The WHAT IS BIOCELL COLLAGEN?

Liquid Biocell Collagen, multi-patented worldwide, provides the vital ingredients Hyaluronic Acid (HA), Collagen and Chondroitin sulfate our joints need in order to rebuild and lubricate them. **Liquid Biocell Collagen** has proven efficacy from medical research, 35 published clinical studies, laboratory studies and human clinical trials.

Medical research and clinical studies demonstrate that Liquid Biocell Collagen is the only clinically proven formulation usable by the human body. Studies prove it can restore Hyaluronic Acid and Collagen at a 60 fold increase in 28 days and prevent the further breakdown of HA and Collagen.

BioCell Collagen II® has earned the highly regarded and important GRAS-designation, Generally Recognized as Safe, under the FDA's regulations of safety through scientific procedures based upon published studies, unpublished studies and other data and information.

The product is clinically proven to stimulate new synthesis of cartilage components for an active, flexible and mobile body. **Liquid Biocell Collagen** contains both highly absorbable Collagen and HA which are needed to rebuild healthy skin from the inside and to enhance hydration and skin volume and maintain these levels in damaged and aging skin. Saggy skin happens as the skin loses collagen, elastin, and hyaluronic acid (HA). As HA decreases, unwanted lines, wrinkles, and deep folds appear. The loss of HA is responsible for dehydration of skin, which leads to thinning and reduction in volume and suppleness. The BioCell Collagen patented manufacturing process reduces the size of collagen, HA and chondroitin sulfate to ensure efficient absorption.

Since 1997, **Biocell Collagen** dry capsules (does not contain the powerful antioxidants) has been safely and effectively consumed by millions of consumers worldwide with no adverse effects. Billions and billions of capsules have been sold by retailers under their own brand names.

Liquid Biocell Collagen is GMP Labs' next generation product: scientifically advanced technology which has taken years of research and development to create, patent and clinically test. This product is light years ahead of having to swallow several large capsules which can remain in the stomach for several hours or a day—just a small amount of **Liquid Biocell Collagen** is many times more powerful and is immediately absorbable.

LIQUID BIOCELL COLLAGEN IS A POWERHOUSE PRODUCT THAT LET'S YOU TAKE BACK YOUR LIFE TO FEEL BETTER AND LOOK YOUR BEST!

Arthritis, sports fatigue, injuries and aging can all cause wear and tear on our joints leading to discomfort, inflammation and pain as well as more serious joint issues. This affects the very quality of our lives and every year it gets worse. Yet few options have been available for joint issues. We have all used the hundreds of supplements with, and spent thousands of dollars for, Glucosamine and MSM and Chondroitin but these have failed to provide

freedom from pain, stiffness and reduced mobility.

According to the Center for Disease Control and Prevention, more than 50 million people over 45 suffer from joint pain and stiffness, arthritis, and wear and tear of joint cartilage; and everyone will experience visible signs of skin aging and skin damage. The exclusive liquid BioCell Collagen II® formulation can help prevent the breakdown of hyaluronic acid (HA), help maintain HA levels for healthy skin, and guard against natural and photo- aging as well as minimize sagging skin.

Liquid Biocell Collagen is the only liquid antioxidant available containing the internationally patented formula of hyaluronic acid, collagen and chondroitin sulfate combined with the highest levels of resveratrol. It is the only clinically proven product that is completely usable by the human body and is made with a rapid absorption process which delivers all the ingredients for immediate use.

THE COMPANY BEHIND LIQUID BIOCELL COLLAGEN

GMP Laboratories of America, Inc. was founded more than 20 years ago and is located in the Anaheim Center for Advanced Technology in California. The company is an international leader and is known worldwide as a custom developer of pharmaceutical grade dietary supplements, vitamins and homeopathic products.

GMP Labs is an FDA certified Good Manufacturing Practices facility and is FDA approved for manufacturing of over the counter drugs. The company provides a complete range of research and development, manufacturing and packaging services in its state of the art 90,000 square-foot premises. They develop and manufacture products for the largest sellers of supplements internationally including Walmart, Vitacost, Olympian Labs, Purity Products, Vitamin Shoppe, GNC, Whole Foods, Source Naturals, Health Logics, and numerous others which are under strict confidentiality. The company has turned down offers from these large retailers for the rights to **Liquid Biocell Collagen.**

HOW LIQUID BIOCELL COLLAGEN IMPROVES OUR HEALTH

It is a scientific fact and biological certainty that Hyaluronic Acid (HA) and Collagen will break down in the body as we age. By the age of 50, research has shown that the amount of Hyaluronic Acid in our bodies is reduced by almost half. This negatively impacts all our joints causing pain and stiffness, loss of flexibility, arthritis, and mobility problems as well as negatively impacts our skin causing loss of firmness, wrinkles, and sagging.

HYALURONIC ACID- The most amazing biological lubricant and vital to the human body:

- HA is naturally present in the human body and found in the highest concentrations in the joints, skin, hair and eyes.
- HA works by acting as a cushion, shock absorber and lubricant in the joints and

keeps the bones from rubbing together.

- Loss of HA and Collagen as we age causes the skin on our face and body to become wrinkled and lined, lose firmness and sag, and for both men and women, hair starts thinning.

PATENT PROTECTION

Liquid Biocell Collagen has no competition because it is protected by several worldwide patents. GMP Labs owns the proprietary process that makes HA, Collagen and Chondroitin Sulfate into the most potent and completely usable form.

THE SCIENCE BEHIND BIOCELL COLLAGEN

Decades of medical research, scientific substantiation, 35 published clinical studies, and the published peer reviewed clinical studies conducted by GMP Labs support the safety and efficacy of **Biocell Collagen.**

I. SAFETY TESTING

- Several toxicology studies demonstrated that Biocell Collagen is well tolerated over 30 times the recommended human dose.
- Human clinical trial proved Biocell Collagen was well tolerated without any adverse effects.
- Human clinical trial evaluating the safety of resveratrol found no serious adverse effects.

II. CLINICAL TRIALS

1) **Human Joint Pain**
   89 subjects were given BioCell Collagen. 80 out of 89 subjects taking Biocell Collagen experienced reduction of pain.

2) **Pilot Human Joint Health**
   16 subjects were given BioCell Collagen. All subjects reported significant reductions in pain and stiffness as well as greatly improved activities of daily life.

3) **Human Joint Health-Multi-Center**
   Largest human study to date of Biocell Collagen in 80 subjects suffering from joint conditions associated with arthritis. Proved safety and efficacy in improving joint condition symptoms, joint stiffness and physical, social & emotional function of a person with osteoarthritis.

4) **Human Skin Study Of Biocell Collagen - Ongoing**
   Early results show: hydrated skin with fewer wrinkles, prevention of the breakdown of HA, helps maintain HA levels for healthy skin, and guards against natural and photo- aging.

WHAT YOU CAN EXPECT FROM LIQUID BIOCELL COLLAGEN

- **According to clinical trials, the benefits of Liquid Biocell Collagen are evident before six weeks. It is recommended taking it twice per day, consistently, for two to three months to experience the product's full benefits and optimal results.**

- **Each 25oz Bottle Contains 30 Grams (30,000mg) of A Standardized Quality Controlled Amount of Biocell Collagen, Resveratrol and Potent Anti-Oxidant 'Super' Fruits: Blueberry, Red Grapes, Strawberry, Apple, Pomegranate, Mangosteen, Açai, Jujube, Lycium (Goji Berry), Nopal, Maqui, and Noni.**

  - 6oz daily is equal to 6000mg of Immediately Absorbable Ingredients

WE KNOW RESVERATROL IS AN IMPORTANT NUTRIENT- CAN'T WE DRINK A COUPLE OF GLASSES OF WINE AND GET ALL WE NEED?

-1 ounce LIQUID BIOCELL COLLAGEN = as much resveratrol as 2 bottles of red wine
-6 ounces LIQUID BIOCELL COLLAGEN = as much resveratrol as 12 bottles of red wine
-1 bottle LIQUID BIOCELL COLLAGEN = as much resveratrol as 48 bottles of red wine

MORE IMPORTANTLY-- no controls or standards exist for the amount of resveratrol in red wine-not by any company or in any type of red wine
--one glass of red wine = 1mg-5mg resveratrol depending on the wine
--one bottle of red wine = 5 mg-25mg of resveratrol depending on the wine

GMP Labs Gains NSF Certified for Sport® Registration

The dietary supplement manufacturer meets NSF's stringent certification requirements for 150 banned substances by professional athletic associations.

Anaheim, CA (PRWEB) December 29, 2011

GMP Laboratories of America, Inc. is now registered as NSF Certified for Sport®. The program is specially designed by NSF International as a focused solution designed to minimize the risk that a dietary supplement or sports nutrition product contains banned substances. The program is officially recognized by the **NFL, NFLPA, MLB, MLBPA, PGA, LPGA, NCAA, and CCES.**

In order to obtain the registration, GMP Laboratories had to meet the NSF's stringent certification requirements. These guidelines were developed through a consensus process which involved regulators, sports industry leaders, and consumer groups. Some of the requirements include product testing for over 150 banned substances, label content confirmation, formulation and label review, production facility and supplier inspections, as well as ongoing monitoring in line with substance prohibitive lists: **NSF Annex A, NFL, MLB, and NCAA.**

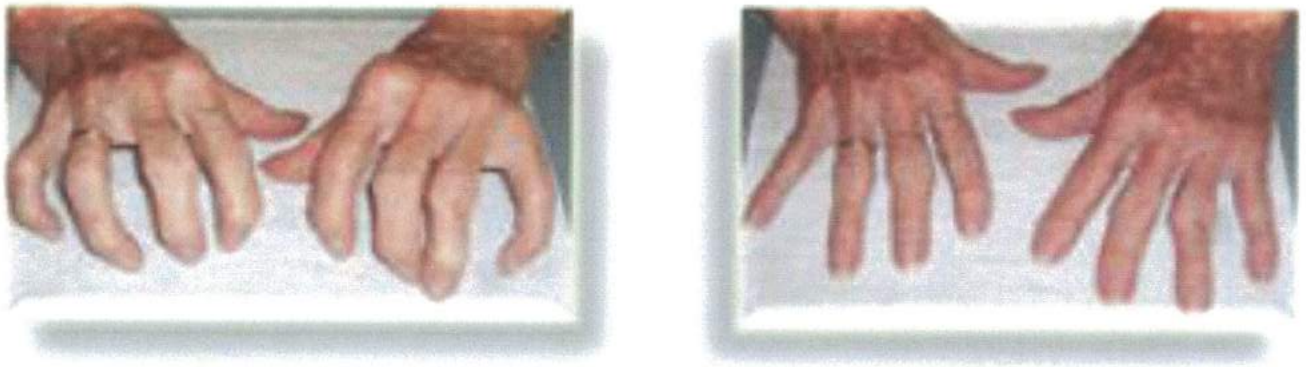
Customers of products that are made at an NSF Certified for Sport® facility can be sure that the products were manufactured with the following preventative measures in mind, to:
1) Protect against adulteration of products,
2) Verify label claims against product contents, and
3) Identify athletic banned substances in the finished product or ingredients.

Suhail Ishaq, president of GMP Laboratories of America, commented on this achievement, "We are proud to expand our growing list of cGMP certificates with the prestigious NSF Certified for Sport® registration. Additionally, our customers now have even more confidence that the sport supplements manufactured at our site are held to the highest standards." GMP Labs' other certificates include cGMP compliance from both NSF International and Silliker Labs.

8 WEEK

Clinical Trial Results

Participants taking BioCell Collagen II® showed increase in mobility and decrease in stiffness



Before **After**

BIOCELL TECHNOLOGY LLC

COMPETITIVE ADVANTAGES OF BioCell Collagen II®

Joosang Park, VP of Scientific Affairs

11/20/2009

BioCell Collagen II® provides highly absorbable, hydrolyzed collagen type II (approx 60%), depolymerized chondroitin sulfate (20%), and hyaluronic acid (10%) derived from a naturally occurring source for individuals looking to support various arthritic joint conditions and to improve aging-associated skin appearance.

Recent research on native and hydrolyzed forms of collagen and glycosaminoglycans (GAGs) such as hyaluronic acid (HA) has provided a convincing support for BioCell Collagen II® as an essential dietary ingredient for healthy aging which may benefit both the joint and the skin. As shown in the Table, these science-based competitive advantages clearly differentiate BioCell Collagen II® from other market-leading collagen-based products.

Table. Competitive Advantages of BioCell Collagen II® Compared to Other Collagen-based Products

	BioCell Collagen II®	Undenatured form	Hydrolyzed Peptides
Collagen type[1]	type II	type II	type I ?
Daily dose for clinical efficacy[2]	1.5 g	40 mg	10 g*
Hydrolysis for higher bioavailability	yes	no	yes
Molecular weight[3]	1.5 - 1.8 kDa	macromolecules	~ 3 kDa*
Absorption and cartilage accumulation[4]	yes	unclear	yes
Chondrocyte stimulation[5]	yes	no	yes
Hyaluronic acid, chondroitin sulfate[6]	yes	no	no
Inhibition of hyaluronidase[7]	yes	no	no
Targeted tissues	joint and skin	joint	joint

1. Major type of collagen in the final products. Type II collagen is a predominant type of collagen in articular cartilage.
2. The content of collagen only is ~800 mg for BioCell Collagen and 10 mg for the Undenatured form.
3. Average molecular weight. Patented hydrolysis for BioCell Collagen II®
4. Oesser et al, 1999, J Nutr, 129:1891-1895
5. Oesser et al, 2003, Cell Tissue Res, 311:393-399. Only hydrolyzed collagen stimulates chondrocytes to produce type II collagen
6. Essential components both for the joint and the skin. Chondroitin sulfate has anti-inflammatory activity.
7. Hyaluronidase-specific

* Based upon the company's presentation at SupplyWest2009 in Las Vegas.

1. Replenishing structural components essential both for the joint and the skin

BioCell Collagen II® provides a naturally-occurring combination of essential structural proteins and GAGs for the health of the connective tissues, whereas other collagen-based products shown in the Table provide collagen or its hydrolysate only as a bioactive ingredient. Aging is associated with decrease in the content and/or integrity of the structural components, and BioCell Collagen II® replenishes them to support healthy structure and functions of the joint and the skin. For example, type II collagen is specific for articular cartilage in the synovial joint forming collagen fibrillar network to provide tensile strength, while various GAGs such as HA and chondroitin sulfate (CS) serve as matrix filler of the cartilage to generate viscoelasticity to provide lubrication and resistance to compressive and shearing forces (see review by Bhosale). BioCell Collagen II® contains hydrolyzed type II collagen, depolymerized CS and

clinically bioavailable HA, altogether promoting structural integrity of articular cartilage in the most common and the most movable joint. Clinical trial demonstrated that BioCell Collagen II® statistically significantly improved joint comfort and mobility by reducing pain and stiffness in people suffering from osteoarthritis (2).

2. Provision of hyaluronic acid, a pleiotropic molecule for the skin

BioCell Collagen II®, not the other products, provides HA and CS. HA, CS, and collagen type I and III are vital structural components of skin the amount of which declines as we age. In particular, HA is not only responsible for moisture retention, suppleness, and elasticity in the young-looking skin but known to stimulate the proliferation of dermal fibroblasts that synthesize all components in the dermis (3, 4). A study on aging dermis showed that dermal fibroblasts experienced aging-associated morphology change in concomitant with collagen degradation and increase in oxidative stress and matrix metalloproteinase (MMP)-1 activity (5). BioCell Collagen II® contains highly absorbable HA, which plasma level increases 60-fold at steady state after oral intake (6). While replenishing the structurally and functionally critical GAGs together with CS, HA is considered to provide more comprehensive support for the dermis through the stimulation of dermal fibroblasts under the tension of the vicious aging-dependent cycle.

On the other hand, photoaging processes are similar to those of intrinsic, chronological aging. Dai et al showed that during photoaging the total cell number and percentage of proliferating fibroblasts in the papillary dermis decreased in concomitance with the loss of HA from the dermis, which contributes to the quiescent phenotype of dermal fibroblasts (7). BioCell Collagen II® may alleviate the effects of photoaging through the provision of HA.

3. Stimulation of chondrocytes in the cartilage by hydrolyzed, not by undenatured, collagen

Chondrocytes are the only resident cells in articular cartilage, responsible for the biosynthesis of all of its structural components. In a similar vein to HA's ability to stimulate dermal fibroblasts, only hydrolyzed collagen, not native form of collagen, has been shown to stimulate chondrocytes (7). Furthermore, hydrolyzed type II collagen derived from chicken sternum was stronger in stimulating the biosynthesis of new type II collagen than hydrolyzed collagen primarily comprising type I collagen (8). This study suggests a possible positive feedback mechanism for the regulation of collagen turnover in the cartilage and also generates an important implication as to how hydrolyzed collagen may restore the degenerating joint.

4. Patented process to hydrolyze BioCell Collagen II® to lower MW for better absorption

To the best of our knowledge, BioCell Collagen II® harbors lowest molecular-weight, type II collagen among collagen-based products with clinical efficacy. This can be interpreted that BioCell Collagen II® is better absorbed into circulation and highly bioavailable at the site of action. Oesser et al showed that hydrolyzed collagen were well absorbed into small intestine and that cartilage was the only tissue that the hydrolyzed collagen was accumulated for long-term (9). This data suggests another possible mechanism for the restoration of damaged articular cartilage.

The advantage of using collagen hydrolysate has been described in comprehensive reviews (10, 11). The authors stated after reviewing medical literature in the PubMed database concerning preclinical and clinical research on collagen hydrolysate, 'A growing body of evidence provides a rationale for the use of collagen hydrolysate for patients with OA.' They added, 'According to published research, orally administered collagen hydrolysate has been shown to be absorbed intestinally and to accumulate in cartilage. Collagen hydrolysate ingestion stimulates a statistically significant increase in synthesis of extracellular matrix macromolecules by chondrocytes ($p < 0.05$ compared with untreated controls).'

5. Anti-inflammatory activity of chondroitin sulfate

The presence of CS further differentiates BioCell Collagen II® from the other products as CS has anti-inflammatory activity (12, 13). CS, the second major content in BioCell Collagen II® but not found in the other products, is another key structural component in the articular cartilage and the skin. A review on chondroitin sulfate in osteoarthritis stated, "We definitively have enough clinical data available supporting the view that oral CS is a valuable and safe symptomatic treatment for OA disease."(14) Its anti-inflammatory effect also implies reduced need for intake of analgesics (e.g. NSAIDs) together with cost-benefits.

6. Inhibition of hyaluronidase by BioCell Collagen II®

What is most unique and intriguing to BioCell Collagen II® is inhibitory activity of hyaluronidase. HA, a key ingredient found only in BioCell Collagen II® among the three products, is present in virtually all tissues and bodily fluids (15, 16). One third of HA is turned over daily by coordinated action of HA synthases and hyaluronidases. Given that hyaluronidase-mediated degradation of HA is implicated in aging, inflammation, and cancer metastasis, BioCell Collagen II® 's ability of inhibiting hyaluronidase may have a profound implication, as discussed below, in terms of aging processes which takes place in the joint and the skin.

Hyaluronidases degrade HA, contributing to aging processes in the connective tissues, in that shortened chain length and decreased polyanionic charge attenuates its ability to bind water, resulting in reduction of volume, turgidity, and elasticity of the skin, which leads to wrinkle formation. In the joint, the loss of HA integrity may lead to the weakened lubrication and dampened resistance to compressive forces. BioCell Collagen II®'s inhibitory activity of hyaluronidase in a dose-dependent manner implies that the effects of chronological aging can be attenuated by ingestion of BioCell Collagen II®, which would lead to increase HA bioavailability over 40 times higher at steady state (6).

Conclusion

Taken together, accumulating scientific data indicates that BioCell Collagen II® has a potential of becoming a paradigm-shifting dietary supplement in that it not only alleviates various arthritis-associated symptoms, but also may help restore the degenerating joint. Moreover, BioCell Collagen II® can provide an additional health benefit for aging skin by providing essential structural elements such as HA and CS for the dermis, and probably by stimulating proliferation and healthy functions of dermal fibroblasts which are responsible for production and regulation of the dermal extracellular network.

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Liquid BioCell™ May Improve Recovery Following Weight Training Exercise: Study

Feb 21, 2015

Supplements containing Liquid BioCell™ may protect the connective tissue of the musculoskeletal system and enhancing recovery from intense exercise, says preliminary data from a proof-of-concept study

Six weeks of intake for the collagen ingredient were associated with an attenuation of deleterious changes in muscle tissue damage and inflammatory biomarkers including creatine kinase, lactate dehydrogenase, and C-reactive protein, according to findings published in the Proceedings of the Eleventh International Society of Sports Nutrition (ISSN) Conference and Expo.

Liquid BioCell™ is described as a unique, patented liquid collagen matrix. The three main constituents, collagen type II, chondroitin sulfate, and hyaluronic acid, are reduced to highly-bioavailable, very low molecular weight forms through a hydrolysis process.

Extending its market-leading position as a key joint and skin health ingredient, this new clinical study in recreationally active healthy subjects provides intriguing dataset suggesting that this patented, research-backed dietary supplement has promising new applications in sports nutrition.

Healthy joints are essential for any sports related activity, from cardio to resistance training. Equally important are the neighboring connective tissues surrounding the joints such as tendons and ligaments, which help facilitate flexibility and movement while protecting against injury. Whether you are aiming for prevention or trying to return to normal training after an injury, you need a consistent supply of connective tissue-specific nutrients that provide the biochemical precursors and building blocks needed to promote optimal joint health.

Liquid BioCell™ has these molecular components which were found to impact key biochemical markers of connective and skeletal muscle tissue damage and enhance stress resilience following intense resistance exercise - without the potential undesirable side effects of pain medications.

Study details

Investigators from the Center for Applied Health Sciences (CAHS) recruited eight healthy, recreationally active people with an average age of 29 to participate in their study. Volunteers were randomized to consume three grams per day of the collagen or placebo for six weeks. All the participants underwent an upper body muscle-damaging resistance exercise challenge on day 43, and a re-challenge three days later.

Results showed that the collagen supplements were associated with the attenuation in increases of creatine kinase, lactate dehydrogenase, and C-reactive protein that were observed in the placebo group.

In addition, bench press repetitions decreased by 60% and 55% at days 43 and 46, respectively, in the placebo group, but this only by 49% and 43%, respectively in the collagen group.

“The preliminary data of this proof-of-concept study suggests that daily intake of BCC for 6 weeks may favorably impact key biochemical markers of connective and skeletal muscle tissue damage and enhance stress resilience following intense resistance exercise,” wrote the researchers. “Supplementation was well tolerated and did not adversely affect markers of health or side effect profiles.”

The company is planning additional trials to corroborate the findings, and they are in the protocol development stage of a follow up larger study.

Contact your Independent Jusuru Representative for more information.

Jusuru

LIQUID BIOCELL LIFE

Skin Trial: A White Paper

Liquid BioCell™ Life Counteracts Both Natural and Photoaging Processes

Liquid BioCell™ Life is an innovative dietary supplement in a proprietary liquid formulation, which effectively promotes healthy aging. It contains three major ingredients, which include patented LiquidBioCell™, resveratrol, and thirteen different fruits that are known to contain high levels of antioxidants, vitamins, minerals, and dietary fibers, etc. Scientific evidence on individual ingredients has been well established to support that Liquid BioCell™ Life can synergistically promote overall health. In particular, multiple human clinical trials on Liquid BioCell™ have substantiated its benefits for active joint and skin beauty (1, 2). Numerous independent studies on phytonutrient-rich fruits imply a wide range of potential health benefits, including a decrease in oxidative/inflammatory stresses and in the risk of various chronic health issues. Additionally, resveratrol is one of the most recognized and widely studied natural compounds due to its potential roles in promoting longevity.

Human Skin Trial

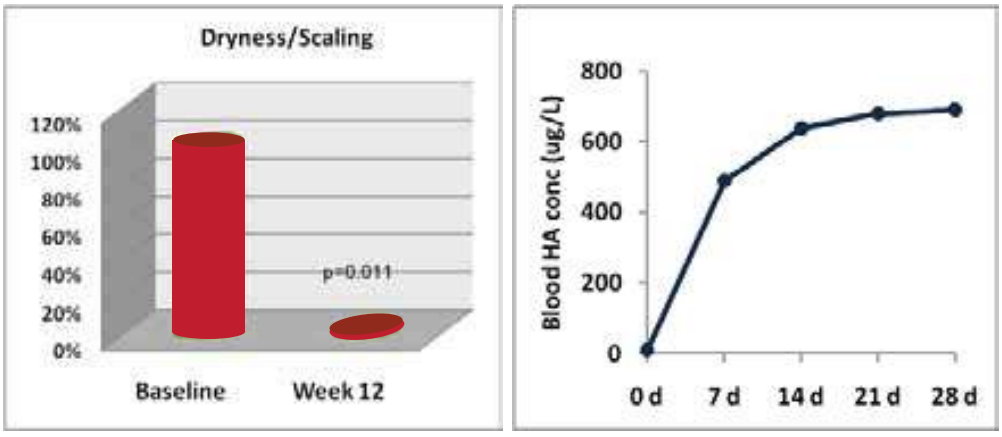
In 2011, Jusuru International, Inc. performed a breakthrough human skin trial that enrolled women in their thirties to fifties undergoing both natural and photoaging processes. This study revealed multi-faceted 'beauty-from-within' physiological mechanisms elicited by daily supplementation with Liquid BioCell™ Life, which is instrumental in counteracting both aging processes. Taking 4 fl. oz. of Liquid BioCell™ Life daily for 12 weeks led to a dramatic reduction of dryness/scaling and to a substantial decrease in wrinkles and deep lines on their faces. Under the skin, Liquid BioCell™ Life caused a significant increase in collagen in the dermis and a significant improvement in blood microcirculation. As a result, the majority of the study participants experienced improvement in hydration/scaling, skin texture, and firmness, as well as reduction of blotchiness/redness.

Youthful Appearance

1. Reduction of dryness/scaling

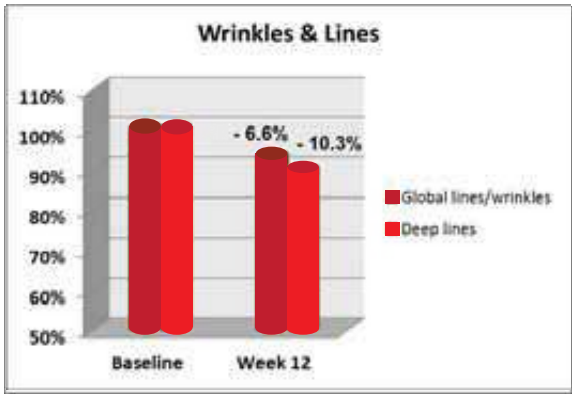
Hyaluronic acid (HA) is present in virtually every tissue in the body and plays a number of diverse physiological functions. For example, its capability of retaining water molecules generates viscoelastic properties, which are critical for the structure and function of the articular cartilage and the synovial fluid of the joints.

The water-holding properties are also essential for the hydration, suppleness, and turgidity of the skin. Unfortunately, its amount and integrity gradually decreases as we age, contributing to skin dryness/scaling and shrinkage of skin volume (3). Photoaging worsens this natural aging process (4).



(Judy, W, SIBR, 2004)

Liquid BioCell™ Life contains Liquid BioCell™, of which major constituents include highly bioavailable HA and biologically active collagen peptides. A previous human study showed that the ingestion of Liquid BioCell™ (1.5 g/day) enhanced the concentration of HA in the blood as much as 60-fold at a steady state (5). It was proposed that the elevated pool of HA would be harnessed to replenish the aging-associated loss of HA in the skin. The current human skin trial demonstrated that daily supplementation with Liquid BioCell™ Life for 12 weeks led to an almost complete disappearance of skin dryness/scaling, as measured by visual/tactile score, as shown above.



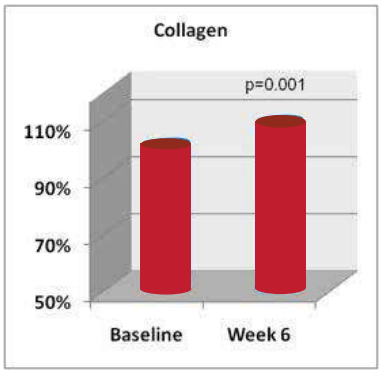
Global lines/wrinkles and deep lines in the full face were measured by a bioinstrumentation technique. Image analysis showed that there was a numerical decrease in these aging-associated parameters. Wrinkles were reduced by 6.6% on average while deep lines by 10.3% at the end of the study. The attenuation of these aging-associated appearances should be underscored by the enhanced hydration and production of dermal matrix molecules, such as collagen (types I/III).

Mechanisms for Beauty from Within

1. Increase in collagen content

The collagen fibrillar network is probably the most critical element for skin beauty because it lays the structural framework for the dermis, the thick layer of the skin. Collagen molecules synthesized from the dermal fibroblasts form an extracellular network in the dermis, into which various ground substances such as HA and dermatan/chondroitin sulfate are embedded.

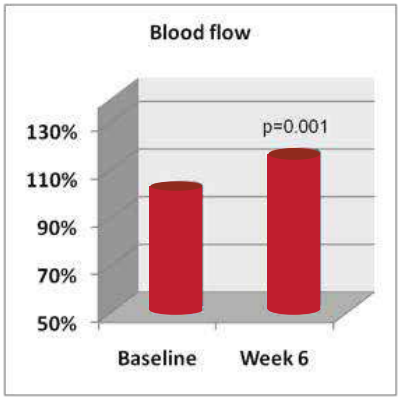
This network provides tensile strength to the dermis and can be degraded by matrix metalloproteinases (MMPs) in response to a variety of stimuli associated with natural and photoaging processes. The loss and breakdown of the collagen fibrillar network is a primary contributor to the generation of wrinkles and fine lines (6).



Daily supplementation of Liquid BioCell™ Life led to a significant increase in collagen content by 6% in the facial skin. Hydrolyzed collagen peptides contained in it may function multiple ways in enhancing the collagen content in the dermis. First, these collagen-derived peptides can supply building blocks for a novel collagen fibrillar network in the dermis. Second, they might stimulate the dermal fibroblasts to produce the essential macromolecules, as studies showed that ingestion of hydrolyzed collagen led to an increase in the number of the dermal fibroblasts concomitant with enhanced density of collagen fibers (7). It is also possible that collagen-derived peptides might interfere with MMPs or collagenases, lessening the degree of aging-associated collagen breakdown.

2. Improvement of Blood Microcirculation

Vascular health should be a basis of healthy and younger-looking appearance because various types of cells residing in the skin need to be nourished well and their waste to be removed effectively. It was interesting that ingestion of Liquid BioCell™ Life led to an enhancement of facial blood flow, as evidenced by a significant increase in hemoglobin content by 14.2%, suggesting that blood microcirculation was improved in the facial skin. Improved microcirculation should support skin homeostasis, helping delay or counteract the appearance of various visible aging signs in the face.



Responses of the Study Subjects

The study participants were asked at the end of the study of the effects of daily consumption of Liquid BioCell™ Life. The majority of the subjects experienced diverse facial skin benefits, which included the enhancement of skin texture, hydration, elasticity and firmness, and the reduction of scaling and blotchiness.

Questionnaire: Liquid BioCell™ Life	Yes (%)
Improving skin texture	88.0
Increase hydration/reduced scaling	80.0
Improving elasticity/firmness	52.0
Reducing blotchiness/redness	52.0
Improving skin tone	44.0
Radiant/luminescent complexion	40.0
Reducing fine lines/wrinkles	40.0

Before and After Images - Examples

Summary

Daily supplementation of Liquid BioCell™ Life (4 fl. oz. daily) led to the following beauty-from-within effects, which underscored the younger facial appearance of the study participants.

- Dramatic reduction of dryness/scaling
- Significant increase in collagen content (types I/III) in the dermis
- Significant increase in blood microcirculation in the facial skin
- Substantial reduction of facial lines and wrinkles

As a result, the majority of the study participants experienced diverse facial skin benefits, which included the enhancement of skin texture, hydration (reduced scaling), elasticity (firmness), and reduction of blotchiness (redness).

Conclusion

Together, with multiple clinical trials of Liquid BioCell™, which demonstrated its efficiency in promoting active joints, this human study provides convincing evidence that Liquid BioCell Life™ is an innovative nutritional supplement, which can support healthy aging through unique and multi-layered mechanisms in promoting youthful appearance, as well as active joints

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* Liquid BioCell is the exclusive highly bioavailable liquid form of BioCell Collagen® TF

FDA Disclaimer

These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

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Louis P. Brady, MD

Louis P. Brady, MD is a graduate of Emory University School of Medicine and Board Certified in Orthopedics. He is an Associate Clinical Professor at the University of Central Florida College of Medicine. According to Dr. Brady, whose work in the area of orthopedics spans over four decades, Jusuru Life Blend is the "most remarkable product to have ever been introduced for joints and the aging process."

"Most remarkable product to have ever been introduced for joints and the aging process."

"The side effects are all good and there is no toxicity according to the toxicology studies that have been done on BioCell Collagen II®, resveratrol, and fruit ingredients."

"Degenerative joint conditions are generally thought to be caused by the slow loss of the normal amount of collagen and/or hyaluronic acid beginning early in life and becoming symptomatic beginning in the third and fourth decade of one's life. This is the same process that occurs in skin causing wrinkles."

"Total joint replacement is a highly successful procedure and will, in most cases; result in return of function and relief of pain. It should; however, be a last resort after all conservative measures have been exhausted. Even though the percentage of complications is very low the risks of any procedure should be fully understood. I have always told my patients, while discussing the risks involved with any procedure, that if it happens to you it is 100 percent."

"I would advise anyone with joint problems to consider a minimum of a three-month trial of Jusuru Life Blend as an alternative to non-steroidal anti-inflammatories. Due to the multiple, so called, "over the counter" products that we are bombarded with on a daily basis that claim to cure almost everything, there is a lot of healthy skepticism. There is usually acceptance of the recommendation after I have explained the rational of my suggestion."

Joosang Park, PhD, MBA

Joosang Park possesses a Ph.D. in cancer biology from Stanford University and earned his MBA at Cornell University. Additionally, he also served as Research Fellow on cancer vaccine development at Harvard Medical School. As VP of Scientific Affairs of BioCell Technology, LLC, he

provides scientific support and research for existing and future products based upon his scientific expertise integrated with business education. According to Dr. Joosang Park, "The ingredients in this dietary supplement are substantiated by science and offer multi-layered benefits for healthy aging. Jusuru Life Blend synergistically supports both joints and skin through replenishing hydrolyzed collagen, HA and chondroitin sulfate in highly bioavailable forms, and is the only liquid nutraceutical to offer BioCell Collagen II®."

"Various studies, including clinical trials, have provided growing evidence that BioCell Collagen II® is a unique healthy aging ingredient that helps promote healthier joints and younger-looking skin at the same time.

Jusuru's patented formulation is constructed not only to augment the dual health benefits of BioCell Collagen II® but to help reduce oxidative stress and inflammation that accelerate the aging process. This trio, BioCell Collagen II®, resveratrol, and twelve fruits, can synergistically promote overall health of all people to support a more active lifestyle with a youthful appearance.

Studies suggest that the patented process to reduce the molecular weight of collagen polypeptides and hyaluronic acids brought about various novel biological properties distinct from undenatured collagen, in addition to remarkable bioavailability of each constituent. More importantly, this hydrolysis step generates more bioavailable building blocks essential not only for the joint cartilage matrix, but for dermal (skin) matrix because macromolecular components- collagen hyaluronic acid, and chondroitin sulfate-found in both of the tissues are synthesized by incorporating these building blocks.

Maintaining a proper amount of hyaluronic acid is critical for healthy skin structure and turgidity because hyaluronic acid is responsible for retaining water in the skin dermis. However, the amount of hyaluronic acid that functions normally decreases in aging skin, resulting in dermal dehydration and eventually in sagging skin and formation of wrinkles. BioCell Collagen II® supplies highly bioavailable hyaluronic acid to attenuate the effect of hyaluronic acid loss caused by natural or photo-aging. Human subjects who took daily doses of BioCell Collagen II saw hyaluronic acid levels increase 60-fold in their blood during the 28 days of the study period.

Typically, liquid supplements have higher bioavailability than a non-liquid form. Bioavailability should be regarded as a more important issue than how much a nutrient is present in a dietary supplement because the nutrient must be absorbed and available at the site of action. Bioavailability is related to the number of steps involved in the

absorption process following oral administration. As a liquid form does not require a dissolution step, it results in fast and highly efficient absorption. Bioavailability of a liquid form may be further enhanced by absorption in the mouth via sublingual mucosa (floor of the mouth) and buccal mucosa (sides and top of the oral cavity).

How soon an individual may get intended health benefits should vary. However, clinical studies of BioCell Collagen II® and anecdotal evidence support that consumers can start to observe the results in several weeks although they sometimes see difference surprisingly earlier. In our clinical trial, significant improvement in the joint across the study subjects was observed several weeks after daily ingestion of BioCell Collagen II®."

Dr. Frank Ryan

(May 21, 1960 - August 16, 2010)

Dr. Frank Ryan was Board Certified by the American Board of Plastic Surgery. He graduated from the University of Michigan in 1982 and from the Ohio State University College of Medicine in 1986. He then completed eight years of post-graduate surgical training at Cedars-Sinai Medical Center, the University of Missouri and UCLA Medical Center. Dr. Ryan has also participated in numerous fellowships, the first of which was a burn reconstruction fellowship at Shriners Hospital for Children. In 1990, he completed a UCLA Division of Plastic Surgery Research Fellowship. Dr Ryan was the UCLA Division of Plastic Surgery Aesthetic Fellow in 1993-1994.

For decades, Dr. Frank Ryan provided aesthetic and reconstructive plastic surgical procedures in his facilities in Beverly Hills, California. Dr. Frank Ryan was certified by the American Society of Plastic Surgeons and was a Fellow of the American College of Surgeons. Dr. Ryan was a member ASAPS, CSPS, CMA, AMA, LACMA, and ASLMS. His clients included a long list of celebrities and he has been featured in countless national news programs and publications including A&E, CNN, VH1, Access Hollywood, The Insider, Good Morning America, Extra, Nightline, E! News, People Magazine, Us Weekly, and Ok! Magazine.

Dr. Frank Ryan was not only a consumer of Jusuru Life Blend, but a passionate advocate of this product. He provided his clients with Jusuru in the waiting room and encouraged them to use the product pre- and post-surgery. His belief that Jusuru Life Blend's results would be immeasurable to sustain health and youthful appearance was so strong that he lead a surge of Beverly Hills physicians to embrace the inside-beauty concept with their practices by incorporating Jusuru Life Blend

into their businesses. Dr. Ryan was also a dear friend and we continue to carry on his spirit to help others with charitable work. See his answers to questions about Jusuru below:

"As we age, we lose HA in our bodies, thus the skin becomes loose, dehydrated and wrinkled. Jusuru Life Blend replenishes our HA levels allowing us to regain soft, smooth, glowing youthful skin. It goes further to prevent the loss of HA, which is critical in either preserving youthful appearing skin or to slow down the aging symptoms.

The collagen and hyaluronic acid in Jusuru Life Blend provide the building blocks of skin and tissue repair; consequently, a regimen of Jusuru Life Blend before and after surgery will aid a rapid recovery. Specifically with regards to skin repair, Jusuru Life Blend plays a significant role and its active components have been thoroughly researched role in skin repair. Higher levels of HA present in the skin will assist in collagen synthesis and organization of the fibril network as the skin repairs itself from incisions or wounds. Further, the product has been shown to increase the rate of collagen synthesis. Antioxidants have also been shown to have anti-inflammatory effects. I recommend it to all of my clients.

Jusuru Life Blend has been the Hollywood secret in helping the most visible personalities look their best. It's a critical part of my overall offering to my patients- it greets them in the lobby in my office."

Richard A. Passwater, Ph.D.

"There are a lot of claims about anti-aging effects from growth hormone, or this herb or that. There is no question that the loss of HA over time is responsible for the visible signs of aging, wrinkled skin, hair loss, voice changes, diminished eyesight, wear-and-tear, osteoarthritis, yet little is known of this wonderful molecule.
A remarkable finding is that these visible aging signs can be reversed with oral HA supplements. HA is the water-holding molecule of the body. It holds water in connective tissues, in between cells. Thus it cushions nerves and ends of bones, provides shape and form to the human eye and skin, and forms a barrier against the spread of disease. One of the pieces of advice I offer in my book is to not to forget to drink water. Glucosamine only yields mild benefits for arthritis sufferers who usually have to take it in high doses (1,500 mg) for prolonged periods of time to experience any results. Just 150-300 mg of oral HA will work better and faster at restoring joint health. Chondroitin sulfate raises the production of HA in the body. Dr. Lester Morrison, a cardiologist at Loma Linda

University in the 1970s, used very high doses of chondroitin sulfate (5,000 mg tapered to 1,500 mg over three or four months) following a heart attack or bouts of angina to successfully rebuild and heal cardiac tissue. Some of these patients also experienced anti-aging benefits, such as renewed hair growth, cancellation of prostate surgery and disappearance of angina. The healing of nerves simply can't take place adequately without the cushioning provided by HA.

I also have observed other profound effects of oral HA. For example, as oral HA refills the eyes, it slightly lengthens the front-to-back length of the eye. This means the focus point of the eyes is altered. Therefore, farsighted people who take oral HA supplements may find their vision improving without glasses. Since all adults become a bit farsighted with advancing age, this has enormous possibilities to keep people out of reading glasses. HA also helps maintain hormone levels. Many women taking oral HA supplements have reported that their hot flashes and symptoms of menopause have abated with use. This is not surprising. Women have been advised that oral estrogen supplementation doesn't prevent disease, and may in fact slightly increase the risk of disease. An alternative to estrogen would be oral HA. Why do women have beautiful skin? Because estrogen elevates HA levels in the skin and body. Once natural estrogen production has ceased (menopause), the natural alternative would be oral HA. There is no argument that the visible signs of aging—baldness, voice change, skin wrinkling and dryness, shrinkage in height, joint pain and dependency upon eyeglasses—all emanate from the loss of HA and not to direct shortages of any hormones like DHEA, or minerals like coral calcium, or any other widely promoted natural remedies."

Clinical Study Shows Hyaluronic Acid in Biocell Collagen II Found To Have Significant Absorption and Bioavailability **Source: BioCell Technology LLC**

NEWPORT BEACH, Ca., – BioCell Technology LLC, the exclusive suppliers of the Patented BioCell Collagen II® ingredient, released results from a double blind clinical study proving that the naturally occurring Hyaluronic Acid (HA) in the BioCell Collagen II® product has significant peak absorption and steady state bioavailability in normal volunteer subjects. Dr. William Judy, senior scientist at SIBR Research, called the study "groundbreaking science" and noted BioCell Technology's revolutionary form of a reduced molecular weight HA manufactured using patented technology, is absorbed and is therefore readily available for use by the body. This is in contrast to previous published studies which showed that other HA forms were not absorbed and thus unavailable for use by the human body,

In a 36-hour peak absorption study using a single dose, BioCell Collagen II® HA significantly increased in the blood in four hours and peaked at a level 7008.62% above control in twelve (12) hours ($P \leq 0.05$). In the blood, HA was rapidly metabolized to two metabolites 1/600th the size of the ingested HA.

In a 28-day steady state bioavailability study using a constant daily dose, after seven (7) days, BioCell Collagen II® HA and its metabolites in the blood became stable, and these metabolites remained significantly increased ($p \leq 0.001$) throughout the balance of the study (HA at 3542.58% above control and HA metabolite at 11890.15% above control).

"This unprecedented study on hyaluronic acid, a key element in the unique matrix of BioCell Collagen II® which consists of Collagen Type II, Chondroitin Sulfate, and Hyaluronic Acid, demonstrates a synergistic effect that allows for the impressive absorption as indicated in these exciting and promising studies. BioCell Technology has expanded its intellectual property profile and has a patent pending to cover oral and topical applications of hyaluronic acid derived from various natural sources for use in dietary supplements and cosmetics," said Suhail Ishaq, Vice President of BioCell Technology, LLC.

By determining the rate and magnitude of HA absorption and its bioavailability, this study using the BioCell Collagen II® product clearly demonstrates that this HA form has the physical characteristics necessary to allow it and its metabolites to move rapidly from the blood to the tissues. These findings further support the previous documented efficacy of BioCell Collagen II® in the management of joint and skin care.

Founded in 1997, BioCell Technology, LLC pioneered the applications of Sternum Collagen Type II, the highest concentrated natural source available of type II collagen. It is the exclusive supplier of BioCell Collagen IITM. BioCell Technology, LLC owns the exclusive rights to market BioCell Collagen II under the United States Patent #6,025,327. BioCell Collagen IITM is a branded trademarked logo that is available for display on its client's labels to market under their own brand name or formulas.

Drs. Trentham and Weiner studied ten people with severe rheumatoid arthritis. They eliminated the standard therapies such as NSAIDS and Methotrexate and fed the patients pure collagen type II derived from chicken sternal cartilage. At the end of the second month, six of the ten patients showed substantial improvement and one showed a complete regression of the disease that lasted 26 months with no side effects. The clinical response in this study was defined as a 50 percent or "greater" reduction in swelling and tenderness, a 50 percent improvement in morning stiffness, as well as an improved 15 minute walk time and grip strength. Thus, there were several objective and subjective criteria for measuring success. These results are profound. As a matter of fact, this study demonstrated a 70 percent response rate of the most severe cases of rheumatoid arthritis with no side effect, with these patients completely removed from standard medications. Even the other 30 percent of the group had significant improvement. And on the basis of this trial, the same doctors conducted a phase II trial on 59 patients with, again, severe active rheumatoid arthritis. Twenty-eight received the collagen type II, while 31 received a placebo pill. What is interesting is that those who were off all drugs and taking collagen type II showed very significant stabilization and improvement, while those on placebos continued to deteriorate. In fact, four collagen type II patients showed a complete resolution of the disease, while no patients in the placebo group displayed any remission. Again, no side effects were detected. We see that oral ingestion of collagen type II may well prove to be the most effective natural means of arthritis management regardless of the form of the disease.

The question becomes, now, how exactly is collagen type II working to produce these incredible results. It appears the mechanism of action is immune suppression through white blood cells located in the GALT. These white cells devour some of the collagen type II and then instruct the rest of the immune system to stop attacking collagen type II, as these white cells identify it as friend and not foe. Once the immune system is alerted to stop attacking the collagen type II, there seems to be a proliferation of T-suppressor cells. These are also known as T8 cells. Ultimately, this decreases the number of inflammatory cytokines that are partly responsible for the inflammatory reaction in rheumatoid arthritis. Part of the other reason, and this is precisely why it works in osteoarthritis, is that collagen type II has such a high concentration of glucosamine and chondroitin, which 30 years of double-blind, placebo-

controlled studies demonstrate a strengthening of the cartilage, as well as an increase in new cartilage cell production. These ingredients also have anti-inflammatory properties and insure maximum water concentration in the cartilage itself, which acts as a cushion during normal physical activity.
Collagen type II also contains proteoglycans that inhibit blood vessel formation in joints and reduce enzyme attacks on the cartilage itself. Thus, there is a rejuvenation of the cartilage producing cells and a decrease in the destructive biochemistry of the joint. Additionally, many of these proteoglycans found in collagen type II support the lubricating fluid of the joint called the synovial fluid. These proteoglycans increase the thickness and lubricating effectiveness of this fluid.
It is extremely important to understand that not all types of collagen are the same, and not all types of cartilage can give you a medicinally effective collagen type II. Collagen type II must be derived from chicken sternal cartilage, only. The reason is very simple. This source of cartilage holds the greatest concentration of joint-saving proteoglycans. When a person who has arthritis begins to take collagen type II, they should wait of a period of eight to twelve weeks before they make a decision as to whether or not the product is working. It takes this length of time in most people to get significant results. I have seen some individuals eliminate pain within one week, but this is the exception, not the rule. Collagen type II should be taken 20 minutes before eating. In lieu of the fact that there are no side effects with collagen type II, this would seem to be the product of choice when suffering from any form of arthritic condition.

In my research, I discovered the medicinally effective ingredient in cartilage is really due to the collagen type II fraction. We know that cartilage is composed of four or five different kinds of collagen. There are 14 different kinds of collagen altogether, but the primary collagen, the most predominant one, the most medicinal collagen, is collagen type II. If collagen type II is derived from chicken sternal cartilage, from chicks six to eight weeks old, it contains the greatest number of anti-inflammatory and joint supporting proteoglycans. These proteoglycans include chondroitin sulfate which has over 30 years of double-blind, placebo-controlled studies indicating that it actually helps to rebuild the cartilage in arthritis joints, is a powerful anti-inflammatory and also supports the joint tissue. Once again, if the product is derived from chicken sternal cartilage, these two components are in highest concentration compared to any other cartilage source. In addition to these, collagen type II also contains a

powerful, newly discovered antioxidant proteoglycan called cartilage matrix glycoprotein (CMGP), which can help reduce the oxidative damage to the joint. In addition to these new discoveries, there are other ingredients in collagen type II that make it more effective than just taking glucosamine or chondroitin by themselves.

Collagen Type II and Heart Disease

Medicines such as aspirin and NSAIDS, as well as natural therapeutics such as fish liver oil or antiarthritic herbs, cannot compete with the overall biochemical power of collagen type II. It may turn out also that collagen type II is one of the most cardio-protective agents ever discovered. From a recent analysis, I've found that Chondroitin sulfate A is a natural proteoglycan that is found in the lining of our arteries, our nasal septa, and even the cornea of our eye, as well as in the collagen tissue throughout the body. Chondroitin sulfate A (CSA) has a powerful antithrombogenic or anticoagulant affect, which, in essence, prevents blood clots and has been shown to reduce the incidence of stroke. In Japan, CSA is used by more than 20,000 people every day and in the 20-year history of its use, no cases of toxicity have been reported.

The next Botox?

FDA Approves Hyaluronic Acid Injections for Wrinkles...Until now, only two other injectable products have been approved for the treatment of wrinkles: Collagen and Botulinum Toxin (Botox [TM]). Several studies suggest that Hyaluronic Acid (HA) may pose a better alternative to the above mentioned products. Although used for generations by women in the Far East to maintain their flawless complexion, HA has recently gained increased awareness in the United States with the FDA)s endorsement of HA injections for wrinkle reduction. Using HA undeniably decreases fine lines, deep wrinkles, crow's feet, and acne scars.

Baby Skin at 40?

Yuzuri Hara ("the Village of Long Life"), a small town in Japan, has long known the benefits of HA. Most residents live well into their nineties but many have managed to keep their skin free of the all-too-familiar signs of aging. According to a documentary on ABC News Primetime, local doctors attribute this wonder to the abundance of HA in the villagers' bodies because of their unique ethnic diet. It is no wonder that HA has become a sought after product, as clinical studies continue to reveal its importance to skin health and appearance.

Scientific Breakthrough, Biocell Collagen II Fights Wrinkles without injections... With the recent discovery of Biocell Collagen II, the benefits of Hyaluronic Acid can be realized without costly and painful injections. The clinically researched and patented technology behind Biocell Collagen II addresses aging where it begins--from beneath the outer layers of the skin. Biocell Collagen stands out from other products as it dually replenishes levels of HA and collagen--internally through a combination of scientifically proven ingredients to virtually plump out the appearance of wrinkles.

Dr. Nelson Lee Novick, M.D., FACP, FAAD, an associate clinical professor of dermatology at The Mount Sinai School of Medicine in New York City and a dermatology clinic chief at The Mount Sinai Medical Center, stated that BioCell Collagen II "is a unique combination of collagen II, hyaluronic acid and chondroitin sulfate (HAs companion that rebuilds cartilage) to nourish the skin from the inside out where topical applications cannot reach ... By restoring facial contours, enhancing moisture levels and reducing wrinkles, this ingredient heals and rejuvenates skin tissue to levels that will erase the [many visibly] distinguishing features between young and old."

Not all HA Products are Created Equal ...The chart below shows the results of a double-blind clinical study proving that the naturally occurring HA found in Biocell Collagen II has superior absorbability and unprecedented steady state bioavailability. Dr. William Judy, senior scientist at SIBR Research, called the study "groundbreaking science" and noted that this revolutionary form of hyaluronic acid manufactured using patented technology--is absorbed and readily available for use by the body. This is in contrast to previously published studies which showed that other HA products were not absorbed and thus unavailable for any benefit. The rapid absorption of the oral delivery system provides results immediately. Biocell Collagen II is a true scientific anti-aging breakthrough. Now that the secret of the Far East is accessible and affordable, millions of

men and women everywhere are taking advantage of this painless technology.

* Plumps out wrinkles and rejuvenates skin appearance
* Supports healthy skin function
* Supports skin youthfulness & elasticity
* Increases skin moisture
* No injections required
* Replenishes levels of HA and collagen with a unique system using dietary supplement
* Provides antioxidant vitamins, minerals & herbs to support healthy aging

1. **Sheldon, E "A randomized double blind clinical pilot trial evaluating the safety and efficacy of hydrolyzed collagen type II (BioCell Collagen II®) in adults with osteoarthritis," Miami Research Associates. April 25, 2003.**

Clinical Study Shows Biocell Collagen II® Effective for Osteoarthritis

The findings of Dr. Eric Sheldon, a clinical research investigator at Miami Research Associates, are derived from a placebo-controlled pilot study in sixteen men and women with OA who received the supplement for an eight-week period. The supplement tested is a unique, patented extract of naturally occurring type II Collagen, Chondroitin Sulfate, and Hyaluronic Acid named **BioCell Collagen II®**.

"This preliminary study suggests that **BioCell Collagen II** has promise in the management of chronic osteoarthritis symptoms," said Sheldon, a rheumatologist and voluntary rheumatology instructor at the University of Miami School of Medicine. "We used a symptom assessment tool that is used routinely in OA drug studies and the results are encouraging." Osteoarthritis, also known as degenerative joint diseases, is the most common form of arthritis.

Sheldon said the data reveal that daily consumption of **BioCell Collagen II** led to clinically meaningful improvements that were significantly superior to the group receiving placebo supplements. Additionally, the **BioCell Collagen II** group had no greater incidence of adverse events or side effects.

2. **William, Judy, "Clinical study shows hyaluronic acid in BioCell Collagen II® found to have significant absorption and bioavailability," SIBR. February, 2 2004**

Clinical Study published in The FASEB Journal Shows Hyaluronic Acid in Biocell Collagen II® Found To Have Significant Absorption and Bioavailability

Dr. William Judy, senior scientist at SIBR Research, called the double blind clinical study proving that the naturally occurring Hyaluronic Acid (HA) in the BioCell Collagen II product has significant peak absorption and steady state bioavailability in normal volunteer subjects "groundbreaking science" and noted BioCell Technology's revolutionary form of a reduced molecular weight HA manufactured using patented technology, is absorbed and is therefore readily available for use by the body. This is in contrast to previous published studies which showed that other HA forms were not absorbed and thus unavailable for use by the human body.

In a 36-hour peak absorption study using a single dose, BioCell Collagen II HA significantly increased in the blood in four hours and peaked at a level 7008.62%

above control in twelve (12) hours (P=0.05). In the blood, HA was rapidly metabolized to two metabolites 1/600th the size of the ingested HA.

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By determining the rate and magnitude of HA absorption and its bioavailability, this study using the BioCell Collagen II product clearly demonstrates that this HA form has the physical characteristics necessary to allow it and its metabolites to move rapidly from the blood to the tissues. These findings further support the previous documented efficacy of BioCell Collagen II in the management of joint and skin care. This unprecedented study on hyaluronic acid, a key element in the unique matrix of BioCell Collagen II which consists of Collagen Type II, Chondroitin Sulfate, and Hyaluronic Acid, demonstrates a synergistic effect that allows for the impressive absorption as indicated in these exciting and promising studies.

3. **Food Chem Toxicol. 2007 Feb;45(2):315-21. Epub 2006 Aug 30.**

Acute and subchronic oral toxicity studies in rats of a hydrolyzed chicken sternal cartilage preparation.

Schauss AG, Merkel DJ, Glaza SM, Sorenson SR.

AIBMR Life Sciences, Inc., Natural and Medical Products Research, 4117 S. Meridian, Puyallup, WA 98373, USA.

Abstract

Two acute and subchronic oral toxicity studies were conducted in rats to evaluate safety of a patented preparation of hydrolyzed chicken sternal cartilage (BioCell Collagen II) containing collagen type II, chondroitin sulfate, and hyaluronic acid. In the acute oral toxicity study, five males and five females of Sprague-Dawley rats were administered a single dose of 5000 mg of the test product per kg body weight and observed for 14 days. All animals survived and exhibited normal body weight gain throughout the study. Macroscopic necropsy examination conducted on day 15 revealed no gross pathological lesions in any of the animals. In the subchronic study, Sprague-Dawley rats (40 males, 40 females) were divided into four same-sex groups (10 animals/group). Animals in each group were administered daily either 0, 30, 300

or 1000 mg of the test product per kg of body weight for over 90 days. All animals survived and showed no significant changes in their body weights and histopathology. Although some differences were observed between the treated and control animals in several parameters, they were generally not dose-related or considered to be of toxicological significance. In conclusion, the results from the two oral toxicity studies with male and female young adult rats indicated that the test preparation from hydrolyzed chicken sternal cartilage collagen (BioCell Collagen II) was well tolerated at all four doses tested.

4. Cellular and Molecular Life Sciences Volume 65, Number 3, 395-413, DOI: 10.1007/s00018-007-7360-z

Hyaluronan synthesis and degradation in cartilage and bone

E. R. Bastow, S. Byers, S. B. Golub, C. E. Clarkin, A. A. Pitsillides and A. J. Fosang

Abstract

Hyaluronan (HA) is a large but simple glycosaminoglycan composed of repeating D-glucuronic acid, beta1-3 linked to N-acetyl-D-glucosamine beta1-4, found in body fluids and tissues, in both intra- and extracellular compartments. Despite its structural simplicity, HA has diverse functions in skeletal biology. In development, HA-rich matrices facilitate migration and condensation of mesenchymal cells, and HA participates in joint cavity formation and longitudinal bone growth. In adult cartilage, HA binding to aggrecan immobilises aggrecan, retaining it at the high concentrations required for compressive resilience. HA also appears to regulate bone remodelling by controlling osteoclast, osteoblast and osteocyte behaviour. The functions of HA depend on its intrinsic properties, which in turn rely on the degree of polymerisation by HA synthases, depolymerisation by hyaluronidases, and interactions with HA-binding proteins. HA synthesis and degradation are closely regulated in skeletal tissues and aberrant synthetic or degradative activity causes disease. The role and regulation of HA synthesis and degradation in cartilage, bone and skeletal development is discussed.

5. IMMUNOMODULATORY AND ANTI-INFLAMMATORY EFFECTS OF CHONDROITIN SULPHATE

Journal of cellular and molecular medicine volume 13, issue 8a, pages 1451–1463, august 2009

Patrick Du Souich[1,*], Antonio G. García[2], Josep Vergés[3], Eulàlia Montell[3]

Abstract

- Biochemistry of chondrotin sulphate
- Mechanism of action of chondroitin sulphate

  Effect of chondroitin sulphate on the chondrocyte

  - Effect of chondroitin sulphate on the synovial membrane
  - Effect of chondroitin sulphate on subchondral bone
- Human use of chondroitin sulphate
  - Chondrotin sulphate in osteoarthritis
  - Chondrotin sulphate in psoriasis
  - Chondrotin sulphate in atherosclerosis
  - Chondroitin sulphate in IBD
  - Chondroitin sulphate in degenerative diseases of the central nervous system (CNS)
  - Other autoimmune diseases that may benefit from chondroitin sulphate

Conclusions

Chondroitin sulphate (CS) is a natural glycosaminoglycan present in the extracellular matrix and is formed by the 1–3 linkage of D-glucuronic acid to N-acetylgalactosamine. In chondrocytes, CS diminishes interleukin-1 **p** (IL-1p)-induced increases in p38 mitogen-activated protein kinase (p38MAPK) and signal-regulated kinase 1/2 (Erk1/2) phosphorylation, and decreases nuclear factor-KB (NF-kB) nuclear translocation and as a consequence, reduces the formation of pro-inflammatory cytokines, IL-1 **p** and TNF-a, and pro-inflammatory enzymes, such as phospholipase A2 (PLA2), cyclooxygenase 2 (COX-2) and nitric oxide synthase-2 (NOS-2). The mechanism of action of CS explains its beneficial effect on the

cartilage, synovial membrane and subchondral bone. On the other hand, *in vivo*, CS given orally prevents hepatic NF-KB nuclear translocation, suggesting that systemic CS may elicit an anti-inflammatory effect in many tissues besides the articulation. There is preliminary evidence showing that in human beings, CS may be of benefit in other diseases where inflammation is an essential marker, such as psoriasis and atherosclerosis. The review of the literature suggest that CS might also be of interest for the treatment of other diseases with an inflammatory and/or autoimmune character, such as inflammatory bowel disease, degenerative diseases of the central nervous system and stroke, multiple sclerosis and other autoimmune diseases.

6. **Curr Med Res Opin. 2006 Nov;22(11):2221-32.**

Collagen hydrolysate for the treatment of osteoarthritis and other joint disorders: a review of the literature.

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ABSTRACT

BACKGROUND: There is a need for an effective treatment for the millions of people in the United States with osteoarthritis (OA), a degenerative joint disease. The demand for treatments, both traditional and non-traditional, will continue to grow as the population ages.

SCOPE: This article reviews the medical literature on the preclinical and clinical research on a unique compound, collagen hydrolysate. Articles were obtained through searches of the PubMed database (www.pubmed.gov) through May 2006 using several pairs of key words (collagen hydrolysate and osteoarthritis; collagen hydrolysate and cartilage; collagen hydrolysate and chondrocytes; collagen hydrolysate and clinical trial) without date limits. In addition, other sources of information, such as abstracts presented at scientific congresses and articles in the German medical literature not available on PubMed, were reviewed and included based on the authors' judgment of their relevance to the topic of the review.

FINDINGS: According to published research, orally administered collagen hydrolysate has been shown to be absorbed intestinally and to accumulate in cartilage. Collagen hydrolysate ingestion stimulates a statistically significant increase in synthesis of extracellular matrix macromolecules by chondrocytes ($p < 0.05$ compared with untreated controls). These findings suggest mechanisms that might help patients affected by joint disorders such as OA. Four open-label and three double-blind studies were identified and reviewed; although many of these studies did not provide key information--such as the statistical significance of the findings--they showed collagen

hydrolysate to be safe and to provide improvement in some measures of pain and function in some men and women with OA or other arthritic conditions.
CONCLUSION: A growing body of evidence provides a rationale for the use of collagen hydrolysate for patients with OA. It is hoped that ongoing and future research will clarify how collagen hydrolysate provides its clinical effects and determine which populations are most appropriate for treatment with this supplement.

7. **Semin Arthritis Rheum. 2000 Oct;30(2):87-99.**

Role of collagen hydrolysate in bone and joint disease.

Moskowitz RW. Case Western Reserve University, Division of Rheumatic Diseases, University Hospitals of Cleveland, OH, USA.

ABSTRACT

OBJECTIVES: To review the current status of collagen hydrolysate in the treatment of osteoarthritis and osteoporosis.
METHODS: Review of past and current literature relative to collagen hydrolysate metabolism, and assessment of clinical investigations of therapeutic trials in osteoarthritis and osteoporosis.
RESULTS: Hydrolyzed gelatin products have long been used in pharmaceuticals and foods; these products are generally recognized as safe food products by regulatory agencies. Pharmaceutical-grade collagen hydrolysate (PCH) is obtained by hydrolysis of pharmaceutical gelatin. Clinical studies suggest that the ingestion of 10 g PCH daily reduces pain in patients with osteoarthritis of the knee or hip; blood concentration of hydroxyproline is increased. Clinical use is associated with minimal adverse effects, mainly gastrointestinal, characterized by fullness or unpleasant taste. In a multicenter, randomized, doubleblind, placebo-controlled trial performed in clinics in the United States, United Kingdom, and Germany, results showed no statistically significant differences for the total study group (all sites) for differences of mean pain score for pain. There was, however, a significant treatment advantage of PCH over placebo in German sites. In addition, increased efficacy for PCH as compared to placebo was observed in the overall study population amongst patients

with more severe symptomatology at study onset. Preferential accumulation of 14C-labeled gelatin hydrolysate in cartilage as compared with administration of 14C-labeled proline has been reported. This preferential uptake by cartilage suggests that PCH may have a salutary effect on cartilage metabolism. Given the important role for collagen in bone structure, the effect of PCH on bone metabolism in osteoporotic persons has been evaluated. Studies of the effects of calcitonin with and without a collagen hydrolysate-rich diet suggested that calcitonin plus PCH had a greater effect in inhibiting bone collagen breakdown than calcitonin alone, as characterized by a fall in levels of urinary pyridinoline cross-links. PCH appeared to have an additive effect relative to use of calcitonin alone.

CONCLUSIONS: Collagen hydrolysate is of interest as a therapeutic agent of potential utility in the treatment of osteoarthritis and osteoporosis. Its high level of safety makes it attractive as an agent for long-term use in these chronic disorders.

8. Osteoarthritis Cartilage. 1998 May;6 Suppl A:14-21.

ANTI-INFLAMMATORY ACTIVITY OF CHONDROITIN SULFATE.

Ronca F, Palmieri L, Panicucci P, Ronca G. -Department of Human and Environmental Sciences, University of Pisa, Italy.

ABSTRACT

The pharmacokinetics of chondroitin sulfate (CS, Condrosulf, IBSA, Lugano, Switzerland) were investigated in rats and in healthy volunteers using CS tritiated at the reducing end and CS labeled with 131I or 99mTc respectively. A rapid absorption of orally administered CS is observed in rats and in humans when the drug is dissolved in water. Lower and delayed absorption is observed when CS is administered in gastroresistant capsules. The absolute bio-availability is 15 and 12% for rats and humans respectively. The CS shows a tropism for cartilagineous tissues in rats and for knee tissues in humans as demonstrated by scintigraphic analysis with 99mTc-CS. Monomers, oligo and polysaccharides produced by enzymatic hydrolysis

of CS appear in the blood and tissues together with native CS. The effects of partially depolymerized (m.m. 3 to 15 kD) and desulfated fractions on human leukocytes were investigated. CS and its fractions inhibit the directional chemotaxis induced by zymosan-activated serum, are able to decrease the phagocytosis and the release of lysozyme induced by zymosan and to protect the plasma membrane from oxygen reactive species. In rats the oral administration of CS significantly decreases granuloma formation due to sponge implants and cell migration and lysosomal enzyme release in carrageenan pleurisy. Compared with nonsteroidal anti-inflammatory drugs (indomethacin, ibuprofen), CS appears to be more effective on cellular events of inflammation than on edema formation. It is noteworthy that CS is devoid of dangerous effects on the stomach, platelets and kidneys. In synovial fluid of patients requiring joint aspiration, treated orally for 10 days with CS (800 mg/day) the hyaluronate concentration and the intrinsic viscosity significantly increased, while collagenolytic activity, phospholipase A2 and N-acetylglucosaminidase (NAG) decreased. These results give an insight into the mechanism of the anti-inflammatory and chondroprotective actions demonstrated by this drug in a number of clinical trials in patients with osteoarthritis.

9. **Osteoarthritis Cartilage. 2008;16 Suppl 3:S19-21. Epub 2008 Jul 31.**

Clinical review of chondroitin sulfate in osteoarthritis.

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Abstract

Symptomatic slow-acting drugs for the treatment of osteoarthritis (SYSADOA; OA) are compounds which are prescribed as drugs in European countries since many years, whereas they are sold as nutraceuticals in USA. In Europe, the publication of the EULAR Recommendations for the Treatment of Knee OA in 2003 has listed oral chondroitin sulfate (CS) as evidence 1A and strength of recommendation A which represents the highest level for a therapeutic strategy. Symptomatic slow-acting drugs are intended to be used as ground therapy for OA; these compounds are not rapidly

acting agents such as Non Steroidal Anti-Inflammatory Drugs (NSAIDs), and their clinical efficacy on algo-functional symptoms can only be demonstrated after a couple of weeks of regular intake. Interestingly, once the administration is stopped, they do show carry-over effects of various durations, from about 3 months with the oral formulations to 6-9 months with intra-articular formulations. The main rationale behind the use of the SYSADOA therapeutic class is the reduction of NSAIDs in the overall drug management of OA disease and therefore consequently to limit the very significant risks of upper Gastro-intestinal (GI) tract erosions, ulcers with bleeding and/or deleterious renal effects in elderly patients. The evidence for clinical efficacy of oral CS as a drug able to significantly improve the algo-functional symptoms of OA disease does come from a set of randomized clinical studies published a couple of years ago. Indeed, it was demonstrated that the drug was effective in knee and finger OA, whereas previous data suggested that hip OA patients could also benefit from it. In addition, oral CS supported the comparison with NSAIDs such as diclofenac sodium in a medium/long-term clinical study in patients with knee OA. A dose-finding study in patients with knee OA did provide strong data supporting the administration of 800 mg of CS orally which had nearly the same effects as 1200 mg/day, whereas the use of a sequential 3 months administration mode, twice a year was also shown to provide the same results as a continuous treatment. The good tolerability and safety aspects of oral CS were largely documented in these CTs. Taking these important points into account, we definitively have enough clinical data available supporting the view that oral CS is a valuable and safe symptomatic treatment for OA disease. More recent data based on a couple of previous trials and two pivotal studies do provide further evidence that oral CS does also have structure-modifying effects in knee OA patients. A couple of other compounds such as hyaluronan, diacerein, avocado and soya unsaponifiables, doxycycline have also been tested with respect to their potential disease-modifying effects. Additional compounds including receptor activator of NF-kappaB (RANK) ligand inhibitors, cathepsin K inhibitors, bisphosphonates are further assessed regarding their potential structure-modifying effect.

10. Am J Pathol. 2009 Jan;174(1):101-14. Epub 2008 Dec 30.

Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin.

Fisher GJ, Quan T, Purohit T, Shao Y, Cho MK, He T, Varani J, Kang S, Voorhees JJ. --Department of Dermatology, Medical School, University of Michigan, Ann Arbor, Michigan 48109-5609, USA.

Abstract

Aged human skin is fragile because of fragmentation and loss of type I collagen fibrils, which confer strength and resiliency. We report here that dermal fibroblasts express increased levels of collagen-degrading matrix metalloproteinases-1 (MMP-1) in aged (>80 years old) compared with young (21 to 30 years old) human skin in vivo. Transcription factor AP-1 and alpha2beta1 integrin, which are key regulators of MMP-1 expression, are also elevated in fibroblasts in aged human skin in vivo. MMP-1 treatment of young skin in organ culture causes fragmentation of collagen fibrils and reduces fibroblast stretch, consistent with reduced mechanical tension, as observed in aged human skin. Limited fragmentation of three-dimensional collagen lattices with exogenous MMP-1 also reduces fibroblast stretch and mechanical tension. Furthermore, fibroblasts cultured in fragmented collagen lattices express elevated levels of MMP-1, AP-1, and alpha2beta1 integrin. Importantly, culture in fragmented collagen raises intracellular oxidant levels and treatment with antioxidant MitoQ(10) significantly reduces MMP-1 expression. These data identify positive feedback regulation that couples age-dependent MMP-1-catalyzed collagen fragmentation and oxidative stress. We propose that this self perpetuating cycle promotes human skin aging. These data extend the current understanding of the oxidative theory of aging beyond a cellular-centric view to include extracellular matrix and the critical role that connective tissue microenvironment plays in the biology of aging.

11. **Am J Pathol. 2007 Nov;171(5):1451-61.**

Chronic ultraviolet B irradiation causes loss of hyaluronic acid from mouse dermis because of down-regulation of hyaluronic acid synthases.

Dai G, Freudenberger T, Zipper P, Melchior A, Grether-Beck S, Rabausch B, de Groot J, Twarock S, Hanenberg H, Homey B, Krutmann J, Reifenberger J, Fischer JW., Molekulare Pharmakologie, Institut für Pharmakologie and Klinische Pharmakologie, Universitätsklinkum Düsseldorf, Düsseldorf, Germany.

ABSTRACT

Remodeling of the dermal extracellular matrix occurs during photoaging. Here, the effect of repetitive UVB irradiation on dermal hyaluronic acid (HA) was examined. C57/BL6 mice were chronically (182 days) irradiated with UVB, and consecutive skin biopsies were collected during the irradiation period and afterward (300 and 400 days of age). UVB caused marked loss of HA from the papillary dermis and down-regulation of HA synthase 1 (HAS1), HAS2, and HAS3 mRNA expression. In contrast, hyaluronidases (HYAL) 1, HYAL2, and HA receptor CD44 were

unchanged. Furthermore, transforming growth factor beta-1 (TGF-beta1) and TGF-beta1-receptor II expression were decreased in UVB-irradiated biopsies, and TGF-beta1 strongly induced HAS1 and HAS2 expression in cultured dermal fibroblasts. Therefore, TGF-beta1 might be one factor involved in UVB-induced down-regulation of HAS enzymes. In addition, total cell number and the percentage of proliferating fibroblasts in the papillary dermis of UVB-irradiated mice were decreased. Down-regulation of HAS2 by lentiviral overexpression of short hairpin RNA in vitro caused inhibition of HA synthesis, DNA synthesis, and migration of dermal fibroblasts. In conclusion, chronic UVB irradiation induces loss of HA from the dermis, thereby contributing to the quiescent phenotype of dermal fibroblasts

12. **Cell Tissue Res. 2003 Mar;311(3):393-9. Epub 2003 Feb 25.**

Stimulation of type II collagen biosynthesis and secretion in bovine chondrocytes cultured with degraded collagen.

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Abstract

The functional integrity of articular cartilage is dependent on the maintenance of the extracellular matrix (ECM), a process which is controlled by chondrocytes. The regulation of ECM biosynthesis is complex and a variety of substances have been found to influence chondrocyte metabolism. In the present study we have investigated the effect of degraded collagen on the formation of type II collagen by mature bovine chondrocytes in a cell culture model. The culture medium was supplemented with collagen hydrolysate (CH) and biosynthesis of type II collagen by chondrocytes was compared to control cells treated with native type I and type II collagen and a collagen-free protein hydrolysate. The quantification of type II collagen by means of an ELISA technique was confirmed by immunocytochemical detection as well as by the incorporation of (14)C-proline in the ECM after a 48 h incubation. Chondrocytes in the control group were maintained in the basal medium for 11 days. The presence of extracellular CH led to a dose-dependent increase in type II collagen secretion. However, native collagens as well as a collagen-free hydrolysate of wheat proteins failed to stimulate the production of type II collagen in chondrocytes. These results clearly indicate a stimulatory effect of degraded collagen on the type II collagen biosynthesis of chondrocytes and suggest a possible feedback mechanism for the regulation of collagen turnover in cartilage tissue.

13. **Int J Dermatol. 1994 Feb;33(2):119-22.**

Hyaluronic acid in cutaneous intrinsic aging.

Ghersetich I, Lotti T, Campanile G, Grappone C, Dini G., Department of Dermatology, University of Florence, Italy.

Abstract

BACKGROUND: In elderly individuals all components of the skin and subcutaneous tissue undergo histologic and ultrastructural changes. The turgidity of the dermis appears decreased, presumably due to altered patterns and levels of glycosaminoglycans (GAGS), especially hyaluronic acid and dermatan sulfate that are the most common. A linear, age-related decrease in the content of GAGS (mainly hyaluronic acid) has been hypothesized in human aged skin.
METHODS: We used the cationic dye Alcain Blue to selectively stain hyaluronic acid within the dermis in old and young subjects to compare ultrastructurally its topography and variations with age.
RESULTS: We demonstrated a progressive reduction in the number of electron-dense granules of hyaluronic acid and of their filaments until they were completely absent in subjects aged 60.
CONCLUSIONS: We propose that the variations of the levels of hyaluronic acid in the dermis in aging could account for some of the most striking alterations of the aged skin, including decreased turgidity, less support for microvessels, wrinkling, and altered elasticity.

14. **Arthritis Rheum. 1998 May; 41(5):938.**

Treatment of rheumatoid arthritis with oral Type II Collagen. Results of a multicenter, double-blind, placebo-controlled human clinical trial

Barnett ML, Kremer JM, St Clair EW, Clegg DO, Furst D, Weisman M, Fletcher MJ, Chasan-Taber S, Finger E, Morales A, Le CH, Trentham DE. Beth Israel Deaconess Medical Center, Boston, Massachusetts 02215, USA.

ABSTRACT

OBJECTIVE: Oral administration of cartilage-derived type II collagen (CII) has been shown to ameliorate arthritis in animal models of joint inflammation, and preliminary studies have suggested that this novel therapy

is clinically beneficial and safe in patients with rheumatoid arthritis (RA). The present study was undertaken to test the safety and efficacy of 4 different dosages of orally administered CII in patients with RA.

METHODS: Two hundred seventy-four patients (274) with active RA were enrolled at 6 different sites and randomized to receive placebo or 1 of 4 dosages (20, 100, 500, or 2,500 microg/day) of oral CII for 24 weeks. Efficacy parameters were assessed monthly. Cumulative response rates (percentage of patients meeting the criteria for response at any time during the study) were analyzed utilizing 3 sets of composite criteria: the Paulus criteria, the American College of Rheumatology criteria for improvement in RA, and a requirement for > or = 30% reduction in both swollen and tender joint counts.

RESULTS: Eighty-three percent of patients completed 24 weeks of treatment. Numeric trends in favor of the 20 microg/day treatment group were seen with all 3 cumulative composite measures. However, a statistically significant increase ($P = 0.035$) in response rate for the 20 microg/day group versus placebo was detected using only the Paulus criteria. The presence of serum antibodies to CII at baseline was significantly associated with an increased likelihood of responding to treatment. No treatment-related adverse events were detected. The efficacy seen with the lowest dosage is consistent with the findings of animal studies and with known mechanisms of oral tolerance in which lower doses of orally administered autoantigens preferentially induce disease-suppressing regulatory cells.

CONCLUSION: Positive effects were observed with CII at the lowest dosage tested, and the presence of serum antibodies to CII at baseline may predict response to therapy. No side effects were associated with this novel therapeutic agent. Further controlled studies are required to assess the efficacy of this treatment approach.

15. **J Cell Physiol. 1998 Dec;177(3):465-73.**

Hyaluronic acid stimulates human fibroblast proliferation within a collagen matrix.

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Abstract

Human dermal fibroblasts suspended in a collagen matrix exhibit a 4-day delay in cell division, while the same cells in monolayer divided by day 1. The initial rates of 3H-thymidine incorporation by cells in monolayer or suspended in collagen were not significantly different. When suspended in collagen, there was a threefold increase in the proportion of cells in a tetraploidal (4N) DNA state compared to the same cells in monolayer. Flow cytometry analysis and 3H-thymidine incorporation studies identified the delay of cell division as a consequence of a block in the G2/M of the cell cycle and not an inhibition of DNA synthesis. The inclusion of 150 microg/ml of hyaluronic acid (HA) in the manufacture of fibroblast populated collagen lattices (FPCL) caused a stimulation of cell division, as determined by cell counting; increased the expression of tubulin, as determined by Western blot analysis; and reduced the proportion of cells in a 4N state, as determined by flow cytometry. HA added to the same cells growing in monolayer produced a minimal increase in the rate of cell division or DNA synthesis. HA supplementation of FPCLs stimulated cell division as well as tubulin concentrations, but it did not enhance lattice contraction. The introduction of tubulin isolated from pig brain or purchased tubulin into fibroblasts by electroporation prior to their transfer into collagen lattices promoted cell division in the first 24 hours and enhanced FPCL contraction. It is proposed that tubulin protein, the building blocks of microtubules, is limited in human fibroblasts residing within a collagen matrix. When human fibroblasts are suspended in collagen, one effect of added HA may be to stimulate the synthesis of tubulin which assists cells.

PLEASE SEE PAGES BELOW FOR LIST OF ADDITIONAL SUPPORTING STUDIES (some of them have been abstracted above)

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HYALURONIC ACID
Molecular Versatility and Implications in Cancer
v.2

BioCell Technology LLC

Joosang Park, PhD MBA

VP of Scientific Affairs

Jan 4, 2011

What is HA?

HA is a large, linear glycosaminoglycan (GAG) polymer with a repeating disaccharide unit of D-glucuronic acid and N-acetyl-D-glucosamine. It is critical for maintaining the integrity of the extracellular matrix (ECM) in various tissues. It can exist in a protein-bound state with aggregating proteoglycans (PGs), and modulate cell adhesion, migration, and proliferation via interaction with cell surface receptors (1).

The enzymes, HA Synthases (HAS) and hyaluronidases (HYL), coordinately regulate local levels and molecular weight (MW) of HA, thereby involved in both normal and pathophysiological phenomena including cell movement, proliferation, inflammation, fertilization, aging, and cancer. Although HA clearly plays an extracellular, structural hydrodynamic role, it also plays an instructive role via cell signaling which requires HA receptors such as CD44 and RHAMM on the cell surface (2).

Varying Sizes and Biological Properties

HA normally exists in varying sizes (2,000-25,000 units with MW of 10^5 - 10^7 Da), and is ubiquitously expressed in tissues, while involved in a myriad of biological functions (2). Understanding normal and abnormal physiological functions in the context of its molecular weight and local concentrations remains a challenge.

Higher MW HA, generally occurring in intact healthy tissues, is normally produced by HAS at the plasma membrane, whereas it is degraded by extracellular PM-tethered HYL that is coordinated with intracellular lysosomal HYLs (3). In contrast, lower MW HA, often reflecting cellular distress, is produced in many malignancies, likely due to heightened HYL activity (3). It remains unclear how these specific HA molecules are generated and maintained. Interestingly, exogenously-administered, low MW (1-3x10^5 Da) HA activated anti-cancer immune responses to inhibit the growth of colorectal cancer cells *in vitro* and *in vitro* (4). Splenocytes isolated from these mice showed significantly higher proliferative capacity when stimulated by tumor lysate-pulsed dendritic cells, and increased tumor infiltrating lymphocytes (TIL) were observed. This result suggested that low MW-HA could be a candidate for therapeutic adjuvants for colorectal cancer immunotherapy.

On the other hand, very low MW-HA such as HA oligomers serve as antagonists of HA-CD44 interactions leading to the suppression of anchorage-independent growth of breast and colon

cancer cells, and may be useful in therapeutic strategies aimed at preventing tumor recurrence from the therapy-resistant sub-populations (CSC) within malignant cancers (2).

HA level is significantly elevated during normal processes as well including embryogenesis, cell migration, tissue turnover, and wound healing (5). Thus, it is not surprising that cancer cells frequently perturb the normal regulation of HA via altered signaling cascades which involve abnormal regulation of the enzymes and receptors (6).

HA in BioCell Collagen II® is Depolymerized to Very Low MW by a Patented Process

HA is a key constituent of BioCell Collagen II®. What differentiates BioCell Collagen II® from other HA-containing products is the size of HA. Using a patented process, its average MW is significantly reduced to 23,000 Da (50-60 repeating units). Our study demonstrated that this facilitated absorption and elevated HA bioavailability in the blood. It is likely that its size becomes even smaller while passing through the digestive system. As discussed above, reduction in its size might have significant physiological implications in terms of anti-cancer immune responses as well as bioavailability.

Can HA cause cancer?

It is clear that HA by itself is not a cancer-causing molecule. There has been no scientific data that implicate elevated HA levels as a cause for any type of cancer. Studies have shown that HA metabolism in cancer cells becomes perturbed in association with abnormal regulation of HAS and HYL, and abnormal interaction with its receptors can contribute to cancer progression (2). It is this altered, malignant physiological environment that elevated HA levels may have a tumor-promoting effect.

Non-linear Complexity of HA Metabolism in Carcinogenesis

It takes complete understanding of HA anabolism and catabolism to understand how HA metabolism participates in each of the three steps of carcinogenesis (initiation-promotion-progression). As of now, there is no scientific data indicating that elevated levels of HA can cause initiation of carcinogenesis.

Although up-regulation of tumor-associated HA is implicated in cancer progression (2), potential contribution of HA from exogenous sources (ex. daily ingestion) to the malignant process needs to be discussed with caution. Recent data showed that treatment with low MW-HA was shown to produce anti-cancer immunity *in vivo* (4). The complexity is added because HA acts through its receptors, CD44 and RHAMM to regulate cell movement and proliferation (7). Alteration in both CD44 and HA expression such as up-regulation of normal CD44 and expression of abnormal CD44 variants have been widely shown in cancers from cancer patients and in animal models (8), suggesting that cancer cells overexpressing HA have already undergone malignant changes in the corresponding signaling transduction pathway.

Many studies demonstrated that over-expression of HA from cancer cells played a positive role in tumor malignancy. In cancers of breast, colon, prostate, and bladder, there seems to be decoupling between the enzymes, leading to elevation of extracellular levels of HA and partially degraded oligomers (3; 9). In addition, HA produced by cancer cells promotes cell migration, aids in the loss of contact inhibition, and offers protection against immune surveillance (9). However, it is interesting that very high levels of HA produced by HAS 2 can inhibit cancer promotion (10). The role of HA in cancer promotion is further compounded by the importance of partial HA degradation in inflammation which is an important factor in the progression of many cancer types (11; 12). Furthermore, HA oligomers, like low MW-HA, from exogenous sources were shown to inhibit cancer growth, in this case, by competing for interaction with CD44 with tumor-derived, endogenous polymeric HA (13).

Even in a local environment where cancer cells are established, involvement of HA in cancer progression remains complicating. For example, over-expression of intact CD44 in cancer cells promoted cancer progression, whereas it was inhibitory in other studies (14). Moreover, HA is not involved in caner progression of rat pancreatic carcinoma cells (15). These studies suggest that responses of established cancer cells to HA appear to be influenced by many factors including organization, concentration, alternative splicing, or post-translational modification of CD44 and/or RHAMM (14).

Inhibition of Hyaluronidase by BioCell Collagen II® is a Tumor-suppressing, not Tumor-Promoting Activity

Hyaluronidase (HYL) is involved in tumor growth, infiltration, and angiogenesis. Like HA, the role of HYL is not linear in that it can be both tumor-promoting and tumor-suppressing depending upon local environments and concentration (6).

HYL as a tumor promoter

Data showed that HYL-1 promoted cancer growth, muscle infiltration, and angiogenesis (14; 9; 16). Blocking HAL-1 expression in bladder and prostate cancer cells suppressed cell proliferation due to cell cycle arrest and decreased their invasive activity (9).

HYL as a tumor suppressor

This concept has been prevalent because elevated HA expression by tumor cells are associated with cancer progression, which would make it easier to explain that an enzyme that degrades HA was a tumor suppressor (6). However, carcinogenesis is a very complicated, multi-step process which involves diverse, aberrantly-regulated molecules. Their actual functions are often displayed differently depending upon local environments.

Recently, the contradictory behaviors of HYL have been understood by data strongly suggesting that the actual function of HYL was concentration-dependent and that tumor cell-derived HYL in genitourinary cancers mainly acts as a tumor promoter (9). Thus, inhibition of HYL by BioCell Collagen II should be regarded as tumor-suppressing, not tumor-promoting, because it would interfere with cancerous cell growth, cancer infiltration into muscle, and neovascularization.

Conclusion

There is no scientific evidence indicating that elevated HA bioavailability leads to initiation of cancer development. In both pre-clinical and clinical studies, no side-effects or toxicities were reported from daily ingestion of BioCell Collagen II® (17; 18), and, interestingly, anti-cancer immune responses were elicited by administration of low MW-HA, leading to inhibition of the growth of colorectal cancer cells (4). Furthermore, BioCell Collagen II® has been safely and effectively used by millions of customers since introduced in 1997.

Studies have shown that the up-regulation and metabolism of HA coordinated by HA synthases and hyaluronidases can be either cancer-promoting or cancer-suppressing, suggesting that defining a physiological context is crucial in understanding their involvement in carcinogenesis. It is also to be noted that HA acts through its receptors such as CD44 and RHAMM and that cancer-promoting effect of aberrantly-regulated HA is exerted through its interaction with altered or over-expressed receptors such as CD44 and RHAMM on ca**n**cer cells which may have undergone changes in their cell signaling cascades (2). For example, in the abstract discussed in the following section, aberrant CD44 variants were shown to be expressed in human breast cancer xenografts in SCID mice and their continuous interaction with HA was required for

cancer cell growth (19). Hyaluronidase treatment caused reduction of tumor progression in concomitant with significant reduction of the abnormal CD44 variants. In contrast, the level of normal CD44 was not affected. These results suggest that elevated expression of HA and its interaction with receptors are differentially regulated between normal and cancer cells.

HA contained in BioCell Collagen II® is depolymerized to very low MW with approximately 50 repeating units. Current data from studies investigating the effects of exogenously administered very low MW-HA and oligomeric HA imply that elevation of HA concentration at cancer lesions after ingestion of BioCell Collagen II® could interfere with interaction between tumor cell-associated HA and CD44, which would lead to the suppression of cancer growth (14; 20). Alternatively, increased production of HA by metastasized cancer cells may provide drug resistance in cancer-bearing subjects, contributing to more malignant progression. In this case, elevated levels of very low MW-HA might interfere with cancer progression by inducing anti-cancer immune responses (14; 4).

BioCell Collagen II® possesses an intriguing hyaluronidase inhibitory activity. Studies showed that hyaluronidase inhibition would lead to two opposing effects depending upon a local environment (9). At concentrations present in tumor tissues, hyaluronidase activity is cancer-promoting because it increases tissue permeability for its extravasation into other tissues. As hyaluronidase inhibition can inhibit cancer progression by interfering with this critical step in cancer metastasis, the effect of BioCell Collagen II® would be cancer-suppressing, not cancer-promoting.

The following pages contain commentary on the abstracts for studies that link expression of HA, its binding to the receptor CD44, and hyaluronidase in various cancer models.

1. Int J Cancer. 2002 Nov 10;102(2):192-7.

Hyaluronidase reduces human breast cancer xenografts in SCID mice.

Shuster S, Frost GI, Csoka AB, Formby B, Stern R.
Department of Pathology, School of Medicine, University of California San Francisco, San Francisco, CA 94143-0511, USA.

A hyaluronan-rich environment often correlate with tumor progression. and may be one mechanism for the invasive behavior of malignancies. Eradication of hyaluronan by hyaluronidase administration could reduce tumor aggressiveness and would provide, therefore, a new anti-cancer strategy. Hyaluronan interaction with its CD44 receptor and the resulting signal transduction events may be among the mechanisms for hyaluronan-associated cancer progression. We have shown previously that hyaluronidase treatment of breast cancer cells in vitro not only eradicates hyaluronan but also modifies expression of CD44 variant exons of tumor cells. We now determine if such effects occur in vivo and if it is accompanied by tumor regression. SCID mice bearing xenografts of human breast carcinomas were given intravenous hyaluronidase. Tumor volumes decreased 50% in 4 days. Tumor sections showed decreased hyaluronan. Intensity of staining for CD44s was not affected, whereas staining for specific CD44 variant exon isoforms was greatly reduced in residual tumors. Necrosis was not evident. Hyaluronidase, used previously as an adjunct in cancer treatment, presumably to enhance penetration of chemotherapeutic drugs, may itself have intrinsic anti-cancer activity. Removing peritumor hyaluronan appears to cause an irreversible change in tumor metabolism. Continuous hyaluronan binding to CD44 variant exon isoforms may also be required to stabilize inherently unstable isoforms that participate perhaps in tumor progression. Further investigation is required to confirm a cause and effect relationship between loss of hyaluronan, changes in CD44 variant exon expression and tumor reduction. If confirmed, hyaluronidase may provide a new class of anti-cancer therapeutics and one without toxic side effects.

2. Clin Cancer Res. 1999 May;5(5):1073-6.

Expression of the hyaluronan receptor, CD44S, in epithelial ovarian cancer is an independent predictor of survival.

Kayastha S, Freedman AN, Piver MS, Mukkamalla J, Romero-Guittierez M, Werness BA.
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Most ovarian carcinomas present at advanced stage, principally as the result of dissemination to peritoneal sites. Standard CD44 (CD44S) is the principal receptor for hyaluronic acid, and in vitro and animal studies have suggested that the attachment of ovarian carcinoma cells to the peritoneal mesothelium involves the interaction between CD44S on ovarian carcinoma cells and hyaluronic acid on mesothelial surfaces. We, therefore, analyzed a series of ovarian carcinomas for the expression of CD44S by immunohistochemistry to see whether expression of this receptor by tumor cells correlated with clinicopathological factors and measures of patient

outcome. Fifty-six fixed, paraffin-embedded primary epithelial ovarian tumors were immunostained with antibody to CD44S. Membrane staining was considered positive, and results were correlated with stage, grade, age, histology, and survival. Twenty-two (39%) tumors were positive for CD44S. There was no correlation between CD44 expression and histological type, grade, age, or stage. However, CD44 expression was significantly associated with survival in both univariate (P = 0.003) and multivariate (P = 0.006) analyses. These results support a role for CD44S expression in the spread of ovarian epithelial cancer and suggest that expression of this molecule is a significant independent predictor of survival in women with this disease.

3. (Unknown source)

As shown in Figure 1, the various types of molecules that interact with hyaluronan can contribute to many of the stages of cancer metastasis.
Hyaluronan synthases (HAS) play roles in all of the stages of cancer metastasis. By producing anti-adhesive HA, HAS can allow tumor cells to release from the primary tumor mass and if HA associates with receptors such as CD44, the activation of Rho GTPases can promote epithelial-mesenchymal transition (EMT) of the cancer cells. During the processes of intravasation or extravasation, the interaction of HAS produced HA with receptors such as CD44 or RHAMM promote the cell changes that allow for the cancer cells to infiltrate the vascular or lymphatic systems. While traveling in these systems, HA produced by HAS protects the cancer cell from physical damage. Finally, in the formation of a metastatic lesion, HAS produces HA to allow the cancer cell to interact with native cells at the secondary site and to produce a tumor for itself. [1]

Hyaluronidases (HAase or HYAL) also play many roles in cancer metastasis. By helping to degrade the ECM surrounding the tumor, hyaluronidases help the cancer cell escape from the primary tumor mass and play a major role in intravasation by allowing degradation of the basement membrane of the lymph or blood vessel. Hyaluronidases again play these roles in establishment of a metastatic lesion by helping with extravasation and clearing the ECM of the secondary site. [2] Finally, hyaluronidases play a key role in the process of angiogenesis. HA fragments promote angiogenesis and hyaluronidases produce these fragments. [3] Interestingly, hypoxia also increases production of HA and activity of hyaluronidases. [4]
The hyaluronan receptors, CD44 and RHAMM, are most thoroughly studied in terms of their roles in cancer metastasis. Increased clinical CD44 expression has been positively correlated to metastasis in a number of tumor types. [5] Mechanistically, CD44 affects adhesion of cancer cells to each other and to endothelial cells, rearranges the cytoskeleton through the Rho GTPases,

and increases the activity of ECM degrading enzymes. [6] Increased RHAMM expression has also been clinically correlated with cancer metastasis. Mechanistically, RHAMM promotes cancer cell motility through a number of pathways including focal adhesion kinase (FAK), Map Kinase (MAPK), pp60 (c-src), and the downstream targets of Rho Kinase (ROK). [7] RHAMM can also cooperate with CD44 to promote angiogenesis towards the metastatic lesion. [8]

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Commentary:

Studies have shown that HA is ubiquitously expressed and an extremely versatile molecule in its size and functions. Thus, when the role of HA is discussed, a physiological context should be defined because the same size and level of HA may produce opposing consequences.

HA is highly expressed in some cancer cells and promotes cancer progression in some malignant environments. However, there is no scientific data to indicate that elevated HA expression from normal cells or heightened availability after daily ingestion can contribute to carcinogenesis. Rather, recent data demonstrated that administration of very low MW HA similar to depolymerized HA in BioCell Collagen II® or oligomeric HA inhibited the growth of breast and colorectal cancer cells (4; 14). Moreover, elevated expression of HA is found in many normal physiological processes which include embryogenesis, wound repair, and tissue turnover. Much higher expression of HA is also found in the skin and cartilage tissues of young people than in aged people, suggesting that HA up-regulation is also associated with normal, healthy physiological processes.

Normal and cancer cells are vastly different in their responses to physiological stimuli. Cancer cells have already undergone a number of genetic and molecular changes and have committed themselves to malignant carcinogenic processes. The potential role of HA in carcinogenesis should be discussed in terms of its interaction with receptors such as CD 44, which is often up-regulated (CD44S) or altered (CD44v) in cancer cells, but not in normal cells. In addition, downstream signaling pathways are awry in cancer cells so that a signal becomes amplified to support cancer cell survival and subsequent malignant behaviors such as metastasis. However, elevated HA expression in normal cells does not have the malignant effect because of normal control of CD44 expression and homeostasis in the regulation of signaling pathways. Thus, elevated levels of very low MW HA in normal individuals resulting from daily ingestion of BioCell Collagen II® will not have any serious harmful effects and instead serve as a pool that can be tapped by cartilage and skin tissues where the amount of high MW HA decreases due to aging or chronic joint conditions.

What is interesting about hyaluronidase (HYL) is that HYL acts as both tumor promoter and tumor-suppressor depending upon its concentration. At concentrations present in tumor tissues, HYL displayed tumor promoter functions contributing to tumor growth, invasion, and angiogenesis, whereas, at much higher concentrations not naturally expressed by tumor cells, it suppresses growth by inducing programmed cell death (9). This result suggests that the eventual effect of HYL inhibition, like that of HA, should be discussed in the context of the specific physiological environment.

The data showing anti-cancer effects of very low MW or oligomeric HA from exogenous sources support that depolymerized (very low MW) HA contained in BioCell Collagen II is cancer-suppressing. And, inhibition of HYL by BioCell Collagen II® is potentially cancer-suppressing because at concentrations produced in cancerous environment HYL is cancer-promoting (9). Thus, it is a scientifically groundless assertion that elevated HA bioavailability by BioCell Collagen II® might cause cancer. Rather, current data support potential anti-cancer effects from daily ingestion of BioCell Collagen II®, although BioCell Technology never claims this particular benefit.

Finally, despite convincing scientific safety and efficacy data supporting health benefits for the joint and the skin, BioCell Technology always advise that those who have any disease including cancer should consult their doctors before taking any dietary supplements as well as BioCell Collagen II®.

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