Restoration of Bony Fundamental Systems Under the Influence of Pulsed Ultrasound

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The action mechanism of ultrasound in fracture healing is not yet known, at present being considered that they act mainly in the inflammatory and repairing phase of the bony tissue. In this study we considered the histological and histomorphometric evaluation of pulsed ultrasound action upon bony fundamental system, given its importance in the fracture restoration. Our study performed on laboratory animals highlights histometrically and histologically the high quality of the bony tissue inside the callus formed under the influence of pulsed ultrasound stimulation.

Keywords: external fundamental system, internal fundamental system, pulsed ultrasound, fracture

The healing of fractures depends on a cascade of complex events: induction phase of osteogenic cells, inflammatory reaction, formation of fibrocartilaginous callus and of bony callus, all these stages succeeding on a period of several months.[1] The existence of some factors that certainly influence the bony tissue healing process, will ultimately lead to the absence of consolidation. Implementation of some complementary techniques that are designed to accelerate the healing processes, reduces the complications rate. The therapies based on the amplification of natural biological phenomena of healing by electromagnetic environments, ultrasounds or osteogenic proteins are enlisted up in these technological developments [2,3].

S.J. Warden [4], following his performed studies, claims that the action mechanism of ultrasound in fracture healing is not yet known. There is a significant number of action modes which have been proposed to explain its effects, but two factors explain best the difficulty to identify the interaction ultrasound - fracture healing. First, fracture repairing derives from a cascade of events that depend on the genetic expression time and on the synthetic sequence of the numerous components. The second impediment would be the difficulty in understanding how the ultrasound and living tissue interact. The hypotheses of ultrasounds effectiveness are based on the 3 phases during the fracture healing: the inflammatory phase, repairing phase and remodeling phase.

The hypotheses suggest that ultrasounds act mainly on the first two phases of the healing process, the micro-movements theory being widely accepted at present [5], the mechanical stress without macroscopic shifting accelerating the fracture healing. [6] In our study we aimed to evaluate the repairing of the bony fundamental system, given that by osteotomy there occurs the destruction of periosteum and blood circulation, important factors in restoring bone matrix.

Experimental part

Materials and method

The study lot consisted of 12 subjects, who underwent osteotomy surgery on the right tibia on day D0, then a plaster bandage was applied. From the sixth day, D6, we applied stimulation with ultrasound of low frequency pulsed 2: 8, with the intensity of 0.5 W/cm², the frequency of 1.5 MHz, 20 min /day, till day D26. Euthanasia was performed on the day D60. There was used as a control group the left tibia of the subjects included in this study. The preparations obtained by polishing technique were examined under a microscope Nikon E 600 and purchased on a computer with a Sony video camera. The images obtained were then processed using Image Analysis Software Lucia G 3.52. For the histometric analysis we used 20X camera lens, on the image taken up and purchased on computer there was defined the number of pixels equivalent to 1 micron. Thus, any errors between the measurements on histological preparations of the control lot and those of the test lot are excluded, and the obtained results can be statistically analyzed. We aimed to achieve a qualitative interpretation of all these parameters on the images obtained on the optical microscope, determining the thickness of the osteonal lamellas which form the external and internal fundamental system-measurements being performed directly by positioning the calipers on each side of the lamellas, but also the number of ducts which belong to the osteoplasts of each lamella. The obtained results were systematized in the form of tables, on lots for which the average was calculated. Data processing was performed using Excel software [38].

The statistical tests were performed with Student Fisher Method (the t-test for populations with unequal dispersion), which has a statistical coefficient Pt smaller than 0.05 and this is quite significant.

Results and discussions

The examination of the sections obtained from the bony tissue by polishing shows the presence of an external fundamental system better represented in the stimulated lot. Its thickness is greater than in the control lot. The concentric bony lamellas are numerous, there can be observed osteoplasts disposed with their long axis in the axis of the bony lamellas, showing on the cross-section an area smaller than the osteoplasts from the central area (fig. 1).

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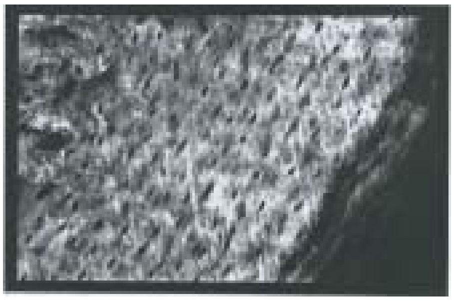
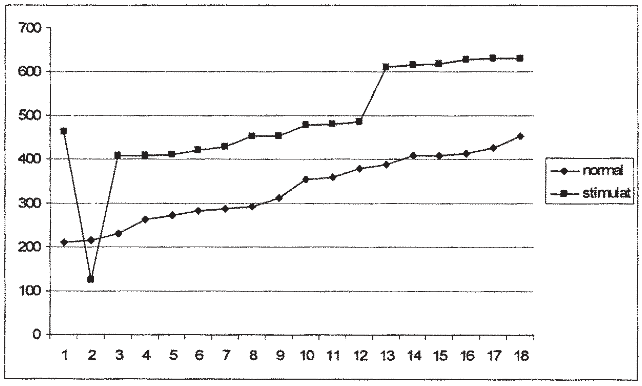


Fig. 1. External fundamental system, stimulated lot. There can be observed numerous bony lamellas and osteoplasts. Polished bone, obj. X10



Graphic 1. Graphical representation of thickness dynamics of the external fundamental system at the control lot and the stimulated one

Table 1

THE TABLE REPRESENTS THE AVERAGE NUMBER OF OSTEOPLASTS AND THICKNESS OF THE BONY LAMELLAS FROM THE EXTERNAL FUNDAMENTAL SYSTEM / MICROSCOPIC FIELD IN THE STIMULATED LOT AND THE CONTROL ONE

Normal bone			Stimulated bone		
Number of lamellas	**Number of osteoplasts**	**Thickness of lamellas**	**Number of lamellas**	**Number of osteoplasts**	**Thickness of lamellas**
10	81	290.91	15	81	420.78
10	59	228.10	13	91	409.66
10	60	281.05	15	93	453.72
10	65	272.76	17	95	410.74
10	66	214.05	17	82	407.4
10	54	209.02	13	94	464.46
14	71	358.68	17	104	480.99
12	81	412.35	19	102	477.69
13	71	409.66	23	105	618.18
10	61	286.04	23	127	630.18
10	85	310.74	23	138	627.15
10	63	262.36	13	121	428.93
12	72	377.69	22	110	610.92
14	71	409.66	19	122	485.62
13	93	453.72	22	119	615.13
13	69	426.94	22	120	629.25
14	86	354.87	19	96	452.74
12	89	387.43	15	98	123.89

Table 2

PERFORMING THE SIGNIFICANCE TEST OF THICKNESS OF EXTERNAL FUNDAMENTAL SYSTEM ON MICROSCOPE FIELD IN THE TWO LOTS

Mean	462.59	314.16
Variance	16593.37	6380.381
Observations	18	18
df	27	
t Stat	4.274838	
P(T<=t)	**0.000107**	
t Critical	1.703288	

Fig. 2. The Volkmann canals can be observed linking the central cortical area of the periosteum. Polished bone, obj X20, stimulated lot

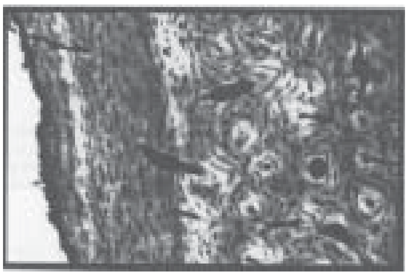


Fig. 3. External fundamental system, Volkmann canals can be observed, linking central cortical area with periosteum. Polished bone, obj X10, control lot

Although the thickness of cortical has approximately equal values in the stimulated bone and in the control lot, there appears an oversize of the external fundamental system (tables 1 and 2, graphic 1). From the morphometric study there can be observed the increase of the number of bony lamellas in the stimulated lot, the average being 18 lamellas versus 11.5 in the normal bone. The average thickness of the external fundamental system is 462.59 μ in comparison to 314.16 μ in the control lot. However, the individual growth does not occur in the thickness of the lamellas, observing that in both groups was between 25 to 27μ.

The external fundamental system arises mainly from periosteum, thanks to its osteogenic internal layer. We evaluated this parameter because the periosteum was cut in osteotomy, the local circulation being destroyed.

The release of fibroblast growth factors are also stimulated through macrophages, thus being encouraged the fibroblasts proliferation. Fibroblast growth factors subserve the forming of the bony precursor cells, leading to an precocious debut of the bone repairing process.

The number of osteoplasts is grown in the stimulated bone simultaneously with increasing the number of bony lamellas. The statistical significance of this parameter in the stimulated lot was: **P(T<= t)= 0.000107**.

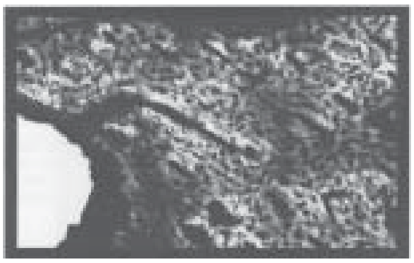


Fig. 4. The Volkmann canals can be seen linking the central cortical area and endosteum and the reduced number of bony lamellas. Polished bone, obj X20, stimulated lot

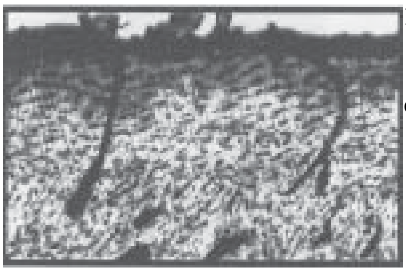


Fig. 5. Internal fundamental system, control lot. The Volkmann canals can be observed linking the central cortical area to endosteum and an even increased number of bony lamellas. Polished bone, obj X20.

The typical image of periosteal surface appears in relation to the vascular system, which in this case depends on periosteal capillaries and presents particularities as in the picture. In the stimulated lot there can be observed a high density of Volkmann canals, which ensures the exchange of nutrients between the periosteum and bony cortical area. The blood vessels on periosteal surfaces are arranged perpendicular on the external fundamental system. (figs. 2,3)

In the case of the internal fundamental system, there can be observed a decrease of width in the stimulated lot in comparison to the control lot (figs. 4,5). The average number of bony lamellas is 7 in comparison to 9.76 in the control lot. The average thickness of internal fundamental system being 123.28 in the stimulated lot in comparison to 181.74 in the normal bone. In this spot, too, there is an increase in the number of Volkmann ducts that connect the haversian systems and bony medulla.

After fracture, the bone itself is damaged, the layer of soft tissue including periosteum and the surrounding muscles are destroyed and numerous blood vessels that cross the fracture line are broken. A hematoma is formed in the medullary canal, between the ends of the fracture and the subperiosteal. The effect of this vascular disaster has a crucial importance. [7] Osteocytes are devoid of the nutrition source and thus they die on the junction portion of the collateral canals. Therefore, the closest places of the fracture site are dead, containing no living cells. Injury of the periosteum, of the bone marrow and other surrounding soft tissue also contributes to the necrotic material in the region. The presence of so large necrotic material in the area, leads to an intense and immediate acute inflammatory response. The general vasodilation and plasma exudation occur, resulting in acute edema which is visible macroscopically in the fracture region [8].

The first step in the repairing phase is identical with reparative processes which are present in other tissues, too. Hematoma organizes itself, probably playing a very small role in immobilization of the fracture and serving essentially as a fibrin scaffold, on which reparatory cells perform their function. There is no relationship between the size of periosteal callus and the size of the hematoma in the healing area. A bigger hematoma which occupies the space between bony endings is undesirable, so it is reabsorbed as soon as possible. The size of periosteal callus strictly reflects the need of stabilization of fracture fragments in the healing process. A high level of external callus indicates the necessity of an additional support beyond the normal contour of the bone, representing a delay in the bone healing, but it is a normal reparative process in warm-blooded animals and in humans [9].

The osteocytes that survive do not take part in the repairing process, they being destroyed during resorption. Anyway, most of the cells directly involved in the fracture healing get into the fracture hot spot together with granular tissue that invade the region from the surrounding vessels [10]. If these cells with reparative role derive directly from endothelium, they are migratory cells or they originate from pre-reticulocytes, seems less important than the fact that repairing is linked to penetration of vascular buds [11]. After osteotomy, either by excessive removing of periosteum or by destroying the intramedullary system, the repairing process must continue through derived vessels of the survival system [12]. The main origin of the blood vessels was the subject of controversy in the past, which means that under normal circumstances, periosteal vessels contributing with the majority of vessel buds/ sprouts in the early stages of a normal bone healing, [13-15] while medullary artery becoming important later in the process. In our experiment, the periosteum was discontinued in osteotomy, the local circulation being thus destroyed, then to assess its recovery, we evaluated the external fundamental system, which arises mainly from periosteum, thanks to its internal osteogenic layer. It can be noted an increase in the number of bony lamellas in the stimulated lot, with average 18 lamellas versus 11.5 in the normal bone. The average thickness of the external fundamental system is 462.59 μ compared to 314.16 μ in the control lot. However the individual growth does not occur in the thickness of the lamellas, observing that these, in both groups were in the range of 25-27 μ.

The ultrasounds definitely influence the degranulation of the mastocytes inside the injured region. The mast cells degranulation stimulates the release of chemical mediators such as histamine, influencing vasodilation, formation of venules and increase in vascular permeability [16].

Proinflammatory effects of ultrasounds accelerate the debut of the inflammatory phase, rapidly filling the liquidian exudate in the interstitial medium. Irradiation by ultrasound generates the adhesion of leukocytes to the endothelial cells. During fracture repairing, this process facilitates the migration of leukocytes from the blood compartment to the injured area, thus eliminating more efficiently the tissue debris as well as the pathogen agents within the lesion. [17] They stimulate the release of fibroblast growth factors with the help of macrophages. It is thus encouraged the proliferation of fibroblasts, being facilitated the collagen synthesis and angiogenesis [15,18,19].

Thus, the fibroblast growth factors have a role in the cartilage growth and help to build the precursor cells of bone formation, leading to precocious debut of the bone repair process.

The collagen reconstruction thus starts from amino acids, which begin to join together by primary valences, creating the axes of the collagen macromolecules. The axes are not straight but folded, or even helical, being necessary 421 amino acid residues to build up the required axis, the axis measuring 12000 A^{o} in length and only 4 A^{o} in thickness. After the axis formation, on it there will be attached lateral chains of amino acids, such as proline, hydroxyproline. The assembly of amino acid macro-molecules in chains is a consequence of amino acids tropochemistry and orientation forces. The chains form protofibrils which join in parallel fascicles, then forming fibrils [20, 21]. In the stimulated lot there can be noted a good remaking of the external fundamental system, which demonstrates that ultrasounds influence periosteal reconstruction, affecting the vasodilation, venules formation and vascular permeability. Thus, the removal of tissue debris becomes more effective as well as the pathogens from the lesion site.

The modeling and remodeling activity of the bone is made by apposition and concomitant resorption, which means the addition to a bony surface of the material originating from resorption of another surface. Between the areas where one surface is active, the other one becomes less active, so that the thickness of cortical to be maintained constant.

Conclusions

In the stimulated lot there can be noted a good recovery of the external fundamental system, which demonstrates that ultrasounds influence the periosteal reconstruction, affecting vasodilation, venules formation and vascular permeability.

Our study performed on laboratory animals highlights histometrically and histologically the high quality of the bony tissue in the formed callus under the influence of pulsed ultrasound stimulation.

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