LASER BIOSTIMULATION IN PLASTIC SURGERY

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In the past 30 years the interest in problems of wound-healing has dramatically increased. Partly this is due to the fact that clinicians would like to understand the nature of the disease they treat. But also this is because the experimental model of wound repair gives the opportunity to study the involved cell system in a better way. My own research on wound healing concerning laser biostimulation has been confined to epithelial surfaces of the body. I would like to restrict my remarks largely to results obtained from investigating repair of the skin.

KEY WORDS Laser-biostimulation Plastic surgery

Introduction

Wound healing is a complex, dynamic procedure. Normal wound repair is characterized by exudative, resorptive, proliferative and reparative processes. For a multiplicity of reasons wound healing may be considerably delayed. After initial findings of our good friend, the late Professor Endre Mester in Budapest with HeNe gas laser wound healing,1 we tried this technique with low incident doses of argon laser, initially on wounds on the dorsum of rats and found that the healing was quicker and that there was practically no infection. Then we treated badly-healing wounds, ulcers and atrophic skin of different aetiology with LLLT using the argon laser with excellent results. The same is true for vitiligo and nerve injury, postherpetic neuralgia, and herpes itself. We found an increasing amount of amino acids, hydroxyproline and L-lymphocytes and heparin-producing mast cells. The histology shows large groups of newly formed cells and active fibroblasts; after some days new vessels are formed and the fibroblasts are producing collagen fibres.

In general, a wound is present in skin when there is a lesion of epithelium, basement membrane, and adjacent connective tissue. Depending on the agent causing the wound and the manner in which it is inflicted, the wound may take the form of an incisional space or a cavity, both of which are lined by tissue undergoing necrosis.

As part of the complex process of wound healing, at different times after wounding different cells are encountered and collagen fibre formation begins. It is only recently that the dynamic nature of soft connective tissue fibre systems, both from biochemical and morphological points of view, has become evident.

Over the years a plethora of terms have been advanced to describe the phases of wound healing. These terms have been derived from different experimental models and by investigators employing a variety of histological and biochemical techniques to assess the healing process. In this paper I will use the generally accepted descriptive terminology of the exudative, resorptive, proliferative, and reparative processes of normal wound healing.

The wound healing process

Immediately following wound formation, an initial unstable wound cover is obtained in which the blood which has filled the lesion eventually clots. The fibrin clot, including entrapped blood cells, forms a scab and provides a preliminary seal. Within the first 24 h after wounding, invading leukocytes and phagocytes signal the initiation of an acute inflammatory response in the margins of the lesions. These cells resorb and digest necrotic tissue and most of the clot.

At the same time mitotic activity in the basal cells of the epidermis is observed. This process will eventually reestablish a continuity of the epidermal layer. After approximately 48 h, the fundamental processes of wound repair and regeneration begin with the formation of granulation tissue. One of the most prominent features of this phase of the healing process is the progressive proliferation of capillary sprout cells, angioblasts and endothelial cells, forming a network of new capillaries in the wound bed. It is these that give the healthy granulation tissue its pink colour. These cells are
apparently the first cells of the healing wound which have the potential to synthesize collagen. Indeed, at this time there is a small but clearly demonstrable reaction of the granulation tissue with antibodies to Type III collagen.\(^2\)

Subsequently the granulation tissue becomes highly vascularized at which time one observes an increase in the reactivity of the tissue with antibodies to Type III collagen. Once capillaries within the granulation tissue have joined to create a continuity of the vasculature throughout the tissue, there occurs a proliferation of fibroblasts. The proliferation of these cells corresponds to the time at which rather large cross-striated collagen fibres become apparent. Immunohistochemical studies indicated that these fibres are comprised largely of Type I collagen molecules. As fibroblasts proliferate and synthesis of collagen proceeds, the resulting fibres occupy an even greater proportion of the lesion volume and compress the thin-walled capillaries leading to the formation of blanched scar tissue which exhibits a considerable degree of tensile strength. The final scar, covered by restored epidermis, appears largely acellular, avascular, and rich in collagen-fibres derived from Type I molecules.

It has been known for several years that healing skin wounds contract during the repair process. Since it is apparent that the abundant collagen fibres of the healing wound cannot of themselves provide the required contractile forces, it is possible that these forces are derived from the proliferating fibroblasts which may be termed myofibroblasts.\(^3\)\(^-\)\(^4\) Even though scar formation in the skin is a remarkable biological process, tissue repair is achieved without complete regeneration of the dermal structures. For instance, the scar tissue does not contain the same quantity of hair follicles, glands and melanocytes.

**Collagen and Collagen Formation**

Collagen formation is the most important event in wound repair and remodeling. Collagen is a triple-helix molecule synthesized from amino acids. It is now well-established that collagen is produced initially as a precursor molecule known as procollagen. The synthetic ribosomes of the endoplasmic reticulum activated amino acids are assembled into polypeptide alpha chains, each composed of about 1000 amino acids.

Hydroxylation of the prolyl and lysyl residues takes place while the nascent alpha chains are being synthesized. While the alpha chains are still associated with the ribosomes or immediately after their release into the lumen of the endoplasmic reticulum, they associate in helical configuration to form procollagen molecules. The procollagen molecules appear to be transported through the lumen of the endoplasmic reticulum, and attachment of carbohydrate is believed to take place in the Golgi complex. Molecular collagen segregated in the Golgi vacuoles is released from the fibroblasts. These vacuoles move to the cell surface and discharge their contents into the surrounding ground substance.

Following secretion of the newly synthesized procollagen molecules into the extracellular spaces, the first known event in extracellular processing occurs. This is the conversion of procollagen to collagen which involves cleavage of peptide bonds in all three pro alpha chains at both the NH\(_2\) and COOH\(^-\) terminal regions of the procollagen molecules. The next major event is fibre formation. Electron microscope as well as X-ray diffraction studies on collagen fibres indicate the molecules are incorporated initially into limiting microfibrillar units which are packed together to form larger fibrils or fibres. Current evidence also strongly suggests that both ionic and hydrophobic interactions are important determinants in specifying the spatial relationship and axial displacement between neighboring molecules in the limiting microfibrillar units.

Under physiological conditions collagen synthesis and degradation are in a state of equilibrium. Every injury leading to death of tissue, including wounding, interrupts this balance. The organism responds to such a defect by forming a scar rich in collagen. This process of collagen accumulation can be caused either by an increase in the number of collagen-synthesizing cells or by an increase of collagen production of a single cell. On the other hand, there are indications that a change in collagen degradation could also regulate this process. For a variety of reasons would healing may be considerably delayed. Several factors such as haematologic disorders, vascular disorders, metabolic disorders, changes of the ground substance or immobilization of fibroblasts, modify the reparative process.

**Laser Therapy of Wounds**

Based on findings by Mester and co-workers\(^1\) on slowly healing ulcers irradiated with HeNe and ruby lasers, and on our own experimental work, we have now used the argon laser to stimulate wound healing in badly healing wounds and ulcers in 33 cases.\(^5\)\(^-\)\(^8\)

Documentation of the stimulating effect of this kind of treatment was carried out by objective measurement of the wound size, photographs, biochemical analysis of specimens of the granulation tissue as well as hydroxyproline analysis in urine and morphological assessments by examination of specimens by light and electron microscopy.

After three argon LLLT treatment sessions, epithelization in about half the area of the ulceration was observed. In badly healing wounds after plastic surgery the wounds were closed after four to
six treatments with excellent aesthetic results and physical properties of the new skin (Figures 1a,b).

**Histology after Laser Therapy**

After laser treatment the newly formed tissue consists of different cells dispersed in an abundant intercellular matrix. The cells were represented by a small number of erythrocytes, leukocytes (often grouped in small clots), fibroblasts, macrophages and single-layered mast cells, which play an important role in wound healing because of histamine production (Figure 2a). Near the margin of the wound the cells are densely packed, a few epithelial-like cells are found and newly formed capillaries can be seen (Figure 2b).

The ultrastructural investigations also show different cells from the margin to the middle of the wound. Most of these cells are spindle-shaped fibroblasts, sometimes in a parallel arrangement, leukocytes, and macrophages. Intermingled with them there are relatively few mast cells. The fibroblasts show a large nucleus with a slightly indented or wavy contour and a well-developed rough endoplasmic reticulum consisting of numerous, mostly narrow, interconnected sacks. Furthermore, the cytoplasm contains numerous mitochondria, occasionally dense bodies and lipid droplets. In fibroblasts near the margin of the wound the ergastoplasm and the Golgi apparatus are much more prominent than in fibroblasts from the centre of the wound. In addition bundles of thin filaments are often visible in the cytoplasm. These bundles are usually located in the cortical areas of the cytoplasm (Figure 2c).

The macrophages are irregular in shape and show a large nucleus, a scant rough endoplasmic reticulum, dense bodies of variable size scattered throughout the cytoplasm, and finger-like projections. In electron photomicrographs, mast cells are seen to have small surface folds or villous projections.

The Golgi complex is well-developed; the endoplasmic reticulum is sparse and the mitochondria are relatively few. The granules are limited by a membrane and display considerable variability in their fine structure.

The formed elements of granulation tissue are embedded in a matrix of ground substance having the properties of viscous solution or thin gel. In the samples of our material the intercellular matrix consists of a fine granular ground substance, in which fibrin aggregates are dispersed. Embedded in this intercellular ground substance we find collagen fibrils randomly arranged in small bundles. Most of the fibrils range in size from 20 to 40 nm. In addition, bundles of thin filaments are often visible in all areas of the wound. Near the wound margin large collagen fibres, 80 to 120 nm in diameter are detected.

**Discussion**

Collagen deposition in normal granulation tissue, as a result of increased collagen synthesis, has been described by many authors before. In agreement with these authors, results from our own laboratory show that collagen synthesis occurs on the third day after wounding and hydroxyproline concentration increases continually. Morphologically, many fibroblasts are observed at this early stage of wound healing. In badly healing wounds these stages are delayed or disturbed.

The recent experiments involving slow-to-heal wounds and their therapy suggests the following preliminary explanation of action mechanism of argon laser beams in LLLT:

1. Bacteriostasis by a thermic or biologic effect or by means of phagocytosis through the increase in macrophages.
Figure 2. (a) The newly formed tissue after laser treatment consists of different cells; from the top to the bottom: a few erythrocytes, leukocytes grouped in small groups, fibroblasts, macrophages and single layered mast cells; near the margin of the wound the cells are densely packed. Furthermore a few epithelial-like cells and newly formed capillaries can be seen.
(b) Newly formed capillaries after laser treatment.
(c) Bundles of thin filaments of collagen visible in the cytoplasm.
(2) Proliferation of fibroblasts followed by an increase in collagen-synthesis.

(3) Increase in number of mast cells, giant cells and leukocytes which are important cells for the granulating phase of wound healing.

(4) Due to the phagocytic capacity of macrophages the pathway for the new vessels is cleared quicker and this leads to enhancement of newly forming capillaries and collagen fibrils.

(5) Furthermore, we believe that epithelial cell mitosis is enhanced by argon LLLT leading to an early firm wound closure with excellent aesthetic results and physical properties of the new skin.

Conclusions

Photostimulation, perhaps better referred to as photobioactivation could be proved to be effective in badly healing wounds by clinical, histological and EM findings. We hope to show in the future a dose and type of laser-reflected effectiveness of LLLT.

References