THE EXTRACELLULAR MATRIX AND THE TRANSEPIDERMAL PENETRATION ABILITY OF EXOGENOUSLY SUPPLIED COLLAGEN DEGRADATION PRODUCTS

In multicellular organisms most cells are surrounded by an environment called the extracellular matrix (ECM), also referred to as the 'intercellular matrix'. The ECM is a complex of interconnected molecules composed of simple proteins and polysaccharides. These molecules combine to make up more complicated proteins that align themselves to make up a semi-ordered network in the intercellular space. The network they form is not impermeable, however it allows amino acids and larger peptide subunits to move through it freely. The ECM significantly affects the location, development, proliferation, organization and metabolism of the cells it surrounds, and plays a vital monitoring role during the transformation of these cells into tissue.

The concept of ECM has not been fully adopted in international scientific literature; the more general term "connective tissue" is often preferred. Connective tissue is considered the most important tissue in the body, and is understood to be a combination of the ECM and the cells that are embedded within it and neighboring it.

The strength, elasticity and general condition of connective tissue is determined by several varieties of collagen and elastin. The principal cells of connective tissue are: fibroblasts, osteoblasts, chondrocytes, macrophages and mast cells.

Protein and polysaccharide gel is the most common physical form of the ECM. It helps ensure a steady supply of oxygen and nutrients to the aforementioned cells of connective tissue. It also plays a central role in clearing toxins and metabolic waste products from the perivascular space (the space that surrounds the blood and lymphatic vessels).

The chief function of connective tissue, as the name implies, is to connect the different systems of the body. The systems responsible for immunity include the organs of the lymphatic system, vessels, blood, smooth muscle tissue, meninges and other fluids in the body (with the exception of the fluids we

excrete and the contents of the gastrointestinal tract).

In order to understand key points made in this publication it is essential to understand the common features of the connective tissue subtypes, which appear very different from each other in a broader biological sense. One obvious shared features is the fact that each tissue—be it bone, muscle, nervous, epidermal or connective tissue—is made up of cells.

Only connective tissues have at their disposal the substance we refer to as ECM. Interestingly, the ECM is generally formed by the same cells embedded in this intercellular matrix, and are nourished by amino acids within the same matrix.

For instance, the ECM of a fibroblast located in the dermis at the interface between the epidermis and the dermis can effectively channel protein waste products back into metabolic pathways. These waste products are formed as a byproduct of the fibroblast *shipyard.* The *shipyard* or *slipway* is a complex system that manufactures collagen, elastin, enzymes and proteins.

The ECM surrounding the cells of the skin is capable of much more than just waste disposal. It has been observed to absorb into and penetrate through areas that surround fibroblasts in the dermis—much like the active ingredients that penetrate the epidermal barrier by "smuggling in" special carrier molecules used in designer cosmetic products. Liposomes are an example of one such carrier. The ECM is also a source of amino acids that are released during degradation of biologically active tertiary collagen (which occurs under normal body temperature) directly applied in the form of a hydrate (a protein gel) onto the epidermis.

The experiments were conducted using **Natural Collagen,** a finished dermocosmetic product manufactured in Poland exclusively for **COLWAY. Natural Collagen** is a solution different than thousands of cosmetic gels, tonics, etc. manufactured the world over.

It is a near-pure **tropocollagen hydrate,** giving it unique cosmetic properties. For example, molecular collagen that binds to an aqueous solution (such as water) retains its triple helix conformation despite being outside its donor's

body (a freshwater fish)!

This product is exclusively made of water-binding proteins, an organic acid (<2%), and short chain fatty acids bound with a simple alcohol (<1.5%). Every molecule in the product can be found naturally in the human body. It contains no fragrances, pigments or preservatives of any kind.

Until **Natural Collagen** the concept of using *living* tissue from a vertebrate in the fight against aging was unheard of in cosmetics. While scientists had shown it possible in lab a setting, to harvest enough *living* tissue and keep it alive long enough to aid in wrinkle prevention proved a formidable task both scientifically and financially.

The news that **Natural Collagen** had succeeded in doing so created an opportunity to impact the cosmetics segment on a global scale. Skeptics sited very little scientific research to back up their claim. For these reasons research studies we initiated to investigate the mechanisms of the transdermal penetration ability of the fish-derived collagen hydrate in early 2005.

The ECM plays an important role in the degradation mechanisms of the tropocollagen products applied topically (onto the epidermis). It has been observed that triple collagen has generally degraded into short peptide chains and free amino acids when applied to the skin under normal body temperature conditions. They mainly penetrate the extracellular spaces; initially along deposits then into voids left by keratinocytes that are live but unable to divide. The penetration is highly effective despite some common but erroneous misconceptions. One is that parallel studies have shown the human epidermis to be impermeable to bovine collagen hydrolyzates, along with other protein macromolecules and their derivatives. Triple collagen differs in its versatile triple helix configuration.

The free amino acids and short peptide chains with lengths up to six amino acids also penetrate the skin appendages. Isolated, free amino acids released during degradation of collagen helixes may seek the most difficult pathway: the transcellular pathway.

Natural fish collagen hydrate, in the form of a gel, is absorbed by individual layers of the epidermis within minutes. This process is accelerated if the stratum corneum is weakened leading to situations where the skin craves collagen. Examples include mechanical/laser/enzymatic peeling, over exposure

to sun, and burns from chemicals and artificial lights.

Products of dissimilation of low-order and soluble collagen proteins are formed immediately upon application of the collagen gel to the skin. Under conditions of normal body temperature the molecules of collagen undergo degradation to structures whose size enables them to successfully migrate through the epidermal barrier. The same is not true for bovine collagen products.

Collagen penetrates the layer of the epidermis that is naturally the least permeable: the cornified layer. The cornified layer contains cornified cells, referred to as keratinocytes, which are essentially impermeable to non-gaseous substances, resulting in transepidermal processes occuring mainly outside the cells.

The micro-peptides released during the degradation of the triple- helical and biologically active fish collagen penetrate the layers of the epidermis easily, despite the numerous difficulties found in transdermal penetration using keratin. The skin is a respiratory organ accounting for 5% of gas exchange in the body.The cornified layer, even without being abraded with peeling products, lets in not only gases but substances like arsenic, iodine, hydrogen sulfide, ichthyol, estrogens, allergens, and high molecular weight substances like heavy metal salts.

The epidermis also absorbs water, fats and organic acids, in particular short chain fatty acids. The epidermis enables the lipophilization of degraded collagen peptides and polyunsaturated fatty acids (e.g. omega-3 fatty acids) - which are the natural carriers found in fish proteins, and help to carry them deep into the skin. Contemporary substance cosmetology has spent 80 years of effort trying to overcome the epidermal barrier using substances that are safe for the body and effective at reducing the skin aging process. Eucerin was the first such substance capable of reaching the ECM through the epidermis (Eucerin is the active ingredient in the cream Nivea).

We now know that the collagen hydrolyzates contained in cosmetics and ointments cannot overcome the epidermal barrier, as they are biologically inactive aggregates.

Biologists who notice the degree of manipulation in commercials site the popular term *collagen* refer to the protein hydrolyzates used in cosmetology as

their *peptide carrion,* although it is quite the opposite. Both scientific literature and practice prove that the epidermal barrier is overcome by active substances packaged in carriers (e.g. liposomes), such as vitamins or coenzymes. We already know that this property is also exhibited by the degradation of products of the collagen superhelix: micropeptide chains and free amino acids.

The epidermal layer, on the other hand, will probably never be penetrated by the widespread components of cosmetics: protein macromolecules that include hydrolyzates obtained from fibrous bovine collagen. They simply are too large, their molecular mass is too high. With respect to lower-order proteins, their hydrolysis renders their component parts (micropeptides and amino acids) biologically inactive and therefore "invisible" to the receptors in the skin.

Put another way, it is too late for fibrils/fibers to exhibit transdermal properties. In terms of the evolution of protein biosynthesis, higher-order collagens are simply too large to make it because collagen fibers that are disintegrated even finely in the process of hydrolysis are not biologically active. They therefore do not act on receptors like the molecules found in the body. They do not penetrate the epidermis (e.g. allergens). We have found that only the degradation (dissimilation) products of the tertiary and soluble collagen are effective in feeding the ECM by overcoming epidermal obstacles such as the basement membrane. Their ability to penetrate the epidermis does not differ substantially from that of transdermal peptides, which have been safely manufactured for many years using chemical engineering technologies.

The amino acids from the topically applied collagen reach the basal layer of the epidermis and foment a revolution. They increase the formation of cytokines—lower-order proteins produced by keratinocytes, in the live cells of the basal layer of the epidermis. In our studies, we were particularly interested in investigating the stimulating effect of amino acids released as a result of the degradation of the topically applied collagen helixes on cytokines, such as the fibroblast growth factor (FGF). The positive effect was unquestionable. We observed interactions manifested by overproduction of cytokines (specifically interleukin-6) by fibroblasts.

The most important point here is that the topical application of tropocollagen hydrate increases the activity of the ECM around the fibroblasts, which leads to increased collagen synthesis. The transport mechanisms of the ECM are the same mechanisms that see to it that all the cells receive the

necessary nutrients. They immediately "take care" of every amino acid that struggles through the basement membrane of the epidermis.

This is a mechanism that has never before been reported in scientific literature. Firstly, it confirms the actual transepidermal penetration ability of collagen. Secondly, it proves that topical application of collagen can affect the processes of accelerated collagen turnover in the skin, which translates directly into delayed aging of the skin (including wrinkle formation). Lastly, it provides evidence to support that the ECM is capable of absorption, thus meeting the needs of the cells it surrounds in the form of amino acids and whole peptide chain fragments. This is found in situations where the cells are separated from nutrient sources such as a membrane or a barrier. The epidermis is therefore, tighter than previously thought.

The effectiveness of fibroblast stimulation to increase the systemic collagen formation as a result of exogenous supplementation with amino acids released during dissimilation of fish collagen still requires very meticulous research. The euphoric testimonials of people who use this method, even if they have been obtaining spectacular results for years, is—in our opinion—still not enough for us to announce the discovery of the 'elixir of youth' in this publication. Its aim was to provide purely objective information. Why the elixir of youth? How else would we call a product that makes it possible to non-invasively cause a constant accretion of collagen in the skin?

Obviously we had to face some strong skepticism on the part of our colleagues—medical doctors and biologists. They pointed out to us that in this situation, textbooks should be revised, particularly to include the functions of the ECM. As a reminder, these functions are currently defined as follows:

- formation of support for organs and tissues,
- acting as a universal biological glue
- involvement in the regulation of water and electrolyte balance
- formation of highly specialized tissue structures: bone, teeth, cartilage, tendon, basal membranes and others

Let's also refresh our memory about the components of the ECM. These are:

- the structural protein collagen,
- the structural protein elastin,

- •glycosaminoglycans,
- •proteoglycans,
- •non-collagenous structural proteins (fibronectin, laminin, osteonectin, tenascin etc.).

The ECM components fall into two groups: the amorphous components and the fibrous components. The amorphous components include two groups of substances: glycosaminoglycans and proteoglycans. Both are composed of polysaccharides and proteins. The consistency of our tissues depends on the contribution of the amorphous components. In the case of the intercellular substance of the blood, the plasma, the amorphous components are virtually non-existent. This is why the blood is liquid. In contrast, the intercellular substance of cartilage is characterized by a very high content of glycosaminoglycans and proteoglycans; hence this tissue has the form of a highly compact, hard jelly.

The other group of ECM components, the fibrous components, contains collagen and elastin fibers. They are formed from the triple helixes biosynthesized in fibroblasts. Initially they take the form of fibrils, and then the form of fibers.

Biologists consider this plump form of connective tissue to be the "traditional" connective tissue. The stretchy elastic collagen fibers and other numerous cells are immersed in these amorphous components of the ECM. The most important of these cells are fibroblasts: a "shipyard" that manufactures collagen up to its tertiary form, reticular collagen, tropoelastin and other components of the ECM, even enzymes.