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Distribution of radiological density in bone regenerate in relation to cyclic displacements of bone fragments

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We asked how bone fragment displacement could influence the distribution of radiological density in bone regenerate formed during the process of bone lengthening. The metatarsi of 21 sheep were lengthened by 20 mm by the Ilizarov method. The bone fragments were externally fixed with a specially designed ring external fixator equipped with linear actuator driver system. The test sheep were divided into three experimental groups: the G1 and G2 groups ($N = 8$) and the GR group ($N = 5$) – the reference group. In the case of sheep from the G1 and G2 groups, the lengthening was supplemented with mechanical stimulation of the regenerate in the form of cyclic bone fragment displacements (*CBFD*s) with the amplitudes of 1 mm (G1) and 2 mm (G1). Mechanical stimulation was applied over 30 days for 1 h per day with a frequency of 1 Hz. Eight weeks after the procedure the sheep were sacrificed in accordance with the required procedures. The analysis of the degree of bone regenerate mineralization involved the studies based on the CT scanning. The analysis of the results obtained is based on the paramenter called the *degree of regenerate mineralization* (*RMD*). The analysis of radiological density was carried out in the selected measurement areas. Such an area was located in three horizontal zones, taking into account the regenerate height, i.e. in its middle part (half regenerate length); the top part, 2 mm from the edge of the proximal fragment; and the bottom part, 2 mm from the edge of the distal fragment. The value of the *RMD* parameter varies significantly, depending on the bone regenerate area. The results obtained show that the *CBFD* = 2 mm accelerates the rate of mineralization of an eight-week-old regenerate. In the case of *CBFD* = 1 mm, the mineralization rate is lower by more than a dozen per cent.

Key words: bone elongation, external fixator, mechanical stimulation, radiological density

1. Introduction

Distraction osteosynthesis is a recognised method of treating limb length discrepancy. This procedure was first described in 1905 [8]. However, biophysiological and biomechanical basis of the method was developed at the end of the 1970s, the beginning of the 1980s by ILIZAROV [15], [16]. Distraction osteosynthesis involves intentional fracture of the treated bone, followed by the gradual spreading apart of the bone fragments at a rate of 1 mm per day. During that time tissue structures are generated in the interfragment region (the bone regenerate), which ultimately differentiates towards bone tissue. This treatment method can be employed due to external fixators mounted on the limb segment being lengthened.

Distraction osteosynthesis is undergoing constant development. The research is being conducted in a number of areas, including: search for the most advantageous method of bone fracture [16], [18] and selection of the lengthening site [15], [19], optimisation of the external fixators' design [3], [9], [31], and increasing the potential of the site of bone formation for regeneration. The last of the above-mentioned issues is particularly important from the viewpoint of mechanobiology of the distraction osteosynthesis process. In many papers, their authors assess the significance of various factors of a mechanical nature as the stimulators initiating and regulating the processes of tissue differentiation. Those

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factors can be itemized as follows: hydrostatic pressure [6], [24], tensile strains [5], [11], [23], volumetric strains [1], [5], [12], [22], and extracellular fluid flow rate [22], [24]. The above-mentioned physical quantities and their values result from the kinematics and dynamics of the biomechanical system that they form: the fragments of the bone being lengthened, soft tissues, and the construction of the stabilizer used in the treatment. The external forces acting on such a system create a certain state of the displacement of bone fragments, generating a specific level of hydrostatic pressure, tensile strain, volumetric strain, or extracellular fluid flow rate. There are known studies demonstrating a significant impact of the displacement of bone fragments on the rate of tissue regeneration in the fracture gap [1], [6], [7], [12], [29], [30]. On the other hand, we still do not know much about the relationship between the displacements of bone fragments and the growth of tissue structure in the bone regenerate formed during the process of the lengthening of limb bones. Considering the differences between the concepts of classic osteosynthesis and distraction osteosynthesis [13], [19] it may be concluded that those processes also differ in their biomechanics.

The purpose of the present paper was to determine the impact of the values of bone fragment displacements on the rate of mineralization of the bone regenerate formed during the process of bone lengthening. There is little description of such cases in an available literature. The analysis of the degree of bone regenerate mineralization involved the studies based on CT scanning. This test technique is used in the analysis and assessment of the degree of mineralization of the site of treatment of skeletal injuries [4], [14], [25], [27], [28].

2. Material and method

The research was conducted on 21 Merino sheep. The sheep were 2–2.5 years old and weighed 48 to 54 kg. The lengthening process of a metatarsal bone of the left hind limb was carried out according to the Ilizarov method. Metatarsal bone was selected due to the favourable anatomical conditions facilitating fixator application. A mechanical bone continuity was broken using the method of closed osteoclasis [18]. After initial fixation of the apparatus on the treated limb segment, the distraction elements were temporarily unscrewed. The bone is drilled many times with a specially prepared Kirschner wire with a flat, triangular point at the level of planned fracture, typically in the region of the proximal metaphysis. The bone is fractured by means of rotational movement of the apparatus rings or by grabbing the fixed limb segment above and below the cut point and performing a movement in the direction of the deviation.

The adopted lengthening was 20 mm, which constituted 15% of the length of the elongated limb. The daily one-mm increase in the length of the elongated metatarsus bone was obtained on a one-time basis at the speed of 0.2 mm/s. All surgical procedures were carried out in accordance with the guidelines approved by the 2nd Local Ethics Committee on Animal Experimentation in Wrocław.

A special research circular fixator was designed for the purposes of the experiment (figure 1). The fixator consisted of two rings (surgical steel 316L) with 95 mm inner diameter and 4 mm thickness. At the level of each ring there were two Kirschner wires with the diameter of 2.0 mm, crossing the ring plane at approx. 90° angle. After their passing through the bone and fixation to the ring, the Kirschner wires were pre-tensioned with a force of approx. 800 N. The rings were connected by means of two spacers, 105 mm in length. The use of the above fixator design ensured high axial stiffness of 620 N/mm. This intentional effect practically completely eliminated the displacement of fragments of the lengthened bone caused by movement of the test sheep. The fixators were equipped with a system ensuring displacement of the bone fragments in a particular direction and with a particular amplitude. For this purpose, the fixator spacers were fitted with line actuators controlled by a microprocessor. The fixators applied enabled: i) automatic bone lengthening at a rate of 1 mm/day until its planned length is reached, ii) application of mechanical stimulation of the regenerate in the form of cyclic displacement of bone fragments of a specific amplitude.

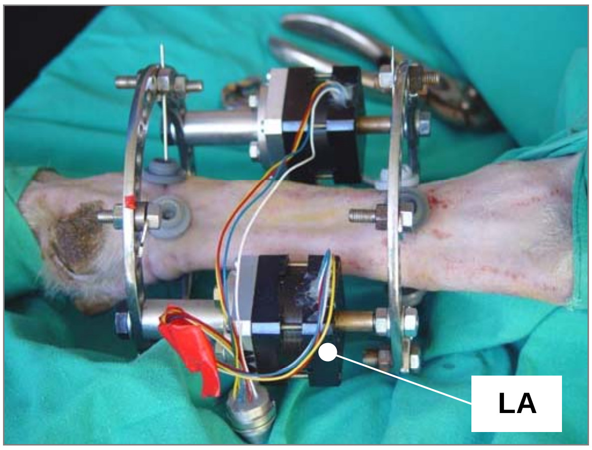

Fig. 1. External fixator on sheep metatarsal; LA – linear actuator

The test sheep were divided into three experimental groups: the G1 and G2 groups (each consisting of $N = 8$ sheep) and the GR group, consisting of $N = 5$ sheep. The GR group constituted the reference group and the sheep in that group were subjected exclusively to the standard lengthening procedure. In the case of sheep from the G1 and G2 groups, the lengthening was supplemented with mechanical stimulation of the regenerate in the form of cyclic bone fragment displacements (*CBFD*) with the amplitudes of 1 mm and 2 mm for the G1 group and the G2 group, respectively. The maximum *CBFD* value was adopted on the basis of clinical observations and the examination of the mechanical properties of external fixators. A patient subjected to lower limb lengthening in the femoral region and moving on crutches loaded the treated limb with the force of 200–250 N [3]. Because the axial stiffness of the Ilizarov apparatus fixed on the thigh ranges from 50 to 80 N/mm [9], [31], axial displacements of bone fragments can be as wide as 4–5 mm. Using the law of model similarity it was assumed that in the case of the lengthening of metatarsal bones of the sheep the corresponding *CBFD* value would be 2 mm. Such a value was adopted as the maximum value acceptable in clinical practice. Stimulation of the bone regenerate with specific CBFD with the frequency of 1 Hz took place each day over a period of 1 hour, which corresponded to 3,600 cycles. The frequency parameters adopted and the number of cycles refer to the results of the research by KASPAR [17] on the selection of the optimal parameters for artificial stimulation of callus. Each time the starting point was the current size of the interfragment gap. The *CBFD* values adopted were applied according to the sinusoidal pulse cycle (figure 2).

Eight weeks after the procedure the sheep were sacrificed in accordance with the required procedures. The samples collected were subjected to, among others, the analysis of the degree of their mineralization by means of the measurement of radiological density.

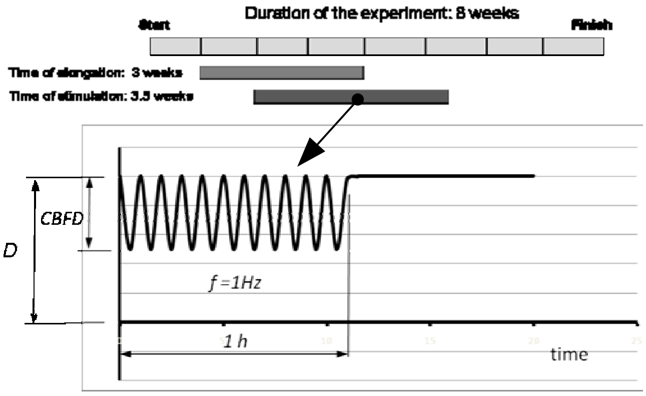

Fig. 2. Schematic experiment schedule

The analysis of the radiological density of the tissue structures forming the bone regenerate was carried out by recording radiographs using computed tomography (CT). The research used a CT/e Dual scanner by General Electric. The output images used 120 kV/20 mA doses, while transverse scans used 140 kV/140 mA doses.

The analysis of radiological density was carried out in the selected measurement areas. Such an area was located in three horizontal zones taking into account the regenerate height, i.e. in its middle part (half regenerate

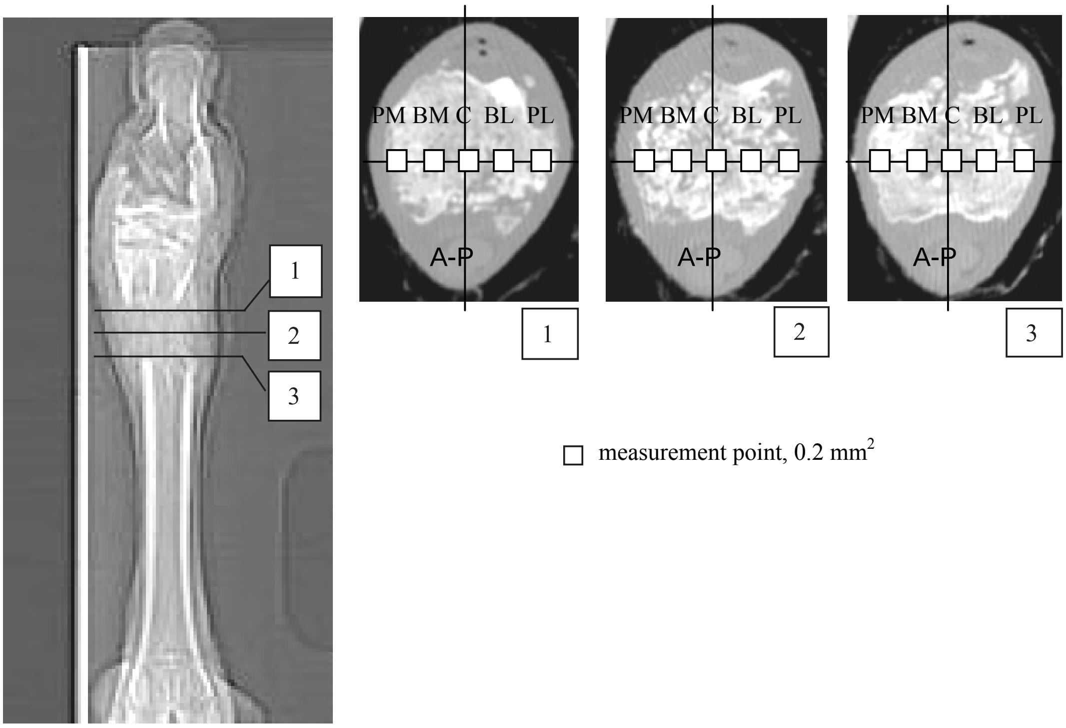

Fig. 3. Typical CT scan of totally elongated metatarsal and localization of measurement areas – CT slice (1, 2, 3)

length); the top part, 2 mm from the edge of the proximal fragment; and the bottom part, 2 mm from the edge of the of the distal fragment (figure 3).

3. Results

In order to standardize the results received, the radiological densities (in units of the Hounsfield scale) determined in the selected measurement areas (HU_{pi}) were compared with the radiological densities determined for the shaft of the same lengthened bone (HU_c), determined 10 mm away from the fracture edge. The analysis of the results obtained is based on the parameter called the degree of regenerate mineralization (*RMD*), defined by:

$$RMD = \frac{HU_{pi}}{HU_c}.$$

The value of the *RMD* parameter varies significantly, depending on the zone of bone regenerate area. The lowest *RMD* values are reached in the middle zone of the regenerate (mean *RMD* = 0.308), where differentiation between the measurement areas is slight (up to 0.07). A distinct lack of significant differences between the experimental groups, i.e. the reference group and the dynamically stimulated groups – G1 and G2 (figure 5), is also of interest.

The *RMD* parameter is far more differentiated in the peri-fragment zones (figures 4 and 6). In the case of the reference group (R), in the central part of the zone, the *RMD* is the lowest and amounts to 0.26. In the proximal sub-fragment part, the *RMD* increases to 0.40÷0.41, and in the external part it amounts to 0.32÷0.37. A similar differentiation of the *RMD* parameter occurs in the distal peri-fragment zone. In the central part of the zone, this parameter reaches 0.22, in the distal post-fragment part it increases to 0.35÷0.40, and in the outer part to 0.23÷0.37.

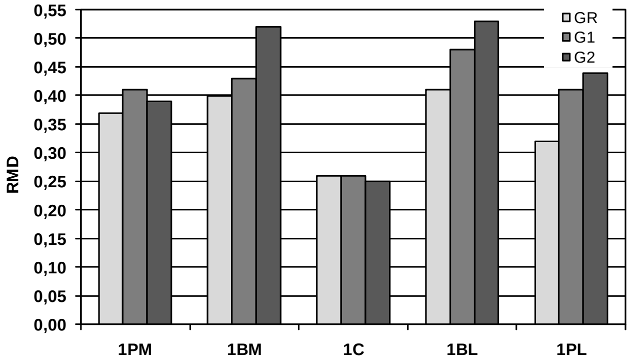

Fig. 4. Comparison of *RMD* values for each group; measurement area 1

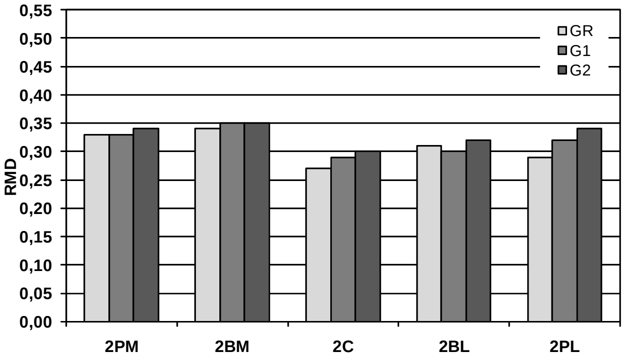

Fig. 5. Comparison of *RMD* values for each group; measurement area 2

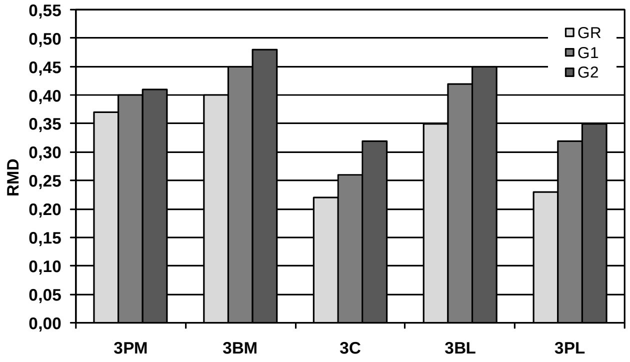

Fig. 6. Comparison of *RMD* values for each group; measurement area 3

In the G1 and G2 experimental groups, the *RMD* values are higher compared with those in the R group. In the sub-fragment part of the proximal peri-fragment zone, the *RMD* parameter determined for the G1 group is higher by 7%÷17.5%, and for the G2 group it is higher by 30% compared with that for the reference group R. In the external part of the group G1, the difference ranges from 11% to 28%, and for G2 – from 5.5% to 37.5%. In the peri-fragment zone, further differences between the *RMD* values obtained are equally important and in the sub-fragment part they oscillate between 12% and 20% for the G1 group, and between 20% and 34% for the G2 group. The corresponding values recorded in the external parts are: for G1, 8%÷39%; for G2, 11%÷52%.

Unlike the peri-fragmental zones, in the middle zone, a dynamic stimulation does not affect significantly the *RMD* parameter.

4. Discussion

The purpose of the study was to determine the impact of bone fragment displacements on the mineralization rate and the biological value of the bone regenerate formed during the process of bone lengthening. The lengthening involved metatarsal bone of the sheep. In the experiment, three *CBFD* levels were analysed: 0 mm, 1 mm, and 2 mm, applied for 1 hour per day. The overall duration of the experiment from the moment of osteoclasis to the collection of regenerate was 8 weeks, whereas mechanical stimulation in the form of *CBFD* was applied for 4 weeks (the start – one week after the beginning of the distraction phase; the end – one week after the completion of the distraction). The degree of bone regenerate mineralization was assessed on the basis of radiological density determined from the images obtained in the tests using a CAT scanner.

The results obtained show a significant differentiation of the mineralization process in the bone regenerate area. In the reference group (R), the lowest value of *RMD* mineralization can be seen in the middle regenerate zone. The process of regenerate mineralization takes place significantly faster in the proximity of bone fragments. In the peri-fragment zones, the *RMD* parameter is higher by approx. 35% than the value obtained for the middle zone. Our results correspond to those obtained by ARONSON et al. [2], who examined the progress of mineralization of the bone regenerate in dogs subjected to lengthening of shank bones. Similar results were also reported by BOER et al. [4] in radiological studies of the regenerate created during the lengthening of shank bones in goat.

Taking into account the mechanobiological aspects of the lengthening process we can conclude that *RMD* differentiation is caused by a systematic increase in the interfragment gap, characteristic of the method of distraction osteosynthesis, and the accompanying changes of biomechanical conditions in the respective regenerate zones. A significant increase in the *RMD* value in the peri-fragment zones is the result of high osteogenic potential in the direct vicinity of the bone fragments. Special role is played here by the periosteum and endosteum containing mesenchymal cells, which in a traumatic situation can transform into bone-forming cells (osteoblasts) [19], [26]. Consequently, in the peri-fragment zones we deal with the processes of intensive proliferation and diversification of tissue structures, which translates into a high rate of mineralization. This is confirmed by the results of a study by KRAWCZYK et al. [18], who analysed in an in vivo sheep model the impact of the way osteotomy was performed on the biological quality of the regenerate. In the zones adjacent to the bone fragments, the analysis detected the significant dominance of the fibrous bone tissue with single osteons, and the bone trabeculae were oriented in the same direction as the performed distraction.

The biological and biomechanical potential of osteogenesis decreases from the bone fragments to the middle regenerate zone. It decreases from the biological viewpoint because in the middle part of the regenerate we deal with a lower concentration of mesenchymal cells and active osteoblasts [26]. On the other hand, the systematic increase in the separation of the bone fragments generates a strain area, which is dominated by tensile strains that slow down the process of tissue differentiation. Using an experimental sheep model, HENTE [13] demonstrated that in an interfragment gap with predominantly tensile strains the cross-section surface area of the generated callus is nearly 25 times smaller than in the case of predominantly compressive strains. Also, the area occupied by the newly created bone tissue is over 10 times smaller if tensile strains occur.

The low degree of mineralization in the middle regenerate zone is caused by the predominance of the fibrous connective tissue that is rich in undifferentiated mesenchymal cells with numerous blood vessels [19]. Therefore, the middle zone, called the distraction zone, exhibits high flexibility practically until the end of the distraction phase, enabling the planned lengthening of the treated bone. During the stabilisation phase, when the compressive strains are dominant, the tissue structures are rapidly reconstructed and mineralized [16], [20], [21].

Comparing the *RMD* values obtained for the reference group with those for the G1 and G2 groups, where dynamic stimulation was applied in the form of cyclic, axial displacements of bone fragments, we can draw a general conclusion that the use of dynamic stimulation during the distraction phase results in an increased mineralization of the eight-week-old regenerate. The higher *CBFD* (2 mm) accelerates the rate of regenerate mineralization compared with the effect obtained at *CBFD* equal to 1 mm. This effect is particularly visible in the peri-fragment zones. In the middle zone, the differences between the groups tested are slight and fall within the limits of statistical error.

In the experimental groups G1 and G2, where axial displacements of bone fragments were applied with a constant amplitude (respectively: 1 mm and 2 mm), a specific level of interfragment strains was generated. It was defined by the ratio of changes in the distance between the fragments to its initial value. In the case of those experimental groups, mechanical stimulation started when the distance between the fragments reached 7 mm. Therefore, we can conclude that at that moment the interfragment strains reach 14% for the G1 group and 28% for the G2 group. However, during the process of limb lengthening the distance between the bone fragments steadily increases, in our case at a rate of 1 mm/day. This means that as the regenerate grew in length, the value of the generated interfragment strains decreased. For example, at the moment the target length was achieved, i.e. 20 mm, the respective strains calculated according to the above-mentioned definition would be 5% and 10%. However, those are only theoretical values. As early as in a distraction phase the tissue structures undergo differentiation at different rates, depending on the regenerate zone. According to the results of the studies presented in the literature [10],[16],[18]–[20] and the results of this work, the differentiation process is most intensive in the peri-fragment zones. Also, those zones show a rapid increase in the mechanical stiffness of tissue structures – they are less susceptible. Therefore, in cases where dynamic stimulation is applied in the form of *CBFD*, the distribution of strains in the regenerate proves to be strongly differentiated. The greatest strains are present in the zones where the tissue structure shows the lowest stiffness, i.e. in the middle zone. Taking the above into account, the presented studies assumed that at the time of *CBFD* application in the middle part of the regenerate – with the lowest stiffness – the actual strains reached approx. 14% (G1) and 28% (G2).

It seems interesting to compare our results with those obtained for a mechanical stimulation of the fracture gap (callus). In their research on sheep, WOLF et al. [29] analysed the impact of cyclic bone fragment displacements (0.2, 0.4, 0.8 mm) on the biomechanical quality of the callus. Their research shows that for a fracture gap with a constant 3 mm width, mineralization of the six-week-old callus is fastest when dynamisation of bone fragments takes place in cycles and the fragment displacements approach to 0.4 mm, which corresponds to the interfragment strains of approx. 13%. The application of twice-big displacements (0.8 mm – 26% strains) results in a slower rate of mineralization. Different results were obtained by YAMAJI et al. [30], who analysed in an experimental sheep model the impact of bone fragment displacements (0.3, 0.7 mm) on the rate of callus formation in a 2 mm-wide fracture gap. The results obtained demonstrated a higher rate of growth of the newly formed bone tissue (in a four-week-old callus) in the case of applying 0.7 mm axial fragment displacements (interfragment strains on a level of 35%). Yamaji et al. also observed the impact of the size of the interfragment gap on the development of bone tissues. Apart from the above-mentioned case of a 2 mm gap, the analysis also concerned a 6 mm-wide gap. The authors of the quoted work conclude that, generally speaking, in the case of a larger fracture gap, with the same cyclic axial displacements of bone fragments, the formation of new bone tissue is significantly slowed down. According to Yamaji et al. this is caused by lower interfragment strains in the callus – the same fragment displacements in a larger fracture gap resulted in, respectively, 5% and 12% strains.

Obviously, a comparison of the results of research on mechanical stimulation of the bone regenerate formed during bone lengthening with the results of experiments on mechanobiology of fractures serves demonstrative purposes. This is mostly due to biomechanical differences between the two processes. The method of conducting the experiment in the two subject fields is also important. In the case of research on fractures, the planned interfragment gap is obtained by the separation of bone fragments or, in the case of larger gaps, by excision of the fragment of the bone shaft [29], [30]. In the gap, that procedure generates a certain ‘shock’ state for the biological matter. In the research into distraction osteosynthesis, the distance between fragments increases gradually, in accordance with the treatment methodology, which in our opinion can have a beneficial effect on the high conformity of the processes taking place during the experiment with the natural progression of treatment.

To sum up, cyclic axial displacements of bone fragments by 2 mm accelerate the rate of mineralization of an eight-week-old regenerate formed after three weeks of the stabilisation phase since the time of achievement of the planned lengthening (20 mm). In the case of 1-mm displacements, the mineralization rate is lower by more than a dozen percent. It should be stressed that the rate of mineralization is not regular in the whole regenerate volume. Therefore, future studies will expand the analysis of the experimental material obtained in such a way as to include histological and biomechanical tests.

References

[1] ARO H.T., KELLY P.J., LEWALLEN D.G., CHAO E.Y.S., *The effects of physiologic dynamic compression on bone healing under external fixation*, Clin. Orthop., 1990, 256, 260–273.

[2] ARONSON J., GOOD B., STEWART C., HARRISON B., HARP J., *Preliminary studies of mineralization during distraction osteogenesis*, Clinical Orthopaedics and Related Research, 1990, No. 250, 43–49.

[3] BĘDZIŃSKI R., FILIPIAK J., *Experimental analysis of external fixators for femoral bone elongation*, Acta of Bioengineering and Biomechanics, 1999, Vol. 1, No. 2, 93–105.

[4] Den BOER F.C., BRAMER J.A.M., PATKA P., BAKKER F.C., BARENTSEN R.H., FEILZER A.J., deLANGE E.S.M., HAARMAN H.J.T.M., *Quantification of fracture healing with thee-dimensional computed tomography*, Arch. Orthop. Trauma Surg., 1998, 117, 345–350.

[5] CARTER D.R., BLENMAN P.R., BEAUPRE G.S., *Correlations between mechanical stress history and tissue differentiation in initial fracture healing*, Journal of Orthpaedic Research, 1988, Vol. 6, 736–748.

[6] CLAES L., ECKERT-HUBNER K., AUGAT P., *The effect of mechanical stability on local vascularization and tissue differentiation in callus healing*, Journal Orthopaedic Research, 2002, 20, 1099–1105.

[7] CLAES L.H., WILKE H., AUGAT P., RÜBENACKER S., MARGEVICIUS K., *Effect of dynamisation on gap healing of diaphysesal fractures under external fixation*, Clinical Biomechanics, 1995, Vol. 10, No. 5, 227–234.

[8] CODIVILLA A., *On the means of lengthening in the lower limbs, the muscles and tissues which are shortened through deformity*, Am. J. Orthop. Surg., 1905, 2, 353–369.

[9] FILIPIAK J., MORASIEWICZ L., *Właściwości biomechaniczne stabilizatora Ilizarowa z hybrydowym układem wszczepów*, Chirurgia Narządu Ruchu i Ortopedia Polska, 2005, 70 (1), 49–56.

[10] FILIPIAK J., KUROPKA P., MORASIEWICZ L., KRAWCZYK A., BĘDZIŃSKI R., KURYSZKO J., WALL A., *Effect of bone fragment displacements on bone regenerate formation during distraction osteogenesis*, Journal of Biomechanics, 2006, Vol. 39, Suppl. 1, S9.

[11] GARDNER T.N., HARDY J.R.W., EVANS M., RICHARDSON J.B., KENWRIGHT J., *The static and dynamic behavior of tibial fractures use to unlocking external fixators*, Clin. Biomech., 1996, 11, 425–430.

[12] GOODSHIP A.E., KENWRIGHT J., *The influence of induced micromovement upon the healing of experimental tibial fractures*, J. Bone Joint Surg. Br., 1985, 67, 650–655.

[13] HENTE R., FUCHTMEIER B., SCHLEGEL U. ERNSTRBERGER A., PEREN S.M., *The influence of cyclic compression and distraction on the healing of experimental tibial fractures*, Jouranl of Orthopaedic Research, 2004, 22, 709–715.

[14] HUGES T.H., MAFULLI N., GREEN V., FIXSEN J.A., *Imaging in bone lengthening*, Clin. Orthop., 1994, 308, 50–53.

[15] ILIZAROV G., *Clinical application of the tension–stress effect for limb lengthening*, CORR, 1990, 250, 8–26.

[16] ILIZAROV G., *The tension–stress effect on the genesis and growth of tissues*: Part II. *The influence of the rate and frequency of distraction*, CORR, 1989, 239, 263–285.

[17] KASPAR D., SEIDL W., NEIDLINGER-WILKE C., BECK A., CLAES L., IGNATIUS A., *Proliferation of human-derived osteoblast-like cells depends on the cycle number and frequency of uniaxial strain*, Journal of Biomechanics, 2002, 35, 873–880.

[18] KRAWCZYK A., KUROPKA P., KURYSZKO J., WALL A., DRAGAN SZ., KULEJ M., *Experimental studies on the effect of osteotomy technique on the bone regeneration in distraction osteogenesis*, Bone, 2007, 40, 781–79.

[19] KURYSZKO J., KUROPKA P., JĘDRZEJOWSKA I., *Distraction osteogenesis and fracture healing. Differences and similarities*, Acta of Bioengineering and Biomechanics, 2000, Vol. 2, No. 2, 83–88.

[20] LOBOA E.G., FANG T.D., WARREN S.M., LINDSEY D.P., FONG K.D., LONGAKER M.T., CARTER D.R., *Mechanobiology of mandibular distraction osteogenesis: experimental analyses with a rat model*, Bone, 2004, 34, 336–343.

[21] MAFFULLI N., CHENG J.C.Y., SHER A., NG B.K.W., NG E., *Bone mineralization at the calloatasis site after completion of lengthening*, Bone, 1999, Vol. 25, No. 3, 333–338.

[22] MORGAN E.F., LONGAKER M.T., CARTER D.R., *Relationships between tissue dilatation and differentiation in distraction osteogenesis*, Matrix Biology, 2006, 25, 94–103.

[23] PERREN S.M., *Physical and biological aspects of fracture healing with special reference to initial fixation*, Clin. Orthop. Rel. Res., 1979, 138, 175–196.

[24] PRENDERGAST P.J., HUISKES R., SØBALLE K., *Biophysical stimuli on cells during tissue differentiation at implant interfaces*, J. Biomechanics, 1997, 30, 539–548.

[25] REICHEL H., LEBEK S., ALTER C., HEIN W., *Biomechanical and densitometric bone properties after callus distraction in sheep*, Clinical Orthopaedics, 1998, 357, 237–246.

[26] RICHARDS M., HUIBREGTSE B.A., CAPLAN A.I., GOULET J.A., GOLDSTEIN S.A., *A marrow-derived progenito cell injections enhance new bone formation during distraction*, J. Orthop. Res., 1999, 17, 900–908.

[27] SALMS M.G., NIKIFORDIS G., KOSTI P., LAMBIRIS E., *Estimation of artifacts induced by the Ilizarov device in quantitative computed tomographic analysis of tibiale*, Injury, 1998, 29, 711–716.

[28] SWENNEN G.R.J., EULZER C., SCHUTYSER F., HUTMMANN C., SCHLIEPHAKE H., *Assessment of the distraction regenerate using three-dimensional quantitative computer tomography*, Int. J. Oral Maxillofac. Surg., 2005, 34, 64–73.

[29] WOLF S., JANOUSEK A., PFEIL J., VEITH W., HAAS F., DUDA G., *The effects external mechanical stimulation on the healing of diaphyseal osteotomies fixed by flexible external fixation*, Clinical Biomechanics, 1998, 13, 359–364.

[30] YAMAJI T., ANDO K., WOLF S., AUGAT P., CLAES L., *The effect of micromovement on callus formation*, Journal of Orthopaedic Science, 2001, 6, 571–575.

[31] YANG L., NAYAGAM S., SALEH M., *Stiffness characteristics and inter-fragmentary displacements with different hybrid external fixators*, Clinical Biomechanics, 2003, 18, 166–172.