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Comparative histomorphometric study of bone tissue synthesized after electric and ultrasound stimulation

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Abstract

The clinical use of the alternative therapies in traumatology is conditioned by the knowledge and understanding of their actions on the bone tissue. The hereby study aims at the comparative assessment of the effectiveness of the direct current and ultrasounds in treating the fractures. Thus, we have proceeded to a comparative histological study of the bone tissue in the fractured area and the biomechanical description and the three-dimensional model of the stimulated bone's behavior by using micro-CT X-rays and the finite element analysis. The findings clearly show that the bone, which has been stimulated during a period of two weeks, has regained its functions, that is 85% of the compression one and 95% of the shearing one. These values prove that 90% of the bone structure has healed.

***Keywords*:** bone tissue, ultrasound stimulation, electric stimulation.

Introduction

The healing of the fractures depends on a series of complex events: the osteogenic cell induction, the inflammatory reaction, the formation of the fribro-cartilaginous callus, the bone callus and the formation of bone tissue, all these stages being spread on a several months period [1, 2]. The existence of certain factors, which directly influence the healing of the bone tissue eventually, results in the lack of setting. Therefore, the implementation of several adjacent techniques, which stand as catalysts of the healing processes, clearly diminishes the complication rate. The therapies based on amplifying the natural and biological healing phenomena using electromagnetic media, ultrasounds or osteogenic proteins are all part of these technological breakthroughs [3].

Materials and Methods

In our study, we used 24 rabbits, New Zealand race, adults, males, with a weight of 3 kg. We have chosen this race because it is frequently used in experimental studies, allowing us to compare the results of our study with results from other published papers.

The model gives us an advantage because, as far as phylogeny is concerned, it is similar to human models; the tibia of the rabbit is similar to the human tibia, with a shape of an italic elongated "S", prismatic in the superior 2/3 part and cylindrical in the inferior 1/3; the difference is that tibia is welded with the fibula in the distal half. Another advantage is that working with this model is relatively easy, because their size permits us to easily obtain tissue samples.

Osteotomy was performed in D0 day and gyps bandage was applied. From the forth day, D4, ultrasound law frequency pulsing stimulation began, 20 minutes/day, 20 days, until day D26. Sacrificing was performed in D45. Samples were taken and prepared by embedding in paraffin, serial sectioning and staining with Masson method and Hematoxylin–Eosin. Parts of the samples were prepared by the polishing technique.

Through bidimensional analysis of the bone tissue and digital processing of color images for morphometry we used the Nikon E-600 microscope from the Histology Laboratory of the Faculty of Medicine, "Ovidius" University of Constanța, and for obtaining the three-dimensional images we used the confocal laser microscope Zeiss LSM 510, from the physico-chemistry analysis laboratory Riken, Japan, using the following parameters: flame pixel – 512×512; scan speed – 25.6 sec/pixel; flame thickness – 0.5 micron; flame number – 71. Samples obtained by polishing technique were examined with the Nikon E-600 microscope and photographed with a Sony video camera.

Results and Discussion

Subsequent to the fracture, the bone itself being deteriorated, the soft tissue coating formed of the surrounding muscles and the periosteum is destroyed and the countless blood vessels in the area are also ruptured. Consequently, the hematoma in the bone marrow canal is formed, between the edges of the fracture and subperiosteal. The effects of its circulatory disaster are crucial [4]. The osteocytes on the junction area of the collateral canals are nutrition deprived and therefore succumb, thus all tissue surrounding the fracture is dead, none of the remaining cells being alive. The damage the periosteum of the bone marrow and other soft surrounding tissues also contributes to the necrotic material in the region. The presence of a necrotic material of such proportions triggers an immediate, intense and acute inflammatory response. This is followed by general vasodilatation and plasma exudation, resulting in a microscopically visible and acute edema in the fractured region [5].

Only part of the osteocytes will survive in the healing process, the vast majority being destroyed during the resorption period. Nonetheless, most of the directly involved cells in the healing of the fracture join the fracture focus at the same time as the granular tissue of the surrounding blood vessels [2]. Should these healing cells be directly derived from the endothelium, migratory cells or derived from the pre-reticulocytes, is actually irrelevant when compared to the fact that the healing is directly linked to the participation of the vascular sprouts. After the osteotomy, either by an excessive deperiostate or by the destruction of the intra-marrow system, the healing must take place through adjacent vessels in the survival system. In the past, the main origin of the blood vessels has been subject to controversy, because, in normal conditions, the periosteal vessels produce the majority of the vascular sprouts in the early stages of the normal healing process of the bone, the medullar artery taking a more important part in the latter process. In our experiment, the osteotomy of the periosteum has been interrupted, thus destroying the peripheral blood circulation in the area, and as for the assessment of the healing, we have evaluated the fundamental external system, which is generally formed by the periosteum, due to its internal layer, the osteogenic one. The number of bone lamellae in the stimulated lot increases by an average of 18 lamellae, compared to 11.5 in the normal bone. The average girth of the fundamental external system is of 462.59 μm, compared to 314.16 μm in the control group. Nevertheless, the individual growth of the girth of the lamellae does not occur, their measurements ranging in both cases from 25 to 27 μm.

The ultrasounds influence the degranulation of the mast cells on the inside of the damaged area. The degranulation of the mast cells determines the release of chemical mediators, such as the histamine, thus influencing the vasodilatation, the formation of venules and increasing the vascular permeability [6]. Thus, the proliferation of fibroblasts is stimulated, facilitating furthermore the collagen synthesis and the angiogenesis.

The polarized light examination of the bone sections from the section which has not been stimulated displays a very low birefringence of the Haversian systems, most of the yet unformed bone structures being monofringent. This comes to demonstrate the lack of maturation of the collagen, its periodicity being also irregular. Normally and also in the stimulated lot, there is a fair birefringence, proven by the existence of the „Malta Cross", which stands as a proof of the maturity of the tropocollagen molecules.

The same effect of early stimulation of the osteogenesis is found in the induction of a direct current by placing electrodes at the ends of the bone fragments [7]. Under the influence of the direct current, the organization of the collagen fibers on several plans is increased, due to the action of mechanical forces which influence the fracture, while in the case of the non-stimulated callus, the display of the collagen fibers is apparently random, having afribrillar areas mixed with areas poor in collagen, where the calcification is slow and uneven, the collagen fibers being heterogeneous and unequal, with the seldom formation of a fibrous tissue between the fractured margins. The newly-formed vessels in the fractured area display an uneven trajectory, are dilated and full of erythrocytes. In the restitution stage corresponding to the tissue recovery and formation of the early bone matrix, in certain areas, the density of the collagen fibers is increased and the osteoplasts along the fibers are usually large, having a double nucleus and an intensely basophile cytoplasm. The mechanism, which controls the behavior of each cell in the healing process, is probably derived from the microenvironment of each cell. The compression or the absence of a tension discourages the formation of the fibrillar tissue. There is no doubt regarding the fact that the variation of the pressure of the oxygen leads to the formation of the bone, the cartilage, the latter being formed in areas where the pressure is low, assuming this is a consequence of the distance between the cell and the capillary vessel [8]. The formed cartilage is eventually resorbed by an undistinguishable endochondral bone-formation process, excepting the lack of organization. The unstimulated group displays two kinds of changes subsequent to hypoxia: in the perilesional area, the osteons start to close, and in the lesion area cartilaginous tissue is formed. The bone will be formed of cells, which receive enough oxygen and are properly mechanically stimulated [9].

The direct influence of ultrasounds on the osteoplasts is obvious, all of the subjects of the ultrasound-stimulated group developed a bone tissue closely resembling the structure of the normal bone, from the macroscopic point of view, the cortical layer of the tibia having fully recovered. The calcium absorption of the bone cells increases, the adenylate cyclase modulates its activity, as well as the transformation and the synthesis of the beta growth factors. *In vitro*, the effects of the ultrasounds can mostly be seen in the differences between the osteoplast cultures, compared to the electric field, which does not directly stimulate the osteogenesis. However, the calcification of the fibro-chondrocytes eliminates all the obstacles of the calcification of the soft tissues.

We have also carried out a morphometric analysis of the surfaces of these osteocyte lacunae, considering the fact that statistics cite differences of 55–84% between the section surfaces, depending on the way the section has been made. An increase of 10% of the surface of the osteoplasts can be observed in the ultrasound-stimulated lot, the individual values in both lots ranging from 21.86 to 98.139.

This increase of the surface of the osteoplasts depicts a positive evolution of the cellular sector, greatly needed for the tissue reconstruction, given the fact that the osteocytes are no longer considered to be mature inactive bone cells.

Because of the studies that have been carried out, it is revealed that the stimulation of the osteoplasts by ultrasounds or direct current results in the formation of numerous larger, almost-even Haversian systems in the unstimulated callus. The morphometric studies indicate the increase of the perimeter, surface and equivalent diameter of the osteons in the stimulated lot.

In the ultrasound-stimulated group, the average perimeter has increased by 15% compared to the control group (the normal bone), while the Havers canals average is 6.44 in the control group, compared to 7 in the stimulated one. In contrast with the non-stimulated lots, an increase in the number of Haversian canals can be seen (Figures 1 and 2), but also a reduction of their diameter, which clearly points out the existence of an underdeveloped internal bone vascular network, which cannot supply a normal level of nutrients (Table 1).

Table 1 – ***The comparison of the Haversian canals stimulated group/unstimulated group in the pulsing ultrasound and direct current stimulation***

	Haversian canals Stimulated group/unstimulated group ratio
Ultrasound	2.17
Direct current	1.71

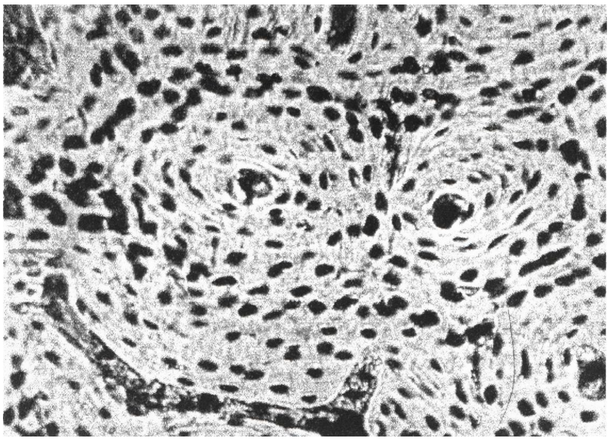

Figure 1 – ***Transversal section on a segment of bone tissue formed after an electrically stimulated fracture (polished bone, ob. ×20) (Mehedinti R et al., 2004).***

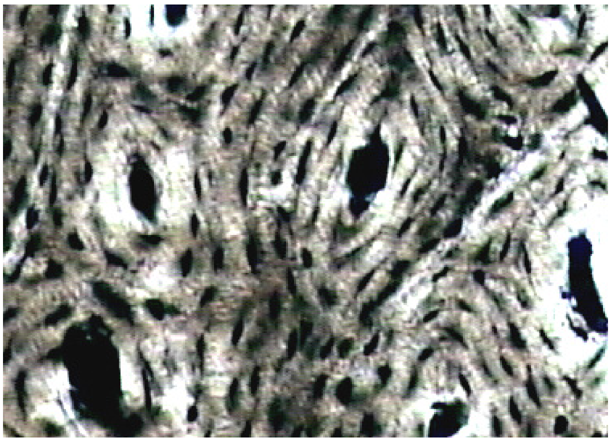

Figure 2 – ***Transversal section on a segment of newly formed bone tissue after an ultrasound-stimulated fracture (polished bone, ob. ×20).***

The Volkmann and Havers canaliculi (which stand as the nourishing layer of the bone) display a functional feature of the vascular elements, an adaptation of every backup tissue. The collaterals, which originate in these Haversian canals, appear to be a network of rhomboid knots whose sharp angles can lead to changes in the blood flow by forming turbulences and the reduction of the plasmatic filtering components in the non-stimulated lots. In the ultrasound or electrically-stimulated groups, the Volkmann canals form even rectangular-shaped knot networks. This type of networks appears in the lengthwise axis of the bone, assuring a reduction in the speed of the blood flow and contributing to the increase in the contact surface of the blood with the vascular wall (Figures 3–6).

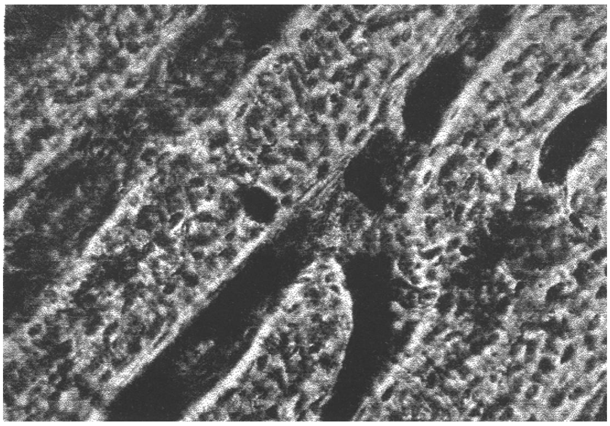

Figure 3 – ***Lengthwise section on an non-stimulated bone fragment. The picture displays certain collaterals belonging to the canals sharp-angle bond to the lengthwise Havers (polished bone, ob. ×40) (Mehedinti T et al., 2004).***

Figure 4 – ***Lengthwise section on an ultrasound-stimulated bone fragment. The picture displays the Volkmann canals, which derive in a sharp angle shape from the lengthwise Havers canals (polished bone, ob. ×20).***

The pulsating ultrasound assures the conditions needed by the bone tissue to heal: it assures the needed blood vessels for the differentiation of the bone cells, prevents possible infections and by the “internal tissue massage” assures the physical conditions required by the stimulation of the bone formation. The callus obtained with the aid of the pulsating ultrasound is of a superior quality, a fact that has been proven by the histomorphometric study carried out.

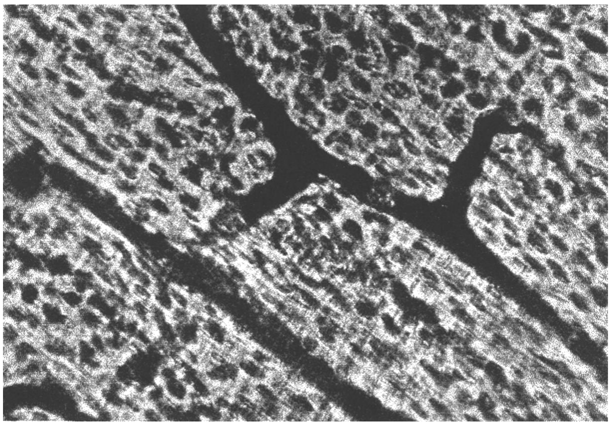

Figure 5 – ***Lengthwise section on a direct current stimulated bone fragment. The picture displays the well-developed Volkmann and Havers systems in 90^0 angles (polished bone, ob. ×40) (Mehedinti T et al., 2004).***

Figure 6 – ***Lengthwise section on a bone tissue fragment belonging to the ultrasound-stimulated lot. The picture displays well-developed Havers and Volkmann systems, having branches in 90^0 angles (polished bone, ob. ×20).***

Conclusions

The results of the study proved that the bone tissue formed under the stimulation of pulsating ultrasounds structures in a spatial-temporal pattern very similar to the one of the healthy bone, also reducing the consolidation time of the fracture.

The stimulated groups display a good recovery of the external fundamental system, which proves that ultrasounds influence the periosteal healing, thus influencing the vasodilatation, the formation of venules and increasing the vascular permeability.

Regarding the stimulated groups, the examination of the stimulated bone sections displays the presence of densely disposed osteons, crossed by a fibrous bone structure, while the structure of the osteons is well organized.

Compared to the non-stimulated groups, an increase in the Harvers canals can be pointed out, which leads to the existence of an underdeveloped bone vascular network in the non-stimulated lots, which compensates by caliber the low number of blood vessels, which cannot deliver the necessary amount of nutrients.

Our findings are comparable with those in the stimulation of the factors using direct current, our method having however the advantage of being non-invasive, therefore not bringing any change to the surrounding soft tissues and thus being considered as a complementary therapy in the prevention of the pseudarthrosis.

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