Enhanced Bone Healing Through Mechanical Stimulation by Implanted Piezoelectric Actuators

Natacha Rosa[1], Fernão D. Magalhães[2], Ricardo Simões[3,4] and António Torres Marques[5]

[1]*FEUP, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n 4200-465 Porto, Portugal*

[2]*LEPAE, Department of Chemistry Engineering, Faculty of Engineering, University of Porto, Porto, Portugal*

[3]*Polytechnic Institute of Cavado and Ave, School of Technology, Barcelos, Portugal*

[4]*Institute for Polymers and Composites IPC/I3N, University of Minho, Guimarães, Portugal*

[5]*DEMec, Faculty of Engineering, University of Porto, Porto, Portugal*

1 STAGE OF THE RESEARCH

This research study from its inception to realization is a three year project. After a year of concept understanding through literature review and familiarity with the theory and terminology underlying this topic, a strategy to overcome this challenge was proposed.

The project is currently in a stage where the ideas and strategies suggested are going to be put into action. The material required is being purchased and the evaluation system prepared.

Albeit, these seems to be an innovative strategy to address this high-impact problem. It is a complex and multidisciplinary issue with very ambitious goals and for which solution proposal presents unpredictable results.

There is still much work ahead and great benefit will be acquired from the exchanges and discussions that will take place at the Doctoral Consortium.

2 OUTLINE OF OBJECTIVES

The main goal of this research work is to develop an actuator device that through piezoelectric mechanical stimulation is capable of accelerating in a controlled manner, the bone physiological fracture-healing process and leads to a reconstructed fracture that approximates normal anatomy.

By decreasing the minimum period of recovery time, we aim to reduce the number of delayed union and non-union cases, which are very common in fractures resulting from high energy trauma, like open fractures. This will facilitate an early rehabilitation and avoid an additional costly surgical intervention.

All these are expected to help reduce the total cost and personal discomfort associated with tibia fractures.

3 RESEARCH PROBLEM

Fractures are one of the most frequent injuries of the musculoskeletal system and from all the long bones in the human body, the tibia is the one in which healing is most problematic. This may be due to the nature of the mechanical loading and biological factors, such as the fact that muscle tissue does not surround the bone (Lacroix and Prendergast, 2002).

Tibia fractures are treated medically, and healthcare cost depends on treatment options, which, in turn, vary by injury type and severity and the presence of complications. There is no universal consensus on the best method of managing these type of injuries. In daily routine, each surgeon develops an individual fracture reduction techniques specific to the different fracture type and they are also challenge to manage the initial fracture using any of the least or non-invasive means available to enhance osteogenesis (Antonova et al., 2013; Smith, 1985; Malizos et al., 2006).

There are two basic histological types of bone healing, depending on the mechanical stability present at the fracture site (Giannoudis et al., 2007).

Primary or direct fracture healing, is rare. The fracture is treated with open reduction, where interfragmentary compression is achieved through the use of lag screw or with a plate placed in compression mode. The fracture fixation in this situation provides absolute stability. There is no movement in the fracture site, and no callus is formed. The fracture heals through the formation of osteonal cutting cones and Harversion remodelling of the compressed cortical (Hak et al., 2010; Giannoudis et al., 2007).

In contrast, secondary bone healing occurs in the vast majority of bony injuries. This type of fracture healing will occur if the fracture site is treated with cast or braces, and intramedullary nail, or plate placed in a bridging mode leading to relative interfragmentary movement. To stabilize the fractured bone during repair, an external callus develops where bone forms intramembranously proximal and distal from the fracture site and endocondrally in the rest of the callus (Giannoudis et al., 2007; Lacroix and Prendergast, 2002).

Biological healing is a complex physiological process that follows a characteristic course divided into three partially overlapping phases: an inflammatory phase (0-3 days after the injury) with formation of early fracture hematoma, an initial inflammatory response characterized by chemotaxis and migration of inflammatory cells and granulation tissue formation; a reparative phase (4 days to months after the injury) with revascularization, soft callus, lamellar bone deposition and hard callus formation, and a remodeling phase (months to years after a injury) which is characterized by changes in bone shape (Kumagai et al., 2012; Mavčič and Antolič, 2012; Claes et al., 2012).

Although, in the last decades, fracture treatment has improved considerably, complications, like delayed union and non-unions with an incidence rate up to 13 %, still occur. Nonunion can be defined as the non consolidation at the fracture site within 6 months. Sometimes, there is no need to wait such an amount of time, and a non union situation can be identified when there is no progress in callus formation at the fracture site at 4 weeks intervals follow up. On the other hand when there are indications of progress in callus formation, it is wise to wait more than 6 months(Antonova et al., 2013; Audigé et al., 2005; Giannotti et al., 2013).

Delayed union and non-unions cases put additional burden on the patient because they prolong the disability and are associated with substantial pain, suffering and morbidity, which is not exempt of risks and potential complications and increases health-care costs. Currently, the assessment of fracture healing and complications detection during the repair process is performed by clinical and radiographic examination, both of which are dependent on the orthopedic surgeon's expertise and clinical judgment (Audigé et al., 2005; Claes et al., 2012).

In Portugal, the reduction of the tibiae open fractures are the sixth most common type of musculoskeletal surgical intervention (NHS-Portugal, 2012). Although, the younger generation is considered the "prime victims" of this type of fracture, by representing a high loss values on the society working force, older adults aged 65 or more, also suffer a large percentage of tibia fractures. Fractures in this age group lead to an acute inpatient stay, post-acute inpatient stay, and home health care as well as outpatient visits and physical and occupational therapy (Antonova et al., 2013; Leung et al., 2009).

The process of fracture healing is impacted by several factors of which some are patient-dependent like nutritional and health-conditions and other depend on external circumstances such as the severity of the trauma experienced. From all the variables affecting the bone healing process, mechanical stimulation has special importance. Mechanical signals can be regulated via stiffness of fixation, rigidity of cast immobilization, control of weight bearing, or even applied loading to the fracture site influence the amount of motion between the bone fragments or forces transmitted across the callus which consequently affects the quality, rate and progression of repair. A compromise should be taken into consideration in order not to interfere negatively with the healing process (Giannotti et al., 2013; Claes et al., 2012).

Several years ago, it was believed that complete immobilization was imperative for successful fracture healing and that the resorptive effect of disuse was necessary to release calcium for callus mineralization. Now, we know that shielding the callus from mechanical stimulus with the use of high stiffness frames can suppress osteogenic response at the pereosteum, and is permissive to a delayed or atrophic non-union, while internal fracture fixation, via plates which are too stiff, can cause osteopenia below the device. At the other extreme, overloading caused by early weight bearing on a fracture protected only by flexible external fixation lead to delayed union. Also decreased frame stiffness stimulates periosteal callus where unstable fixation avoids the invasion of blood vessels and the differentiation of pluripotential tissue into bone, leading to the formation of avascular tissues with higher failure strains, such as cartilage or fibrous tissue and fractures presenting signs of impair, hypertrophic non-union cases or delayed healing in most cases require open orthopaedic intervention. Frames with low stiffness may also result in high pin/bone interface stresses that induce local absorption and are associated with pin loosening (Goodship et al., 2009; Bailón-Plaza and van der Meulen, 2003; Boerckel et al., 2012).

It is also important to highlight the additional difficulty in defining the exact end point of fracture repair which also hampers clinical studies (Claes et al., 2012). In the study developed by (Bacon and Goodship, 2007), after evaluating healed-bone magnitude and orientation through the use of neutron experiments they suggested that "normal" bone matrix is not present even after 22 months of healing and that this may question the apparent ability of bone to repair without a scar.

As mentioned previously, the length of healing time is an important parameter with direct implications on the patient's physical and emotional well-being and it also represents an additional cost to the health care system. In 2007, the average direct costs for treating tibial fracture nonunions were around 32.660 dollars in UK and when considering all categories of care (except emergency room costs) expenditures were more than 2-fold higher on delayed union patients cases than in those with considered union (Antonova et al., 2013; Malizos et al., 2006; Wu et al., 2013).

Based on the conclusions obtained in some research studies (Wu et al., 2013; Heckman and Sarasohn-Kahn, 1997), the delays in fracture healing cost more money than early intervention to shorten the time of fracture recovery. Hence, there seems to be important economic advantages, besides all the other patients well-being benefits, on mechanically stimulating bone fracture to accelerate the healing process in such a way that the need for fracture fixation is reduced to the minimum necessary period of time.

4 STATE OF THE ART

Although, the curing process of a fracture is a phenomenon with biological features, it is directly related to the medical treatment carried out, namely how rapidly the bone can heal and return to normality (Roseiro et al., 2013). Hence, in these section we are going to evaluate the latests tendencies and developments in the medical and research fields.

4.1 Current Clinical Procedures

The common clinical practices used to stimulate bone regeneration and avoid delayed union cases include the use of different bone-grafting methods (such as autologous bone grafts, allograft and bone-grafts substitutes), supply of osteoprogenitors and mesenchymal stem cells to the fracture site and local application of growth factors, which are sometimes accompanied by a therapies such as low-intensity pulsed ultrasound and electrical stimulation. The above mentioned bone-healing stimulation methods, are associated with several drawbacks and limitations to their use and availability (Dimitriou et al., 2011; Stevenson, 1998)

Autologous is the gold standard bone graft material, because it is obtained from the patient's own tissue. But the necessity of sample harvesting requires an additional surgical procedure, with well-documented complications and discomfort for the patient. This strategy has the additional disadvantages of quantity restrictions and substantial cost.

An alternative is allogeneic bone grafting, which is obtained from human cadaver or living donors. Unfortunately, this method presents issues of immunogenicity and rejection reactions, possibility of infection transmission and it also has high costs associated (Dimitriou et al., 2011).

There is also the possibility of using bone grafts substitutes made of synthetic or natural biomaterials. But at present there are no heterologous or synthetic bone substitutes available that have superior or even the same biological or mechanical properties compared to bone, hence this is still an ongoing study field (Dimitriou et al., 2011).

Growth factors are natural potent inducers of encochondral ossification. Therefore, a number of bone growth factors (especially BMPs because these are the most potent osteoindictive molecules) are clinically being used to accelerate normal bone healing ectopically or are injected into the fracture site. However, there are several issues about their use, including safety where supraphysiological concentrations of growth factors are needed to obtain the desired osteoinductive effects, the high cost of treatment, and more importantly, the potential for ectopic bone formation (Bailón-Plaza and van der Meulen, 2003; Dimitriou et al., 2011).

With respect to the bone regeneration by local mesenchymal stem cells application strategy, it is fair to say that the role of these cells in fracture repair is still in its infancy, largely due to a lack of studies into the biology of mesenchymal stem cells *in vivo* in the fracture environment.

Low-intensity pulse ultrasound is a fracture therapy that uses a source of mechanical energy transmitted as high-frequency acoustic pressure waves into the biological tissue. The data in the study developed by (Kumagai et al., 2012), suggests that this technique accelerates fracture healing by stimulating the recruitment of osteogenic progenitors cells to the site of bone formation near the fracture site. Although, this is a well known therapy that enables fractures to be treated without surgical invasion, this technique is limited to the amount of residual periosteum at the fracture site.

Bone's piezoelectric behavior and ”streaming potentials” were the fundamental basic concepts that led to the development of electrical bone growth stimulator devices. There are three main clinical methods for bone electric currents administration: direct current, capacitive coupling and inductive coupling (Griffin and Bayat, 2011).

In direct current treatment, an electrical current is produced between a cathode implanted at the fracture site and the anode in the soft tissue nearby. When using this procedure, patient's compliance is minimal. However, drawbacks include the invasive nature of this technique, possibility of infection, risk of soft tissue reaction, prominent or painful implants, and the potential for lead breakage or electrode dislodgement (Griffin and Bayat, 2011; Goldstein et al., 2010).

The capacitive coupling technique consist in generating an electric field between two capacitor plates placed on the opposite sides of the fracture. Unlike the direct current method, this technique is non-invasive but its 24 hours day use creates the potential for decreased compliance and skin irritation from the capacitor plates (Goldstein et al., 2010).

In inductive coupling procedure, a coil attached to an external power source is placed on the fracture site skin surface to generate a magnetic field, which consequently induces a electrical field. The primary advantages of this procedure is that it is non-invasive and painless. However, patients compliance may be a limiting factor in the success of this treatment (Goldstein et al., 2010; Griffin and Bayat, 2011).

Besides all the advantages shown by the clinical electrical stimulation methods described, the mechanism by which the stimulatory effect enhances bone healing still remains unclear. These procedures have also been contraindicated when the fracture gap is wider than 0.5 cm (Griffin and Bayat, 2011; Zamora-Navas et al., 1995).

When after the application of the common clinical practices, the bone healing presents signs of failure, a second costly surgical intervention, aiming to stabilize the fracture, is inevitable (Antonova et al., 2013).

All the clinical methods currently being used to accelerate bone repair present important limitations. This is most probably due to the still surprisingly lack of information available concerning bone regeneration in humans *in vivo* (Dimitriou et al., 2011). Hence, it seems clear that there is a need to develop novel alternatives to complement the standard clinical methods used for tibia fracture-healing regeneration.

4.2 Research

Now-a-days, the concept that proper loading conditions are crucial for bone repair is well accepted. And also that the mechanical stimulation of bone-healing depends on the type, magnitude, rate, duration and timing of initiation of the loading. But in the research field there is a challenge to identify the mechanical environment which is both safe and enhances the repair process. In an attempt to establish the relevant window of bone-repair mechanical stimulation, *in vivo* internal loads acting in long bones during daily activities were considered of special interest in fracture healing research (Wehner et al., 2009; Leung et al., 2009). Albeit, several excellent papers on bone-healing accelerating methods have been written, due to the limited space we will only mention the ones we believe are more relevant.

The immediate consequence of mechanical loading is strain, which is a small deformation throughout the calcified matrix, 1 $\mu\varepsilon$ equals 1 μm of deformation per meter of length (Aarden et al., 2004). Several *in vivo* studies (Kunnel et al., 2002; Minary-Jolandan and Yu, 2009; Duncan and Turner, 1995; Mavčič and Antolič, 2012) showed that the application of static loads to bone tissue has no effect on bone formation. It was estimated that with the cyclic loading type of treatment 27 % of the healing time was saved in comparison to constant compression. Also, both strain magnitude (or amplitude) and strain rate are considered essential parameters in the stimulation process. According to (Rubin et al., 2001), there seems to be a relation between the strain magnitude and the strain rate in cortical bone.

(Fritton et al., 2000) showed that when counting of the daily (12 to 24 hours) strain events, large strains (higher than 1000 $\mu\varepsilon$) occur relatively few times a day, while very small strains (less than 10 $\mu\varepsilon$) occur thousands of times a day. Moreover, in a study developed by (Rubin et al., 2001), very lower magnitude values - less than 10 $\mu\varepsilon$ - combined with high-frequency physiological strain rate (10 to 100 Hz) showed to be capable of stimulating bone growth by doubling its formation rate. These findings allow concluding that low-amplitude high-frequency postural strains due to muscular contractions could be more effective than high-amplitude low-frequency strains due to locomotion in maintaining bone mass. Such behavior might explain why astronauts in a microgravity environment, where the need to maintain posture is absent, lose bone mass despite rigorous exercise or why 3 h/day of quiet standing has been shown to prevent bone loss in bed rest patients (Fritton et al., 2000).

Based on the fact that fracture healing is a regenerative process of osseous tissue, several studies started considering the possible advantages of applying low-magnitude high-frequency strain stimulus to the bone-healing acceleration process. *In vivo* systems for applying loading, such as whole-body vibration and individual limb compressive were tested in order to successfully show the stimulation ability of low-magnitude high-frequency strain stimulus to induce callus formation and mineralization, and hence accelerate fracture healing.

A beneficial effect of low-magnitude high-frequency vibration was reported by (Leung et al., 2009). In their study, osteotomized rat tibiae with intramedular kirschner wires, were stimulated by a vibration platform with 35 Hz, actuating 20 min/day for 5 days a week during a total period study of 9 weeks. This type of stimulating showed a positive osteogenic effect through the formation of larger amount of callus, accelerated callus remodeling and fracture site healing, comparatively to the non-stimulated ones.

(Goodship et al., 2009) reinforce the idea that mechanical stimulus do not need to be large to positively influence the fracture-healing process. In their study, they applied a short duration (17 minutes), extremely low-magnitude (25 $\mu\varepsilon$), high-frequency (30 Hz) interfragmentary axial displacement on a 3 mm osteotomized mid-diaphyseal sheep tibia, using for that purpose a ferroactive shape-memory alloy incorporated into the body of the external fixator. These experiment showed the beneficial effect of low-amplitude high-frequency interfragmentary axial displacements which proved to be able to accelerate the process of bone healing.

In another study, developed by (Tarniţă et al., 2010), osteosynthesis was stimulated by using biocompatible shape memory alloy nitinol-based staples. The staples continuously ensured the return of the pre-strained plate to its original shape and this effect remains as long as the original shape was not reached. The major disadvantage of this solution is the high cost of the medial plate, which is entirely constructed of nitinol, as well as the highly complex procedure of decoupling this central piece.

Indeed, bone tissue is extremely sensitive to physical signals. When load is applied on bone, first it pressurizes the interstitial fluid around the osteocytes, before the fluid is driven to flow. Then the interstitial fluid within the lacuna and canaliculi is driven to flow through the thin layer of non-mineralized pericellular matrix surrounding the osteocytes cell bodies and their dendritic process, toward the Harversian or Volkmann's channels. During mechanical stimulation, since bone is not a continuum material, microstructural inhomogeneities will result in inhomogeneous microstructural strain fields and local tissue strains will be magnified in association with microstructural features (Aarden et al., 2004; Rath Bonivtch et al., 2007; Klein-Nulend et al., 2013; Duncan and Turner, 1995).

The flow of interstitial fluid through the lacunocanalicular network induces shear stress on the cells membranes and provides the mechanism by which osteocytes sense the very small *in vivo* strains of the calcified matrix (Bacabac et al., 2004). According to (Ajubi et al., 1996), osteocytes react to pulsating fluid flow shear stress as low as of 0.5 to 0.02 Pa. These findings may help to explain the fact that low-magnitude, high frequency stimulus can be sense by bone cells during the healing process.

Despite the significant efforts observed in literature, a quantifiable causal relationship between the rate of healing and mechanical stimulus has never been discovered. But, in the latest research studies there seems to be a point of common agreement on the potential of the osteogenetic response to low-magnitude high-frequency strain stimulus in the bone-healing acceleration process. One of the great limitation on the research studies that try defining an appropriate loading profile on bone healing is that although our understanding of bone regeneration at the cellular and molecular level has advanced enormously, coordinate relations between these mechanical variables and a large number of responses at the molecular and cellular level, in conjunction with physiological ones, creates the complex pathways of bone healing that need to be more exhaustively examined (Giannoudis et al., 2007; Bailón-Plaza and van der Meulen, 2003; Comiskey et al., 2010).

Although a variety of clinical procedures and studies have been developed to try to accelerate the fracture healing process, gaps remain in the search of a practical cost-effective strategy that enhances bone healing with well-defined specifications and regimes in each particular patient at a given point in time(Mavčič and Antolič, 2012).

5 METHODOLOGY

In this research study, by recognizing the risks of overloading the healed tissue and inspired by the potential benefits of the omnipresent very low-magnitude, high-frequency stimulus in the human bone functional regime, we decided to complement the commonly used external fixator healing technique with a short period local stimulation, using a small sized piezoelectric actuator in contact with the bone fracture.

The idea is to use an external fixation which confers considerable rigidity to the bone except in the fracture gap regions where it will be mechanically stimulated with a small size piezoelectric actuator thus permitting controlled interfragmentary strain of the fracture.

Based on the strong anabolic effect of low-magnitude, high-frequency mechanical stimulus on bone healing process (see section 4), and considering that during quiet standing, very small ($\ll$5), high frequency strains persistently bombard the skeleton (Huang et al., 1999), we decided to start by testing extremely low magnitude - 5 $\mu\varepsilon$ - and high frequency 20 to 60 Hz - cyclic interfragmentary motion induced by the piezoelectric actuator for 5 days per week for the same short period of 17 minutes - which was used as in prior mechanical stimulation of fracture repair studies performed on animals and humans (Goodship et al., 2009) - on *in vivo* sheep bone models. The low levels of displacement also avoids any type of risk of mechanical failure of the fixation device.

According to the literature (Gardnera et al., 2000), inter-fragmentary stimulus presents a higher influence when applied soon after injury. Therefore, we intent to start applying the piezoelectric stimulus 5 to 7 days after the surgical intervention. The imposed strains will not be detrimental to the tissue differentiation within the callus, since besides the fact that the initial strain magnitude is already very small, stiffness during fracture healing is predicted to increase gradually over time as healing occurs. In the beginning there is only granulation tissue in the fracture gap ending up with a callus ossification which is when the healing is defined as successful. When that happens, the external fixator is removed. Based in (Gardnera et al., 2000) study, at 4 weeks, the central callus barely changed, the adjacent and peripheral callus calcified rapidly and are able to support compressive loads by 8 weeks. Between 8 and 12 weeks minimal changes in the material properties and shape should be expected and from 12 to 16 weeks, the adjacent and peripheral callus could increasly bear compressive load. The typical healing period of human tibia is 16 weeks.

The ultimate strain which can be withstood also decreases from very high strains in hematoma and granulation tissue, decreasing to mature bone which can be damaged by as little as 2% strain. It is believed that fracture healing will not occur when the strain at the fracture gap exceeds 10 %. There is no harm expected as the healing phases progresses (Goodship et al., 2009; Perren, 1979; Hak et al., 2010; Wu et al., 2013; Lacroix and Prendergast, 2002).

The use of a piezoelectric actuator in this particular situation presents several advantages when compared to other superior motive power actuators in terms of size, driving speed, and control of microscopic displacement. For example, hydraulic actuators have excellent force and displacement capacities but only at very low frequencies. Shape memory alloy actuators are similar in that they can generate a large displacement and force, but their actuation frequency is extremely poor. On the other hand, electromagnetic actuators have good frequency range. Linear induction actuators have excellent force and stroke output; however, they are generally heavy and require significant electrical current. Piezoelectrics are known for their excellent operating bandwidth and can generate large forces in a compact size (Tanaka, 1999; Niezrecki et al., 2001).

The piezoelectric actuator reduced size allows its implementation with minimal additional alteration of surrounding tissue, causing no discomfort to the patients and may be applicable to a range of fixation devices.

By analyzing the principal parameters that characterize any linear actuator - like displacement, force, frequency, size, weight, electrical input power and price - and bearing in mind that a compromise needs to be done in the piezoelectric selection, since actuators which usually perform well in some of these categories are typically poor in others, a few candidates will be selected and tested in the scope of this research.

Before *in vivo* testing is performed on sheep midshaft tibia, the piezoelectric actuators selected will be tested *in vitro* through their montage on human and sheep wet bone fragments fixed as a cantilever beam. We choose to use wet bone samples since dry bone cannot bear acceptable results as considering a living tissue. A good possible evaluation technique would be scanning laser doppler vibrometry which is able to perform accurate of displacement and strain fields.

At the same time, a proper coating polymer must be selected, to ensure biocompatibility of the actuator and integrity during service. The polymeric coating should be more flexible than piezoelectric actuators to not affect adversely the piezoaction. The piezoelectric actuator with and without a polymeric biocompatible coating will be tested *in vitro* according to International Standard (ISO 10993-5, 2009) for cell adhesion, cell proliferation and viability. Based on the literature review, the *in vitro* tests should be performed by using an osteoblastic cell culture. These *in vitro* tests will allow evaluating the level of material cytotoxicity, but area limited to acute studies of the effects of toxicity due to the relatively short lifespan of cultured cells and it does not guarantee that the actuator will behave in a biocompatible manner.

Later, the actuator will be studied *in vivo* in experimental animals. So far, the sheep is the prime choice for animal model since the general mechanisms of bone repair seem to be similar to human. They are docile, easy to handle and house, relatively inexpensive, available at a large numbers and they also spontaneously ovulate. The assessment of fracture healing could be performed by histological, imaging (for example, computed tomography images) and biomechanical testing (for example determination of fracture stiffness) (Claes et al., 2012; Saffar et al., 2009; Gao et al., 2013).

6 EXPECTED OUTCOME

Enhancement of fracture healing has been one of the major goals in modern fracture management because it's economic and clinical importance for the health care system and patient recovery and regain of functions after fracture (Leung et al., 2009; Bailón-Plaza and van der Meulen, 2003).

We do believe that if the natural healing process is not compromised by the presence of the piezoelectric actuator for example due to a toxicity reaction - and if ideal biological (i.e. in terms of traumatized tissue revascularization and the inflammatory process) and mechanical conditions for repair are created accelerated fracture healing may be achieved. The low amplitudes of the signals created by the actuator appear to be well below those which may cause risk to the regenerate tissue.

While the health-care system is under increasing financial burden, addressing bone healing in a timely manner, may not only free up scarce health-care resources and save money, but also improve patient outcomes.

This type of stimulation seems to ensure a low risk to the fracture gap which oppositely may arise during functional loading with low rigidity external fixator.

We expect to develop a piezoelectric actuator capable of accelerating bone healing on tibia mid-shaft fractures and during this process gain a better understanding of the mechanical stimulus parameter values needed to accelerate bone healing and hopefully contribute in some way to explain the still not totally understood complex bone healing process.

ACKNOWLEDGEMENTS

We acknowledge support from the Foundation for Science and Technology (FCT), Lisbon, Portugal, through Project PEst-C/CTM/LA0025/2013 and research grant SFRH/BD/87089/2012 (NR).

REFERENCES

Aarden, E. M., Nijweide, P. J., and Burger, E. H. (2004). Function of osteocytes in bone. *Journal of Cellular Biochemistry*, 55(3):287–299.

Ajubi, N., Klein-Nulend, J., Nijweide, P., Vrijheid-Lammers, T., Alblas, M., and Burger, E. (1996). Pulsating fluid flow increases prostaglandin production by cultured chicken osteocytes - a cytoskeleton-dependent process. *Biochemical and Biophysical Research Communications*, 225(1):62–68.

Antonova, E., Le, T. K., Burge, R., and Mershon, J. (2013). Tibia shaft fractures: costly burden of nonunions. *BMC Musculoskeletal Disorders*, 14(1):42.

Audigé, L., Griffin, D., Bhandari, M., Kellam, J., and Rüedi, T. P. (2005). Path analysis of factors for delayed healing and nonunion in 416 operatively treated tibial shaft fractures. *Clinical Orthopaedics and Related Research*, 438:221–232.

Bacabac, R. G., Smit, T. H., Mullender, M. G., Dijcks, S. J., Van Loon, J. J., and Klein-Nulend, J. (2004). Nitric oxide production by bone cells is fluid shear stress rate dependent. *Biochemical and Biophysical Research Communications*, 315(4):823–829.

Bacon, G. and Goodship, A. (2007). The healing process for fractured tibia bones of sheep studied by neutron diffraction. *Journal of Applied Crystallography*, 40(2):349–353.

Bailón-Plaza, A. and van der Meulen, M. C. (2003). Beneficial effects of moderate, early loading and adverse effects of delayed or excessive loading on bone healing. *Journal of Biomechanics*, 36(8):1069–1077.

Boerckel, J. D., Kolambkar, Y. M., Stevens, H. Y., Lin, A. S., Dupont, K. M., and Guldberg, R. E. (2012). Effects of in vivo mechanical loading on large bone defect regeneration. *Journal of Orthopaedic Research*, 30(7):1067–1075.

Claes, L., Recknagel, S., and Ignatius, A. (2012). Fracture healing under healthy and inflammatory conditions. *Nature Reviews Rheumatology*, 8(3):133–143.

Comiskey, D., MacDonald, B., McCartney, W., Synnott, K., and O'Byrne, J. (2010). The role of interfragmentary strain on the rate of bone healinga new interpretation and mathematical model. *Journal of Biomechanics*, 43(14):2830–2834.

Dimitriou, R., Jones, E., McGonagle, D., and Giannoudis, P. V. (2011). Bone regeneration: current concepts and future directions. *BMC medicine*, 9(1):66.

Duncan, R. and Turner, C. (1995). Mechanotransduction and the functional response of bone to mechanical strain. *Calcified Tissue International*, 57(5):344–358.

Fritton, S. P., J McLeod, K., and Rubin, C. T. (2000). Quantifying the strain history of bone: spatial uniformity and self-similarity of low-magnitude strains. *Journal of Biomechanics*, 33(3):317–325.

Gao, J., Gong, H., Huang, X., Fang, J., Zhu, D., and Fan, Y. (2013). Relationship between microstructure, material distribution, and mechanical properties of sheep tibia during fracture healing process. *International Journal of Medical Sciences*, 10(11):1560.

Gardnera, T., Stoll, T., Marks, L., Mishra, S., and Knothe Tate, M. (2000). The influence of mechanical stimulus on the pattern of tissue differentiation in a long bone fracturean fem study. *Journal of Biomechanics*, 33(4):415–425.

Giannotti, S., Bottai, V., DellOsso, G., Pini, E., De Paola, G., Bugelli, G., and Guido, G. (2013). Current medical treatment strategies concerning fracture healing. *Clinical Cases in Mineral and Bone Metabolism*, 10(2):116.

Giannoudis, P. V., Einhorn, T. A., and Marsh, D. (2007). Fracture healing: the diamond concept. *Injury*, 38:S3–S6.

Goldstein, C., Sprague, S., and Petrisor, B. A. (2010). Electrical stimulation for fracture healing: current evidence. *Journal of Orthopaedic Trauma*, 24:S62–S65.

Goodship, A. E., Lawes, T. J., and Rubin, C. T. (2009). Low-magnitude high-frequency mechanical signals accelerate and augment endochondral bone repair: Preliminary evidence of efficacy. *Journal of Orthopaedic Research*, 27(7):922–930.

Griffin, M. and Bayat, A. (2011). Electrical stimulation in bone healing: critical analysis by evaluating levels of evidence. *Eplasty*, 11:303–353.

Hak, D. J., Toker, S., Yi, C., and Toreson, J. (2010). The influence of fracture fixation biomechanics on fracture healing. *Orthopedics*, 33(10):752–755.

Heckman, J. D. and Sarasohn-Kahn, J. (1997). The economics of treating tibia fractures. *Bulletin Hospital for Joint Diseases*, 56(1):63–72.

Huang, R. P., Rubin, C. T., and McLeod, K. J. (1999). Changes in postural muscle dynamics as a function of age. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 54(8):B352–B357.

ISO 10993-5 (2009). Biological evaluation of medical devices Part 5: Tests for *in vitro* cytotoxicity.

Klein-Nulend, J., Bakker, A. D., Bacabac, R. G., Vatsa, A., and Weinbaum, S. (2013). Mechanosensation and transduction in osteocytes. *Bone*, 54:2.

Kumagai, K., Takeuchi, R., Ishikawa, H., Yamaguchi, Y., Fujisawa, T., Kuniya, T., Takagawa, S., Muschler, G. F., and Saito, T. (2012). Low-intensity pulsed ultrasound accelerates fracture healing by stimulation of recruitment of both local and circulating osteogenic progenitors. *Journal of Orthopaedic Research*, 30(9):1516–1521.

Kunnel, J., Gilbert, J., and Stern, P. (2002). In vitro mechanical and cellular responses of neonatal mouse bones to loading using a novel micromechanical-testing device. *Calcified Tissue International*, 71(6):499–507.

Lacroix, D. and Prendergast, P. (2002). Three-dimensional simulation of fracture repair in the human tibia. *Computer Methods in Biomechanics & Biomedical Engineering*, 5(5):369–376.

Leung, K. S., Shi, H. F., Cheung, W. H., Qin, L., Ng, W. K., Tam, K. F., and Tang, N. (2009). Low-magnitude high-frequency vibration accelerates callus formation, mineralization, and fracture healing in rats. *Journal of Orthopaedic Research*, 27(4):458–465.

Malizos, K. N., Hantes, M. E., Protopappas, V., and Papachristos, A. (2006). Low-intensity pulsed ultrasound for bone healing: an overview. *Injury*, 37(1):S56–S62.

Mavčič, B. and Antolič, V. (2012). Optimal mechanical environment of the healing bone fracture/osteotomy. *International Orthopaedics*, 36(4):689–695.

Minary-Jolandan, M. and Yu, M.-F. (2009). Nanoscale characterization of isolated individual type i collagen fibrils: polarization and piezoelectricity. *Nanotechnology*, 20(8):085706.

NHS-Portugal (2012). Hospital morbidity - Perliminary data. Portugal: National Health Service.

Niezrecki, C., Brei, D., Balakrishnan, S., and Moskalik, A. (2001). Piezoelectric actuation: state of the art. *The Shock and Vibration Digest*, 33:269.

Perren, S. (1979). Physical and biological aspects of fracture healing with special reference to internal fixation. *Clinical Orthopaedics and Related Research*, 138:175–196.

Rath Bonivtch, A., Bonewald, L. F., and Nicolella, D. P. (2007). Tissue strain amplification at the osteocyte lacuna: a microstructural finite element analysis. *Journal of biomechanics*, 40(10):2199–2206.

Roseiro, L. M., Neto, M., Amaro, A., Leal, R. P., and Samarra, M. C. (2013). External fixator configurations in tibia fractures: 1d optimization and 3d analysis comparison. *Computer Methods and Programs in Biomedicine*, 113:360–370.

Rubin, C. T., Sommerfeldt, D. W., Judex, S., and Qin, Y.-X. (2001). Inhibition of osteopenia by low magnitude, high-frequency mechanical stimuli. *Drug Discovery Today*, 6(16):848–858.

Saffar, K. P., JamilPour, N., and Rajaai, S. M. (2009). How does bhe bone shaft geometry affect its bending properties? *American Journal of Applied Sciences*, 6(3):463.

Smith, G. K. (1985). *Textbook of small animal orthopedics*. Lippincott Williams & Wilkins.

Stevenson, S. (1998). Enhancement of fracture healing with autogenous and allogeneic bone grafts. *Clinical Orthopaedics and Related Research*, 355:S239–S246.

Tanaka, S. M. (1999). A new mechanical stimulator for cultured bone cells using piezoelectric actuator. *Journal of Biomechanics*, 32(4):427–430.

Tarniţă, D., Tarniţă, D., Popa, D., Grecu, D., TarniŢĂ, R., Niculescu, D., and Cismaru, F. (2010). Numerical simulations of human tibia osteosynthesis using modular plates based on nitinol staples. *Romanian Journal of Morphology and Embryology*, 51(1):145.

Wehner, T., Claes, L., and Simon, U. (2009). Internal loads in the human tibia during gait. *Clinical Biomechanics*, 24(3):299–302.

Wu, N., Lee, Y.-c., Segina, D., Benjamin, N., Boulanger, L., Murray, H., and Wilcox, T. (2013). Cost savings associated with the use of electrical bone growth stimulation to treat diabetic patients in the us with fracture nonunion. *Journal of Diabetes & Metabolism*, 4:4.

Zamora-Navas, P., Verdera, A., Lorenzo, R., Ayuso, J., and Reina, M. (1995). Electrical stimulation of bone nonunion with the presence of a gap. *Acta Orthopaedica Belgica*, 61:169–176.