Low Intensity Pulsed Ultrasound for Bone Augmentation A Synchrotron Radiation X-Ray micro-CT evaluation

LAURA CRISTINA RUSU, CRISTINA-MARIA BORTUN, BOGDAN HOINOIU, MEDA-LAVINIA NEGRUTIU, LAVINIA ARDELEAN, ANGELA CODRUTA PODARIU, COSMIN SINESCU*

University of Medicine and Pharmacy "Victor Babes" from Timisoara, Faculty of Dentistry, Dental Medicine Department 1, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

Low-intensity pulsed ultrasound (LIPUS) exposure was used as a treatment procedure for healing the fractures in case of animals and clinical studies. The healing period of time is shorter for this method and the healing rate is considerably reduced. In this study were used rats on which was made bone augmentation. The augmentation materials were implanted in the medullar cavity of femur. The interested area was X-ray evaluated for 12 weeks. After 84 days the rats were euthanasia and the bone area treated through augmentation was exarticulated and investigated with synchrotron radiation X-Ray micro-CT. The scans were processed with a designed software and the images became 3D reconstructions. The samples where the Low Intensity Pulsed Ultrasound was used for Bone Augmentation revealed a good quantity and quality of a new bone formation in the area of the induced defect. An accurate analyze evaluated the method of augmentation procedure. The specific mechanism by which low intensity pulsed ultrasound technology accelerate bone healing remains unknown. In terms of the physical mechanism, this technology may exert a mechanical force on cell in soft tissue at the fracture gap. Evaluation of the bone grafting material/bone interface with noninvasive methods such as microCT using the synchrotron radiation could act as a valuable procedure that can be used in the future for usual research procedures.

Key words: low-intensity pulsed ultrasound, synchrotron radiation X-Ray, micro-CT, 3D reconstruction, bone augumetation

Low-intensity pulsed ultrasound (LIPUS) exposure has been used as a treatment procedure for healing the fractures both in animal models and as well as in clinical studies. LIPUS shortens the normal fracture-healing process of the tibia and radius, a fact pointed out by several clinical studies [1–3]. Animal studies have also shown that LIPUS exposure increases maximum resistance to failure and stiffness in diaphyseal fractures in rats [4–6]. Recently, Azuma et al [7] pointed out that LIPUS hurries up the rat femoral fracture healing. Mayr et al [8] reported that LIPUS exposure induced healing of delayed unions at a rate of 91% and healing of nonunion at a rate of 86%. In vitro, the molecular pathway that mediates this action is gradually being clarified. LIPUS exposure increases aggrecan messenger RNA levels and proteoglycan synthesis in chondrocyte cultures [9, 10] and calcium incorporation in cultured bone cells [11] and modulates transforming growth factor synthesis and adenylate cyclase production in osteoblasts [12]. It is still not very clear which is the process based upon LIPUS exposure initiates bone healing in nonunion. The aim of this study is to establish whether LIPUS exposure initiates bone healing in rat nonunion fracture models.

LIPUS is a form of mechanical power that can be transferred into living tissue as acoustic intensity waves. The micromechanical strains which these intensity waves are producing in living tissue can become biochemical occurrence at the cellular level [13–15]. This may occur through several possible mechanisms. The compression of micro-bubbles or cavitations and acoustic streaming could have a direct effect on cell membrane permeability [11,16,17]. Cation channels can be activated by the effect of mechanical pressure at the cell surface [18]. Ultrasound may also affect the attachment of the cytoskeleton to the extracellular matrix [19]. Wang et al [6] pointed out that LIPUS stimulation enhanced the mechanical properties of the healing callus. We also regard LIPUS as a form of mechanical stress, which initiates bone healing in rat nonunion fracture models.

Experimental part

50 Wistar rats, 6 months old from the Animal House of the "Pius Branzeu" Center for Laparoscopic Surgery and Microsurgery with an average weight of animals at surgery of 300g, were used in this experiment. The bone augmentation materials were implanted bilaterally in the medullar cavity of the femur of each animal. For the entire experimental period of time the animals will be kept two or three in a cage with an unlimited supply of fresh water and rodent pellets.

Anesthesia of the animals is achieved with Isofluran in concentration of 5% and O_2 at 1L/min, for induction in the anesthesia chamber. After this first procedure the animal is connected throw a facemask to an open breath circuit of anesthesia which inflows Isofluran at 1% and O_2 at 1L/min. The hindquarter on the experimental side is shaved, and the entire dorsal aspect of the animal is disinfected. Surgery is performed under sterile conditions. Access to the periosteum is made throw a 3 cm longitudinal incision of the skin on the lateral part of the thigh region, in the cranial third, followed by the blunt dissection of the quadriceps muscle with exposure of proximal diaphysis of femoral bone. All the maneuvers are performed under the microscope and the tissues are handled with microsurgical instruments to prevent as much as possible the tissue destruction.

* email: minosinescu@yahoo.com; Tel: 0722280132

Closure of the surgical site is undertaken with Monosyn 5.0 continuous suture in 2 planes of the muscles and separated stitches of the skin with Prolene 5.0 thread. Antibiotic prophylaxis during the surgery is performed with Cefazolin + Gentamicin to prevent any infection with any Staph, strep, Gram(-) bacilli or anaerobes. Postoperatively all the animals receive 5 days long analgesic medication with Buprenorphine (fig.1).

Fig. 1. Aspects from the surgical procedres

Follow up period of the animal consist in daily clinical examination for 21 days with evaluation of the general clinical status (heart rate, respiratory rate, body temperature, mucosal appearance and healing of the incision, posture and locomotion).

Animals are x-rayed using dental equipment for evaluation of the healing process at 2, 4, 6, 8, 10, and 12 weeks, aiming to reveal the influence on the site of bone defect, reaction in the area, vascular reaction and radio-opaque changes.

All the samples were treated with low intensity ultrasound: 200 millisecond burs at sine wave of 1.5 MHz repeating at a frequency of 1.0 kHz with the intensity of 30 mW/cm^2. The right femur with the augmentation material was exposed to ultrasound for 20 min while the left femur was used as a no treated control. The treatment period was finished at 84 days after the surgical intervention.

All rats are euthanatized (Thiopental overdose) at 84 days after surgery. The tight is exposed through an extended lateral incision. Soft tissues were removed with preservation of the periosteum. After exarticulation of the knee and hip joints, the femurs are removed and the specimens with new bone formation region will be gross examined, emphasizing the changing, after that is being prepared, together with bone structure for microCT and microscopy evaluation.

A synchrotron radiation X-Ray micro-CT experiment was performed at the SYRMEP Beamline of the ELETTRA Synchrotron Radiation Facility (Trieste, Italy fig. 2). The 1200 radiographic projections were acquired with a beam energy of 29 keV over 180° with a pixel size of 9 μm. A sample – detector distance of 15 cm was considered in order to have both absorption and phase-contrast signal, for a better viewing of the interfaces. The entire investigation could be supervised during the procedure (fig. 3).

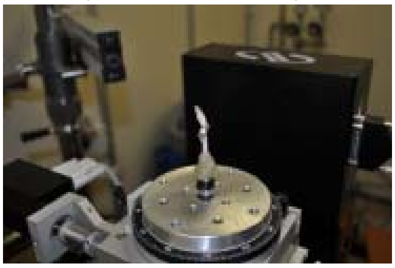

Fig. 2. Micro-CT investigations using the Synchrotron Radiation at the SYRMEP Beamline of the ELETTRA Synchrotron Radiation Facility (Trieste, Italy)

Fig. 3. The evaluation of the microCT investigation

The tomography reconstruction was performed by means of the common filtered back-projection method.

For 3D visualization, data volumes were rendered directly without decomposing them into geometric primitives. A commercial software - VGStudio MAX - was used to generate 3D images and to visualize the distribution in 3D of different constituents.

Results and discussions

The samples where the Low Intensity Pulsed Ultrasound was used for Bone Augmentation revealed a good quantity and quality of a new bone formation in the area of the induced defect (fig. 4). Also the amount of the remaining augmentation materials is smaller compared with the samples where the ultrasound technology was not employed (fig. 5).

VGStudio Max software gives a comprehensive 3D visualization of the reconstructed specimen, allowing the segmentation of the grey histogram, in order to visualize

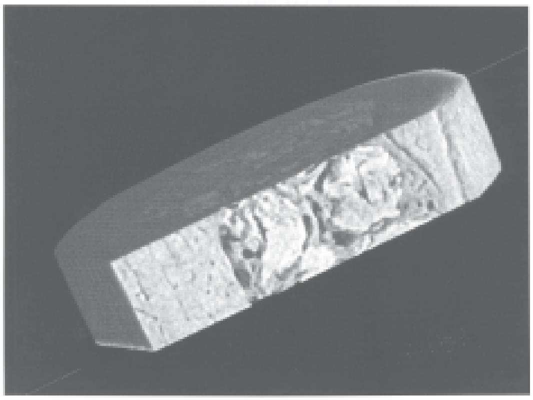

Fig. 4. VGStudio Max software for 3D visualization of the reconstructed femur with Low Intensity Pulsed Ultrasound for Bone Augmentation

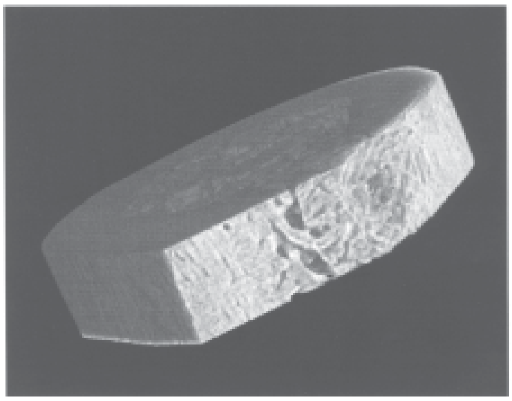

Fig.4. VGStudio Max software for 3D visualization of the reconstructed femur without Low Intensity Pulsed Ultrasound for Bone Augmentation

only the phases of interest in the imaged volume. It allows a direct view of three orthogonal axis (Axial, Sagittal and Frontal), together with the 3D image that can be rotated or slices in any direction for a good visualization of the morphology of the reconstructed specimen.

Conclusions

The specific mechanism by which low intensity pulsed ultrasound technology accelerate bone healing remains unknown; however, in terms of the physical mechanism, this technology may exert a mechanical force on cell in soft tissue at the fracture gap.

Evaluation of the bone grafting material/bone interface with noninvasive methods such as microCT using the synchrotron radiation could act as a valuable procedure that can be used in the future for usual research procedures.

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