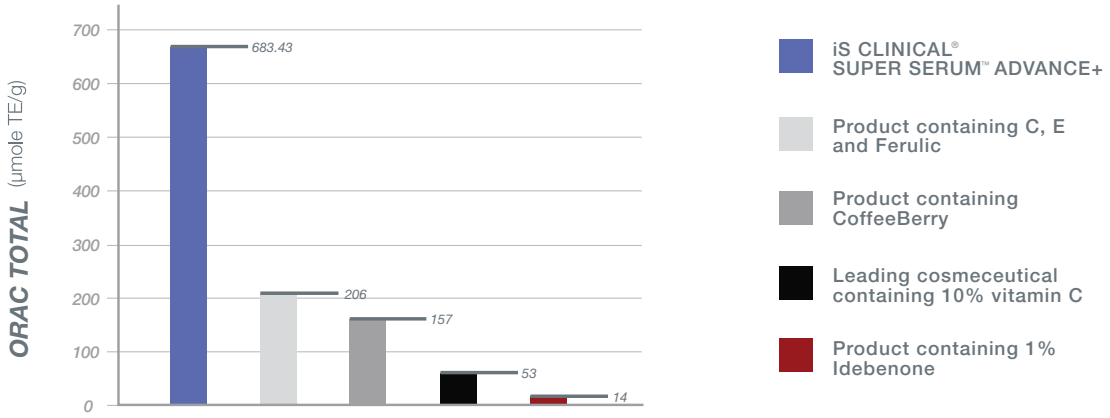
- **Significantly Increased Antioxidant Protection:** SUPER SERUM™ ADVANCE+ is clinically proven to provide significant antioxidant protection.

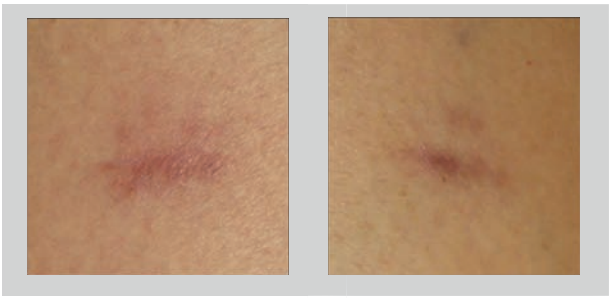
Comparative Antioxidant Strength



ORAC (Oxygen Radical Absorption Capacity) measures total lipophilic and hydrophilic antioxidant capacity
Testing independently performed by Brunswick Labs.

- **Reduction in Scar Tissue:**
  SUPER SERUM™ ADVANCE+ is extremely effective at reducing scarring and associated hyperpigmentation. In a study to test improvements in scar healing, the test subject was cut on each thigh with identical wounds. Nothing was applied to the thigh on the right (control). SUPER SERUM™ ADVANCE+ was applied twice daily to the thigh on the left for 3 months and for another 3 months once daily. The photos below were taken at 3 months and at 6 months. Scarring evident on the treated side is considerably less than the control. In addition, smoother texture and more even pigmentation are noted on the treated side.

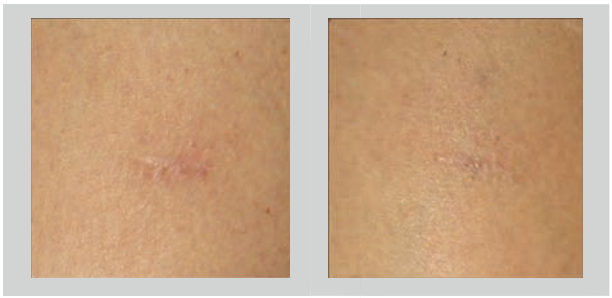
Scar maturation: 3 Months



3 months. Control

3 months with SUPER SERUM™ ADVANCE+ applied twice daily

Scar maturation: 6 Months



6 months. Control

6 months with SUPER SERUM™ ADVANCE+ applied twice daily for the first 3 months and once daily for the following 3 months

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Histology for Scar Maturation Study

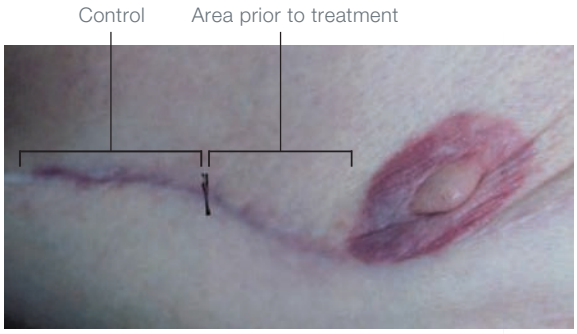
Identical full-thickness incisions were made. One incision served as the control. SUPER SERUM™ ADVANCE+ was applied to the other incision twice daily for 3 months and then once daily for the following 3 months. Both areas were biopsied (incisional biopsies) and sent to a dermatopathologist for examination. The histologic comparison of the control and treated sides is given below in a table:

	CONTROL	TREATED
INFLAMMATION	55-65 chronic inflammatory cells per perivascular space	Less than 50 chronic inflammatory cells per perivascular space
COLLAGEN	Collagen bundles in disarray, gaps between bundles	Collagen bundles regular and orderly, parallel bundles
SCAR SURFACE	Scar surface more elevated above skin surface, orthohyperkeratosis present	Stratum corneum fully basket-weaved, with same appearance as adjacent non-scarred area

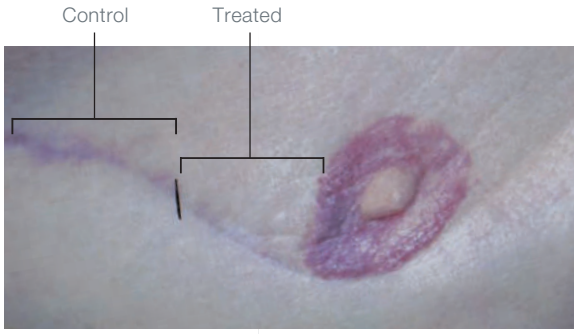
As the above table illustrates, the scar treated with SUPER SERUM™ ADVANCE+ had less chronic inflammation, much more orderly collagen bundles, a flat surface without hyperkeratosis and a fully basket-weaved stratum corneum that appeared like the stratum corneum in the adjacent non-scarred area.

In another study showing improvements in scar healing, product application began 10 weeks after mastectomy. Product was applied to one-half of the incision and the other half of the incision served as control to which no product was applied. Digital photos were taken after one month of twice daily use with SUPER SERUM™ ADVANCE+. The treated side shows less inflammation, smaller overall scar area (including length and width) and a much less visible scar. This is particularly significant since product use began 10 weeks after the surgical procedure.

Scar Maturation



10 weeks after surgery.
Before application of SUPER SERUM™ ADVANCE+



14 weeks after surgery.
Treated side has 4 weeks of
SUPER SERUM™ ADVANCE+ application

More Efficiently Treats Hyperpigmentation:
ADVANCE+ L ascorbic acid is clinically proven to more effectively inhibit the production of melanin and reduce the appearance of pre-existing melanin, resulting in the correction of hyperpigmentation and overall lightening of the skin. The combination of ADVANCE+ L ascorbic acid, Arbutin, and mushroom extracts in SUPER SERUM™ ADVANCE+ works safely and synergistically to inhibit and correct melanin production even more efficiently than before.

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"The clinical line of products stand out from the rest. SUPER SERUM made an extraordinary difference in my skin, and my skin is used to prescription-strength products. All the professional staff in this office has seen significant results. The texture and clarity of my skin and improved dramatically."

Dr. Gerald N, Goldberg, Dermatologist

SUPER SERUM™ ADVANCE+®

3.0 pH +/- 0.5 15ml, £72 30ml, £112

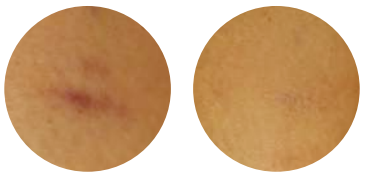
ANTI-AGING I BRIGHTENING I PROTECTIVE

Clinically proven and featuring our scientifically advanced Vitamin C time release technology, with Copper Tripeptide growth factors combined with a powerful blend of our most powerful botanical antioxidants, this formula offers exceptional anti-aging and reparative benefits especially with scar tissue.

BENEFITS

- Reduces appearance of fine lines & wrinkles
- Promotes wound healing & reduction of scar tissue
- Significantly increases collagen production
- Safely lightens & treats hyperpigmentation
- Provides superior anti-oxidant protection

CLINICALLY PROVEN TO REDUCE SCAR TISSUE WITH SUPER SERUM ADVANCE+



3 months 6 monts

KEY INGREDIENTS	INGREDIENT BENEFITS
ASCORBIC ACID (vitamin C) 15.0%	Scientifically-advanced, stabilized form of L ascorbic acid providing time-released, continuous delivery. Potent antioxidant with photo-protective and anti-inflammatory properties. Stimulates collagen synthesis and promotes wound healing.
ASIATICOSIDE, ASIATIC ACID AND MADECASSIC ACID (centella asiatica) 0.5%	Potent antioxidant that stimulates collagen synthesis and improves microcirculation, capillary flow and vascular tone. Promotes wound healing and reduction of scar tissue.
CALCIUM PANTOTHENATE (vitamin B5) 0.5%	Essential element required for enzymatic functions within all cells. Enables the metabolism of carbohydrates, proteins, and lipids, increasing cellular integrity and efficiency.
ARBUTIN 0.5%	Essential element required for enzymatic functions within all cells. Enables the metabolism of carbohydrates, proteins, and lipids, increasing cellular integrity and efficiency.
POLYPORUS UMBELLATUS (MUSHROOM) EXTRACT (source of kojic acid) 0.5%	Inhibits tyrosinase enzymes that produce melanin, thus controlling hyperpigmentation. Also exhibits powerful anti-bacterial, antimicrobial and antibiotic properties.
COPPER TRIPEPTIDE HGF GROWTH FACTOR (proprietary)	Small peptide chain that encourages and directs cell maturation and development. Promotes healing of the pathological effects of aging, encouraging healthy dermal restructuring and healing of wrinkles and stretch marks.

Stockist Enquiries: isclinical.co.uk For further information, images and samples please contact Jenny Pabila: +44 (0) 7971 032 570 or jennypabila@nichepublicrelations.com