International Journal of Sports Medicine

Supported by the German Society of Sports Medicine

Present State of Knowledge on Processes of Healing in Collagen Structures

Prof. Dr. J.C.H. van der Meulen

fibers that is what you expect in a marathon runner but in the operated leg he had 80% fast twitch fibers, that is what you would expect in a sprinter or a weight lifter for instance. This of course is catastrophical for a top athlete as you can easily realize. We have, however, seen in a good cross country skier that when he was admitted for a serious knee injury he had over 80% slow twitch fibers in his operated leg but one month later the percentage of slow twitch fibers had dropped to 57% but after hard training it finally came up to 84% six months later. We have therefore started with an intensive rehabilitation of our athletes after injuries and after operations. We are providing them complete pain relief after surgery by the means of a continuous epidural analgesia with diluted local anesthetic solutions. We institute physical therapy already 2 hours after surgery or as soon as the patient is pain-free after an injury. We have abandoned the old closed cast and are only using partially movable cast braces in the postoperative period and with this very vigorous treatment we have been able to prevent some of the negative effects of trauma and surgery on the muscles of our athletes. How soon can they then return to sport. Well, the cross-country skier I mentioned a minute ago was operated with both a medial and lateral arthrotomy for a serious knee injury. He was given this vigorous rehabilitation. He was given a mobile cast brace one week after surgery and started training cross country skiing two weeks after surgery. At six weeks after surgery he challenged me to one kilometer of cross country skiing and beat me badly which of course does not prove very much. Two months and two weeks after this operation, however, he finished second in an international cross country competition. Three months and two weeks after surgery he won an international competition in Austria. Some people may think it is dangerous or harmful to institute such an early treatment or that torn ligaments may not heal normally. A lot of research has shown movements enhance healing of ligament so that they really get stronger from early motion if it can take place under protected conditions. The actual cross country skier was followed up in an interesting way two years after surgery. He participated in the Olympic Games in Lake Placid last year and he won an Olympic gold medal on 15 km of cross-country skiing. He immediately then re-turned to Scandinavia and won the Holmenkolmen-race and then went back to Sweden and won all the four different distances in our Swedish championship of cross-country skiing.

I am expecting a glorious future for sports medicine. Already today sports medicine has improved the quality of general medical care. In my own specialty, orthopedic surgery for instance, the final goal of orthopedic treatment has always been to make it possible for the injured person to return to his work. For the athletes that is not enough. The athletes have much higher demands than the ordinary persons have. They want to be able to run equally fast or faster or to jump equally high or higher than they did before their injury. This has forced us to develop new and better surgical techniques. This has forced us to change and to improve our methods of rehabilitations and one can therefore say that thanks to these high demands which the athletes pose upon us sports medicine doctors, that the general patient is going to gain a lot from it because he will also get this improved new treatment. Likewise the research in sports physiology on how to train also is of benefit for clinical exercise testing and physical rehabilitation. The studies on nutrition for athletes have helped to find out what a hard working laborer should eat and drink while he is working. I foresee great developments in these different fields of sports medicine. Developments that certainly will benefit whole the field of medicine.

One further important field of sports medicine that I have not mentioned yet is to analyse accidents carefully and thereby try to find out measures to prevent new injuries. After all, prophylaxis is always the best treatment.

On behalf of the World Sports Medicine Federation (FIMS) I would like to welcome you all to this conference on the muscle in sports. I hope you will enjoy these days and that you will learn and that you will get an opportunity to meet the different researchers, to talk with them, to give them impulses, because that is after all what these international conferences are about. I would also like to welcome you in Vienna in June next year where the World Sports Medicine Federation arranges the 22nd World Sports Medicine Congress.

Dr. E. Eriksson, Section of Trauma, Dpt of Surgery, Karolinska Hospital, S–10401 Stockholm

Present State of Knowledge on Processes of Healing in Collagen Structures

Prof. Dr. J.C.H. van der Meulen

Introduction

The greatly increasing number of sport injuries among both youth and older people and the diversity of these injuries necessitates an individual treatment of the patient and his or her injury. In order to be able to give this individual treatment, however, it is necessary to have a basic knowledge concerning the healing process of a wound. We can all indicate the exact moment when this process begins, namely at the time of trauma. However, it is difficult to say when recovery is complete. This contribution, in which the various phases of the healing process of wounds are described, makes it clear why this is so.

1. Reaction phase

The reactions of the body to a wound are the same as those occurring with inflammation, the symptoms being rubor, calor, tumor and dolor. The redness and warmth are caused by dilatation of the vessels, the swelling by exudate, and the pain by a number of factors, including pressure on nerves, action of chemical products (histamine, serotonin and bradykinin), and ischaemia.

Following trauma, this classical syndrome is preceded by a short period (of approximately 10 minutes) of vasoconstriction. This is when the intrinsic and extrinsic coagulation mechanisms are brought into action, the primary purpose of which is to close off the damaged vessels and then to activate the inflammatory mechanism by means of chemical signals. The vessels in the immediate neighbourhood of the wound, particularly the venules, dilate and the capillary permeability is increased. Exudation of plasma occurs, accompanied by haemoconcentrations; the blood flow slows down, and there is margination of leukocytes. The leukocytes adhere to the wall and then migrate between the endothelial cells, so that within a few hours already the damaged area is saturated with granulocytes and macrophages, which are then guided by chemotaxis to the point where they are most needed. They are used in the clearing away of dead tissue (blood platelets and cells), and of foreign particles and bacteria, both by ingestion (phagocytes) and digestion (lysosomes).

What are the chemical mediators which set the stage for enacting the complicated scenario of the processes of vascular dilatation and chemotaxis, and how are they activated? It appears that not only are humoral factors at work in the effector systems, but also cellular factors, involving the action of the products of a number of cells.

Humoral factors

The effector system (Fig. 1) at present known to be involved in the healing process are all characterized by the "cascade effect": each successive factor is activated by its predecessor. Four systems are important for an understanding of the heal-ing process: the kinin system, the fibrinolytic system, the intrinsic coagulation system, and the complement system.

The kinin system, the fibrinolytic system and the intrinsic coagulation system have in common the fact that they are activated by the Hageman factor (XII). This factor is in turn activated by contact with a negatively charged surface (such as collagen), the rough intima of a vessel, or a foreign body (such as glass). The complement system is activated by antigen-antibody interactions.

Kinin system: In the kinin system, kallikreinogen (a non-active proteolytic enzyme) is first converted into kallikrein. This enzyme releases bradykinin from globulin, a kininogen. Release of bradykinin is accompanied by vasodilatation and an increase of capillary permeability.

Intrinsic coagulation system: Here, three important phases can be differentiated: an initial phase, in which thrombin plastin is formed, a second phase, in which prothrombin is converted into thrombin under the influence of thromboplastin and Ca^{++}; and a third phase, in which the trombin converts soluble fibrinogen into insoluble fibrin. Once it has been formed, fibrin appears to eliminate thrombin and thus to prevent the formation of further superfluous coagulation.

Fibrinolytic system: In this system, breakdown of fibrin occurs under the influence of plasmin, which is activated by plasmogen. The resulting peptides also cause an increase in capillary permeability and chemotaxis.

Complement system: Of the nine components of the complement system (C_1–C_9), C_2 or C-kinin, and C_{3a} and C_{5a} (two anaphylatoxins) are important. C-kinin has a direct vasodilator effect; C_{3a} and C_{5a} have an indirect vasodilator effect via the mast cells, and also cause chemotaxis.

Cellular factors

Important mediators in the healing process (Fig. 1) are produced by the following cells:
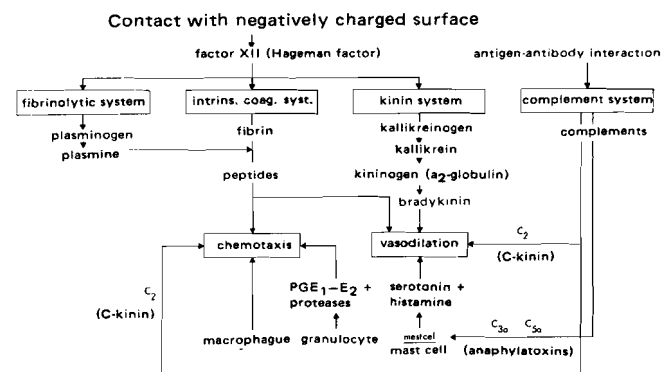

Fig. 1

Mast cells: Histamine and serotonin are formed: the granules of these cells. Both these products have important vasodilator effects. They are released into the circulation by degranulation a process which is aided by the complement components C_{3a} and C_{5a}.

Granulocytes: The prostaglandins E_1 and E_2 are formed particularly in these cells, and these two substances appear to have vasodilator and chemotactic properties. Proteases are also activated in the lysosomes found in these cells, and they in turn also have a function to fulfil when the cell membrane is lysed.

Macrophages: One of the most important functions of these monocytes, and one to which more attention will be paid when we discuss wound repair, appears to be the production of chemotactic substances whose purpose is to attract other macrophages.

2. Regeneration phase

Elimination of debris

Deprivation of oxygen leads to cell death. This statement illustrates in a simple fashion the essence of trauma. Tissues are injured and the blood vessels which supply them are torn. The function of any cells which are still viable is threatened. Before the body can repair the damage by regeneration of tissue, further damage as a result of infection must be prevented, and the debris must be cleared away. This is achieved by the granulocytes and the macrophages, which have arrived at the site in the meantime.

There are 20 to 50 billion granulocytes present in the circulation, and an even greater number held in reserve in the bone marrow. They offer protection against bacterial invasion by means of phagocytosis (Fig. 2).

Phagocytosis is, however, possible only when op onification of these bacteria has occurred, opsonification being the process by which a cell is rendered suitable for phagocytosis by

opsonin, an antibody in the plasma. Protective antibodies are found in the wound exudate as a result of previous contact with these micro-organisms. These combine with the surface antigen to form the antigen-antibody complex, which activates the complement system. The cascade of enzymatic reactions which now follows, leads finally to membranolysis of the bacteria which are thus prepared for phagocytosis.

After the bacteria have been ingested and the walls of the granulocyte have formed a phagosome, this phagosome fuses with the lysosome already present, which contains a large number of hydrolytic enzymes such as lysozymes and lipases, proteases and nucleases. These enzymes thus end up in the phagolysosomes formed by the fusion (degranulation), and destroy the bacteria. In addition to these lysosomal enzymes, granulocytes also produce the enzyme collagenase, which is capable of breaking down collagen.

The granulocyte is the predominant cell in the wound for the first few days following trauma, but from the fifth day the macrophage dominates. These cells are large and mobile and, like granulocytes, are capable of surviving in a poorly nourished area. They ingest macromolecules, converting them to amino acids and sugars. They function as the digestive tract of the wound and hold a key position in the healing process in that they remove the damaged tissue by means of phagocytosis and the production of lysosomal enzymes. In addition, they produce substances which attract other macrophages to the wound by chemotaxis, and activate the formation of endothelial cells and fibroblasts. Their number is increased by the administration of vitamin A, but corticosteroids have an inhibiting action.

Both granulocytes and macrophages can survive in the wound by virtue of their anaerobic metabolism. Both produce collagenase, and both are activated by the ingestion of bacteria and other substances to produce lactic acid. Repair of the tissues is only possible when the wound has become clean, and then only to a limited extent, without complete restoration to the original state.

In man — unlike the salamander, which can regrow an amputated limb — the capacity for regeneration is limited to a few cells, which comprise the endothelial cells, the fibroblasts and the epithelial cells.

Regeneration of endothelial cells

Oxygen deprivation in a wounded area can only be relieved by neovascularization. Capillary buds form in the walls of functioning vessels surrounding a wound, stimulated by hypoxia and activated by macrophages. These buds grow and combine with other similar offshoots to form a capillary loop. In principle, this neovascularization can occur in any of three ways: by the bridging of a large tissue defect; by forming a connection to a previously untapped circulation, as occurs in transplantation; and by forming a connection with other functioning vessels by apposition in a primarily closed wound.

Initially, the basal membrane is still incomplete in these new vessels, and leakage occurs. Only later, when the new vascular network has been strengthened with collagen gel by the fibroblasts, is it able to withstand increasing pressure.
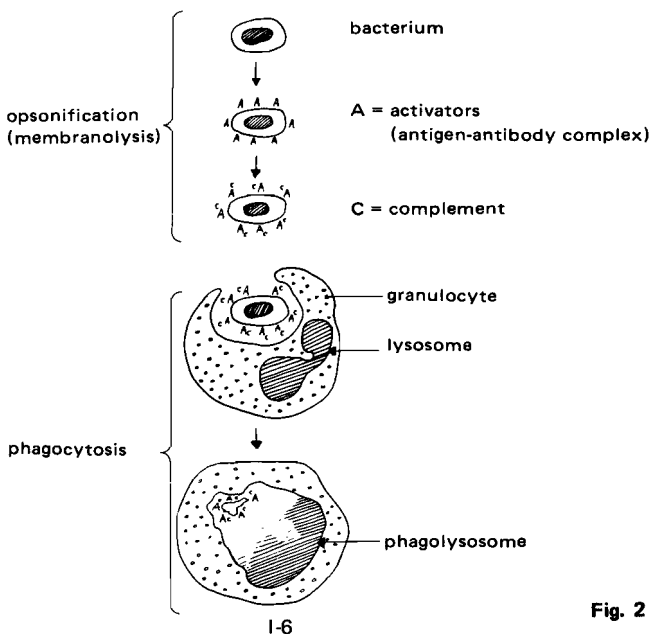

opsonification (membranolysis)

bacterium

A = activators (antigen-antibody complex)

C = complement

phagocytosis

granulocyte

lysosome

phagolysosome

I-6

Fig. 2

Regeneration of fibroblasts

Fibroblasts are formed in perivascular cells. They are mobile at the site of the wound, and migrate along the fibrin threads and other fibres. The migrating fibroblast is directly followed by newly formed capillaries which possess a plasminogen activator. The enzyme plasmin thus formed then breaks down the fibrin network via the fibrinolytic system. The fibroblast begins production of collagen and ground substance within a few days of the trauma under certain circumstances.

Collagen synthesis is stimulated by lactic acid and vitamin C. Lactic acid is formed by granulocytes and macrophages. Vitamin C in its reduced form is only found in a hypoxic environment. The two prerequisites, hypoxia and acidosis, are both satisfied in the wound area. However, oxygen is necessary for the production of collagen, since only part of the collagen molecule can be made in its absence. The solution to this problem appears to be relatively simple. Like the macrophage and the epithelial cell, the fibroblast is a facultative anaerobe. This means that any excess of oxygen available is used by the cell. The PO_2 in the region of the fibroblast therefore remains low and can thus stimulate collagen synthesis unaltered.

Interactions between macrophages, fibroblasts and endothelial cells can be illustrated using a "wound module", which illustrates the succession of events in the wound edge (Fig. 3). The macrophages, which clear the way, lie to the fore of the module. They probably give the signal to the fibroblast to follow, and feed this cell with the amino acids released by the breakdown of the macromolecules. The young fibroblasts cannot begin the formation of collagen until the macrophages have produced sufficient lactic acid, and until oxygen, amino acids, glucose, vitamins and metals such as zinc, copper and iron, can be delivered by a new vascular system. The fibroblasts are therefore followed in turn by capillaries, the most distally functioning of which lie at a distance of 50—75 m from the wound edge.
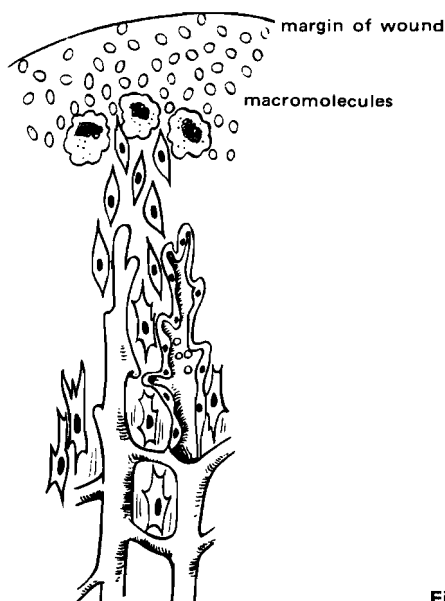
When the walls of the capillaries in this granulation tissue can withstand the arterial pressure and thus become more functional, and when two capillary loops with different pressure systems join up, more oxygen can be delivered from the system with the higher pressure. The front-line fibroblast, which is deprived of oxygen, then reacts by producing more collagen, which forms a matrix allowing the macrophage to advance further. The young fibroblasts then follow in their turn as far as the supply line will allow. The others remain behind to manufacture firm connective tissue.

The synthesis of collagen begins with the lining up of a number of amino acids according to the code stored in the deoxyribonucleic acid (DNA). Messenger RNA provides the correct sequence, and transfer ribonucleic acid (tRNA) transports the various amino acids to their place in the α-chain which occurs on the ribosome (Fig. 4). When assembly is complete, the ration of amino acids is as follows: one-third glycine, one-third X-Y-Z.

Hydroxyproline and hydroxylysine are still absent. These substances are unique because they occur nowhere else in the body; they are made on the ribosome by hydroxylation of proline and lysine by the enzymes proline hydroxylase and lysyl hydroxylase (Fig. 5). These enzymes are active in the supply of oxygen and of cofactors, such as vitamin C, α-ketoglutarate and iron.

The next step in the synthesis of collagen (see also Fig. 4) is the assembly of three α-chains (two similar α_1-chains and one different α_2-chain). Each α-chain represents a right-hand helix. Together, the three α-chains form a left-hand superhelix. The rather shorter tropocollagen is formed next from this procollagen molecule after glycosylation in the Golgi apparatus, and after separation of the terminal registration peptides.

This molecule, which is 3,000 A long and 15 A wide, is excreted into the extracellular space where aggregation occurs. The molecules range themselves in a sequence whose characteristic is that one molecule overlaps the next by a quarter of its length (quarter staggering). As yet, these microfibrils are not strong. The chains in the molecule and the molecules themselves are held together by weak hydrogen bonds, and disaggregation occurs in a saline solution. This situation changes within a few days. The solu-

margin of wound

macromolecules

Fig. 3

Synthesis of ground substance in Golgi's apparatus under addition of protein

8. aggregation of collagen fibres to collagen vessels in bundles of vessels

7. aggregation of tropocollagen to collagen fibres

6. tropocollagen passage in extracellular direction

5. glycolisation

1. amino acide including proline and lysine

2. assembly of polypeptide chains

3. hydroxylation of proline and lysine

4. assembly of three hydroxylated polypeptide chains to a tropocollagen molecule
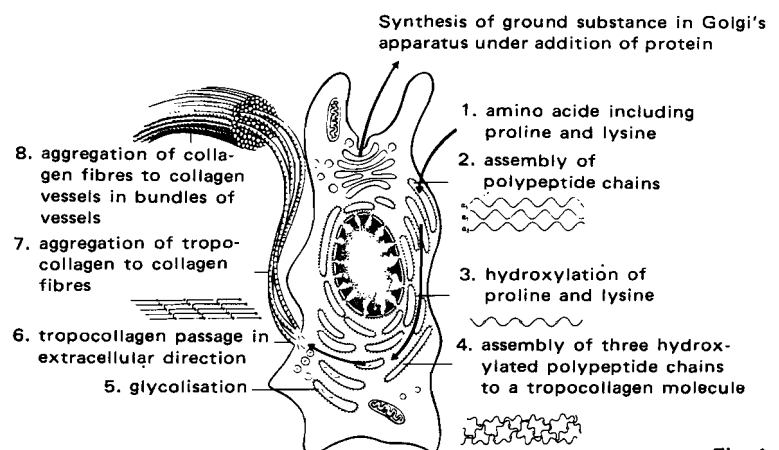
Fig. 4

bility of the collagen decreases, while the stability of the intra- and intermolecular bonds, and thus the strength of the fibres, increases.

As far as is known, the process is as follows: Normal connective tissue contains aldehydes, which are formed by oxidative deamination of lysine (deamination being the splitting off of the amino group NH_2 from the organic bond). The deamination process is catalyzed by the enzyme lysyl amino-oxidase (LAO), an enzyme which only acts in the presence of copper ions. Aldehydes which react with each other form an intramolecular bond. Intermolecular bonds are formed by the reaction of aldehydes with other amino groups. The larger the number of cross-bonds, the stronger the collagen fibres.

Collagen lysis

Collagen is lysed by the enzyme collagenase, which is present in the wound from the start, being brought there by the granulocytes and macrophages. It is capable of breaking down the tropocollagen molecule in the triple helix, unlike the proteolytic enzymes, which only break down collagen when it has been denatured and broken down into α-chains. Local collagenolytic activity can be found up to approximately 7 mm from the wound edge.

Because lysis is a destructive process, less energy is used than in the synthesis of collagen. In extreme oxygen deprivation or extreme deficiency of protein or vitamins, lysis continues whereas synthesis stops.

3. Remodelling phase

This phase, which partially overlaps the regeneration phase, is characterized by reduction in size of the wound surface, an increase in the strength of the scar, and alteration of the fiber structures.

As soon as regeneration of granulation tissue has occurred, contraction takes place, a process which continues for as long as the elasticity of the surrounding fibres allows. There has for years been uncertainty as to the cause of this phenomenon, but recent investigations have shown that fibroblasts are responsible. These cells appear capable of transformation to myofibroblasts, which can form intercellular bonds by means of desmosomes, and furthermore are able to contract due to the presence of contractile proteins (actomyosin). In fact, these cells, which are involved in many processes concerned with contraction of connective tissue, behave like smooth-muscle fibres.

During the first three weeks following the injury, the quantity of connective tissue in the wound and the strength of the fibres increase considerably. At the end of this period, the quantity of collagen stabilizes at a given level. The strength of the fibres, on the other hand, continues to increase for several months. There is thus no relation between the quantity and strength of the collagen. The increase in strength can be explained by a further increase in the number of cross-bonds, and the replacement of old molecules by new ones in another pattern.

This is made possible by a continuous turnover of collagen although the quantity remains constant: new fibres are formed and old ones broken down. The rate of this turn-

over in the first three months definitely appears to be related to the increase in strength of the scar.

Four months after an injury, the turnover of collagen is still considerable. However, it is not only the rate of turnover which determines the nature of the scar but also the level at which the balance is struck between synthesis and lysis. When synthesis of collagen predominates, solid hypertrophic scar tissues is formed, while a loose scar tissue results when the reverse is true.

For the nature of the product formed we are mainly dependent on a number of factors which influence the turnover process in such a way as to produce differentiation in the scar, varying from loose and areolar to stiff and polarized. These are: the age of the patient; the type of tissue; the quantity of scar tissue; the age of the scar tissue; the direction of the scar; the site of the scar; the pressure on the scar; and the traction.

Discussion

Sport injuries generally are not serious. Damage to tissues is usually slight and the healing capacity great. In spite of this, the healing process often takes longer than is desirable. The patient's desire to take up his sport again at the highest possible level as quickly as possible conflicts with the nature of this healing process which takes time. This is somewhat less for the younger patient and somewhat more for the older patient, but always time is required. If the injured structures are prematurely or excessively exposed to strain, the healing process may be prolonged. If this occurs too late, function recovery may be retarded. Both extremes should be avoided. Therefore, when diagnosing and treating a sport injury, it is always useful to ask the following questions:

1. What is the relation between the cause of the injury and its effect?
   When a slight injury has serious effects, it may be assumed that the damaged tissue was less resistant than might be expected under normal circumstances, and, therefore, some suffering was present before the injury took place, for instance through excessive strain.

2. What is the nature of the injury?
   In case of a muscle or tendon injury, early mobilization will promote the ability of these structures to glide in relation to their surroundings and the tensile strength of the scar tissue formed, even when an operation is required to recover structural continuity. On the other hand, premature mobilization might result in disruption or dehiscence of newly formed capillaries and collagen fibres. Therefore, the advantages and disadvantages of putting strain on the structure at an early stage should be carefully weighed.

3. How serious is the injury?
   Whenever, during the healing process of the wound, extensively damaged tissue is replaced by scar tissue, treatment will be longer and more intensive, while at the same time restitutio ad integrum often no longer can be realized due to insufficient revascularization and regeneration.

Therefore the intent of treatment should always be:

1. To heal the wound.
2. To restore anatomical relations between injured and non-injured tissue.
3. To maintain the normal function of the non-injured tissue.
4. To prevent putting excessive strain on the area, in case of insufficient healing, i.e. revascularization of the injured tissues.

Regardless of the nature and gravity of the injury, knowledge of the healing process of a wound will enable us to carry out an individual treatment schedule in a more responsible way. One step further and the sport physician treating the injury will be able to use his knowledge of muscle and tendon metabolism in order to indicate the stress tolerance limits of these structures and thereby maximum individual performance under certain circumstances (for instance, cold, warmth, age, etc.).

Still a bit further and he will be able to predict which patients may and which patients may not expect to experience degenerative changes to bone or cartilage under certain circumstances.

Abstract

This article summarizes present knowledge concerning the healing process of wounds, while drawing attention to several consequences of sport injury treatment.

Dr. J.C. v. d. Meulen, Acad. Ziekenhuis Dijkzigt, Afd. Plastische Chirurgie, Dr. Molewaterplein 40, NL-3015 GD, Rotterdam