EFFECTS OF LDM®-ULTRASOUND ON THE SKIN. BIOPHYSICAL BASES

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INTRODUCTION

Ultrasound is a sound wave with a frequency of over 20.000 Hz. Sound waves are normally longitudinal waves in which the medium particles oscillate in the direction parallel to the wave propagation. At a frequency of 1 MHz or higher, which is usually used in a physical therapy applications, the particles oscillate with an amplitude of less than 0.02 µm. This value is much smaller than the typical cell size (app. 10 µm), however it is bigger than the cell membrane thickness. This particle movement is relatively slow; their speed is app. 10 cm/s. The particle acceleration can at the same time reach values of 1.000 km/s^2 and more. In such a wave the pressure oscillations are especially high; they can be many times higher than the atmospheric pressure.

The most important parameters of an ultrasound wave are the intensity (sound energy which is irradiated into the body per second and per square centimetre; unit of measurement W/cm^2) and the frequency, which defines inter alia the penetration depth of the ultrasound wave into the body. The state of the art in the aesthetic field are ultrasound waves with frequencies of 1 MHz and 3 MHz with penetration depths (50%) of 3 cm and 1 cm respectively, which will thereby be absorbed not in the skin but much deeper.

There are some well documented effects of ultrasound on the cells and tissues, e.g.

- Stimulation of cell proliferation /1/,
- Stimulation of collagen synthesis /2/,
- Modulation of the membrane structure, its permeability and functions /3/.

The majority of these scientific investigations were made *in vitro*. With dermatologic as well as aesthetic applications it is however important to know, which epidermal and dermal structures can be affected by ultrasound and under what circumstances the mechanical effects of ultrasound will be of special importance.

FIBROCYTS AND FIBROBLASTS

Ultrasound waves of usual therapeutic intensity show no significant morphological alterations in the skin /4/. They can however cause pronounced changes in the cellular structures of the skin.

Fibroblasts and *Fibrocytes* are the most important cells of the connective tissue. Fibroblasts are responsible for the building of the extracellular matrix and are very synthetically active. They synthesise not only the protocollagen molecules but also the collagenase which is responsible for the collagen cleavage. On the other hand, the fibrocytes are responsible for the maintenance of the metabolism and much more present in metabolically inactive connective tissue. The number of fibroblasts and fibrocytes as well as their ratio in the connective tissue characterise the synthetic activity of this tissue.

A short-time application of ultrasound in relatively low intensities does not significantly change the collagen production or the ratio of fibroblasts/fibrocytes in the tissue. A longer treatment can however increase the number of fibroblasts with a simultaneous decrease in the number of fibrocytes /4/. This demonstrates that therapeutic ultrasound can under special conditions stimulate the differentiation of fibrocytes into fibroblasts. This differentiation is important for the skin regeneration and wound healing and can inter alia also be responsible for the skin tightening after ultrasound treatment.

Since the ultrasound doses needed for this effect are relatively low, it can be deduced that from the biophysical point of view the mechanical stimulation but not the thermal effects are of prime importance for such a transformation. An amplification of the mechanical effects in an ultrasound wave through application of the new LDM®-Technology can be of essential advantage compared to the treatment with conventional ultrasound.

For such stimulation the frequency of applied ultrasound is also of fundamental importance. For example, it is known, that the special fibroblast stimulation factor produced by macrophages (which among other things also control the number and the activity of fibroblasts in the tissue) can be better stimulated with an ultrasound frequency of 3 MHz. At the same time the release of this factor from the cells is better under the application of ultrasound with a frequency of 1 MHz /5/. Only the combination of different frequencies can optimally regulate both processes.

REPLICATIVE SENESCENCE

It is well known that *postmitotic* (the cells which cannot divide) and *mitotic* cells (which can divide) maturate differently. The mitotic cells (the most important examples in the skin are the keratinocytes and the fibroblasts) can show an important effect named *replicative senescence*.

The potential number of cell divisions of differentiated cells is limited; the later produced postmitotic cells will be accumulated in the skin and they can be essentially responsible for the maturation of the skin structure by aging. These cells also produce inter alia special enzymes and accumulate lipofuscin – the age pigment. The capacity of senescent fibroblasts to produce and contract the collagen gels is very restricted.

Such an accumulation of the postmitotic cells in the skin can be reached not only through a chronological skin aging but also through the regular stimulation of the skin regeneration. The differentiation of the new fibroblasts from the fibrocytes under ultrasound treatment can help to reduce the negative effect of senescent cells on the skin. This opens the possibility to improve the skin characteristics according to the Skin Rejuvenation procedure.

CROSS-LINKS IN THE CONNECTIVE TISSUE

Collagen is one of the most important components of the connective tissue. Different tissue types contain different collagen molecules und have thereby different structures. A quantitatively und qualitatively correct assembling of these structures is responsible for the structural and functional tissue integrity. The formation of adequate cross-links between the collagen molecules is of primary importance for the mechanical stability of the connective tissue. These cross-links in the hierarchical collagen structure can however be built in different ways. According to the number of connected collagen molecules, there are bi- and tri-functional connections. It will be differentiated between the *skin-type* (predominantly formed by so called lysine residuals) and *skeletal-type* (predominantly formed by so called hydroxy-lysine residuals) cross-links in a collagen structure.

In a healthy skin normally only 10% of the cross-links are of the hydroxyl-lysine type. This percentage can however dramatically increase by different skin alterations as e.g. scleroderma, scar formation, dermatoliposclerosis, etc. Typical for these diseases is the excessive deposition of collagen in the skin (or some other organs) with a consequent tightening of the tissue. In the healthy skin there is only a small amount of bi-functional (immature) cross-links; the majority are the mature tri-functional lysine-type cross-links /6/.

The type I collagen molecule is made up of three polypeptide chains, which are connected through *intramolecular* cross-links. In a healthy skin these are weak and reducible covalent aldehyde bonds; in the aged skin the latter will be replaced through multivalent irreducible cross-links. Five such collagen molecules aggregate into a parallel pattern to form microfibrills, which can further aggregate into fibrills and then into collagen fibers. These fine hierarchical collagen structures with their cross-links between different stages of the tissue organisation are responsible for the skin reactions to mechanical, thermal and chemical factors.

Unstable intramolecular cross-links can be reduced through the application of heat or through mechanical pressure. In the course of this the highly organised structures will be converted into gel-like and therewith less regular structures. This can cause the "shrinkage" of the collagen along its main axis with a simultaneous swelling on the transverse axis.

This structural collagen change microscopically looks like a "skin tightening" effect. Its effectiveness depends on many different factors, e.g. type of the tissue, patient age, pH-value and the moisture in the tissue, mechanical forces, etc. Especially important are the mode of the heat as well as the art of the mechanical forces application /7/.

With the LDM®-Technology additional mechanical forces arise, which can easily shake and break the cross-links between the collagen molecules. Consequently, the LDM®-Technology can demonstrate stronger tightening effects compared to conventional ultrasound applications. The application of LDM® in combination with an ultrasound frequency of 10 MHz leads to a better concentration of these forces in the skin area.

EXTRACELLULAR MATRIX

The extracellular matrix is of big importance for the connective tissue; it is not only responsible for the functioning but also for its structural stability. The most important components of this matrix are the glycosaminoglycanes (GAG). GAGs can bind water and thereby form gels with a high turgor, which increases the extracellular spaces and so enables better transport of metabolites. This ground substance is not firm and can thereby quickly react to environmental changes through additional storage or release of water.

The ultrasound with its strong and rapid pressure oscillations causes a dramatic reduction of the gel viscosity through destruction of cross-links in the connective tissue. Such a gel-sol transformation is known as thixotropy. These gel-structures will be built back after some rest period. This property of the gel is often used in technique, e.g. with paints: the paints have to be fluid on painting and have to harden immediately after the pressure on the brush is withdrawn. Similar effects can also be seen for the gelatine after heating. Gelatine is fluid at higher temperatures and is consequently in a sol-phase; after some cooling time the gel-phase will be built back.

During chronological aging and UV skin damaging the concentration of GAGs and their distribution in the matrix are strongly changed. The most important feature of the redistribution is as follows: the GAGs are not situated between the collagen and the elastic fibers as it is the case in the normal skin, but rather directly on the elastic fibers' surface in the superficial layers of the dermis. Consequently much less water can be bound in the tissue. The extracellular spaces remain small, the internal pressure in the tissue, which is responsible for the fresh skin look, is decreased and the metabolism, which proceeds there, can be reduced appreciably.

A strong mechanical shaking of the tissue through LDM® is of advantage for the recovery of the normal matrix