Influence of high-frequency cyclical stimulation on the bone fracture-healing process: mathematical and experimental models

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Mechanical stimulation affects the evolution of healthy and fractured bone. However, the effect of applying cyclical mechanical stimuli on bone healing has not yet been fully clarified. The aim of the present study was to determine the influence of a high-frequency and low-magnitude cyclical displacement of the fractured fragments on the bone-healing process. This subject is studied experimentally and computationally for a sheep long bone. On the one hand, the mathematical computational study indicates that mechanical stimulation at high frequencies can stimulate and accelerate the process of chondrogenesis and endochondral ossification and consequently the bony union of the fracture. This is probably achieved by the interstitial fluid flow, which can move nutrients and waste from one place to another in the callus. This movement of fluid modifies the mechanical stimulus on the cells attached to the extracellular matrix. On the other hand, the experimental study was carried out using two sheep groups. In the first group, static fixators were implanted, while, in the second one, identical devices were used, but with an additional vibrator. This vibrator allowed a cyclic displacement with low magnitude and high frequency (LMHF) to be applied to the fractured zone every day; the frequency of stimulation was chosen from mechano-biological model predictions. Analysing the results obtained for the control and stimulated groups, we observed improvements in the bone-healing process in the stimulated group. Therefore, in this study, we show the potential of computer mechano-biological models to guide and define better mechanical conditions for experiments in order to improve bone fracture healing. In fact, both experimental and computational studies indicated improvements in the healing process in the LMHF mechanically stimulated fractures. In both studies, these improvements could be associated with the promotion of endochondral ossification and an increase in the rate of cell proliferation and tissue synthesis.

Keywords: bone healing; low-magnitude high-frequency mechanical stimulation; mechano-biological model

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1. Introduction

Fracture healing is a complex process that involves decision-making in many aspects, such as the optimum external fixator to use or the specific treatment to follow. As this process is mechanically driven, both experimental and computational studies have been performed in an attempt to improve the understanding of this process. In fact, external fixators have usually been designed from biomechanical [1] and clinical [2] considerations; similarly, treatments for bone healing are based on either mechanical or biological processes [3].

This mechanical and biological coupling has motivated the development of more complex computational models that are normally known as mechano-biological models [4]. These models have succeeded in predicting the outcome of fracture healing and distraction osteogenesis under different loading regimes or external fixators. This highlights the close relationship that is needed between engineers and clinicians. For a complete review of fracture-healing biology and models, see Geris et al. [5].

Nevertheless, mechano-biological models have not normally been used to define and drive treatments or experiments. Therefore, in this study, as a further step we propose the design of a new bone-healing treatment based on the prediction of a mechano-biological model [6]. This treatment was applied in a sheep animal model in order to evaluate its functionality. With this aim, we initially and briefly describe the use of computer biomechanical-based models for the design of clinical treatments and protheses. Next, mechano-biological-based models and applications are widely revised, distinguishing between tissue differentiation theories and their application to bone healing and distraction osteogenesis. And, finally, a revision of the influence of the stimulation type (mechanical, biophysical, biochemical or low-magnitude high-frequency (LMHF) mechanical stimulation) on the course of bone healing is also discussed.

2. Background

(a) Biomechanical computer design of clinical treatments and implants

Biomechanical principles have been widely used in the design of implants and orthopaedic treatments such as fracture healing. Here, only a brief revision of some biomechanical computer models is shown. Models have evolved from the first analytical developments [7,8] and experimental studies [9] to the use of computer-oriented techniques such as the finite-element method. Finite-element models, probably initiated by Brekelmans et al. [10], have become increasingly widespread in recent decades [11,12]. In fact, finite-element models have been used in orthopaedics in the design of prostheses such as for the knee [13], hip [14], pelvis [15], femur [16], tibia [17], hand [18] and foot [19]. In all these studies, the stress and strain states in one or several fixed time points are analysed to correlate them with the suitability of the implant or treatment.

(b) Mechano-biological models of bone healing and their applications

Given that this study aims to demonstrate the potential to drive experiments through mechano-biological models, a wide revision has been developed.
This revision has been divided by distinguishing between theories for tissue differentiation and evolutionary models of bone healing and distraction osteogenesis.

(i) Tissue differentiation theories

Many researchers have attempted to establish the relationship between the mechanical level of an undifferentiated tissue and the ultimate tissue phenotype formed. Pauwels [20] proposed the first rigorous mechano-regulation theory which hypothesized that the invariants of the strain and stress tensors guided the differentiation pathway. Later, a simpler idea, strain-based, was proposed by Perren [21] and Perren & Cordey [22] to predict the evolution of tissues in a bone fracture gap. They developed the concept of ‘interfragmentary strain’. Also based on the ideas of Pauwels, Carter [23] proposed a combination of octahedral shear stress and hydrostatic stress for his differentiation theory [24]. Unlike Pauwels, Carter [23] recognized the influence of vascularization and proposed that low oxygen tension favours chondrogenesis. Claes & Heigele [25], using an elastic finite-element model, proposed a mechano-regulation theory similar to that of Carter [23], but presented in quantitative terms. Unlike the previous models, Prendergast et al. [26] developed a mechano-regulation concept assuming that tissues are poroelastic, and thus comprise both fluid and solid phases. They proposed a mechano-regulation pathway regulated by two biophysical stimuli, the octahedral shear strain of the solid and the interstitial fluid velocity relative to the solid. Finally, Gómez-Benito et al. [27] and García-Aznar et al. [28] proposed a mathematical model exclusively driven by the second invariant of the deviatoric strain tensor (proportional to the octahedral shear strain), assuming that tissues are poroelastic.

(ii) Evolutionary models of bone healing and distraction osteogenesis

The previous tissue differentiation theories have been successfully used in several computational studies to reproduce the main patterns of fracture healing in specific mechanical environments. For instance, Lacroix & Prendergast [29] have developed a bone-healing model based on the mechano-regulation theory previously formulated by Prendergast et al. [26]. This model was then taken up by Andreykiv et al. [30], Isaksson et al. [31], Byrne [32] and Boccaccio et al. [33]. Gómez-Benito et al. [27] and García-Aznar et al. [28] succeed in simulating the main processes that occur during fracture healing and improved on existing numerical models of fracture healing by providing a mathematical formulation of callus growth.

All these models are related to mechanical stimuli, which are definitely perceived by cells whose biological sensing and signalling activities are implicitly assumed but not directly considered. In contrast, other mechano-biological models have introduced biological factors in addition to the mechanical ones. For instance, Ament & Hofer [34] proposed a biomechanical model based on a set of fuzzy logic rules to describe the tissue transformation during healing. They introduced a biological factor that accounted for the vascularization in addition to the mechanical stimulus (the strain energy density). This approach has been taken up by Simon et al. [35,36] and Shefelbine et al. [37], who simulated, using finite-element analysis and fuzzy logic, fracture healing, based on local mechanical...
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factors, as described by Claes & Heigele [25], and the local vascularity. Recently, Chen et al. [38] introduced the supply of nutrients to the previous computational algorithm. Bailón-Plaza & van der Meulen [39] presented the first two-dimensional temporo-spatial mathematical model of the bone-healing process that considers the effect of growth factors. This model was later modified to incorporate the effects of mechanical stimulation on cell differentiation [40] and angiogenesis and directed cell migration with a continuous mathematical model [41] that has been recently improved by introducing a deterministic hybrid modelling framework [42].

Fracture and distraction repair proceed through the same cellular healing process [43], and, therefore, most existing mechano-biological models of distraction osteogenesis are based on existing models of fracture healing, either on the mechano-regulation theory presented earlier by Prendergast et al. [26,44–46] or on that proposed by Gómez-Benito et al. [27,47,48].

(c) Experimental analysis of the influence of the load type in bone healing: low-magnitude high-frequency mechanical stimulus

Although it seems that there exists an optimal mechanical environment for each specific fracture to heal [49], more insight is needed in order to define the type, magnitude and time history of the optimal mechanical load for each kind of fracture. Different authors have tried to identify the influence of the mechanical environment on fracture healing, by determining the effect of the different load types (axial, bending, shear and torsion) on the outcome of the process. Nevertheless, we should mention the difficulty in avoiding an axial load component due to the animal’s weight; this compression component will represent a stimulus for the healing process [50]. In addition, the axial interfragmentary movement reduces the gap size, promoting the healing process [51]. Finally, it is important to underline the difficulty in quantitatively comparing the different loading conditions. For example, when a bone is under compressive motion, the gap distance is shortened; in contrast, tensile motion lengthens it [52].

(i) Axial loads

It is generally accepted that some amount of axial movement between fracture fragments stimulates callus formation and favours the quality and quantity of the new tissue [53]. In contrast, excessive interfragmentary movement delays the healing process and may result in the non-union of bone fragments, causing, in some cases, pseudoarthrosis [51].

The effect of tensile axial loads on the process of distraction osteogenesis has been widely studied. Through the application of tensional stress, a dynamic microenvironment is created, with the formation of tissue parallel to the traction vector [54]. This environment stimulates changes at the cellular and sub-cellular levels [55]. There is a prolongation of angiogenesis with an increase in tissue oxygenation and an increase in the proliferation of spindle-shaped fibroblast-like cells. This type of spindle-shaped cell and its secreted collagen, mostly type I, becomes longitudinally oriented along the axis of tension. The collagen fibres of the fibrous interzone seem to play a critical role in bone formation in tensile environments by providing mechanical stability and facilitating the formation of new bone.
of early bone spicules [2,56]. It has also been observed that these cells can express osteocalcin, osteopontin and alkaline phosphate, which is evidence of some osteoblastic differentiation.

(ii) Shear loads

Bishop et al. [57,58] compared the bone-healing process under pure interfragmentary torsional shear, pure axial compression and no applied motion. The torsional load group resulted in more intercortical mineralized callus and bridging than the axial group, and more callus area than the non-stimulated group. Through this experiment, they analysed the effect of octahedral normal and shear strains on fracture healing. The mass of the periosteal callus in the animals subjected to a history of torsional load was twice the mass of the periosteal callus in the animals subjected to axial load. This fact may justify the choice of the second invariant of the deviatoric strain tensor as the mechanical stimulus that controls the mechano-biological processes involved in bone regeneration.

Nevertheless, the influence of shear interfragmentary movements on fracture healing is still controversial [59,60]. On the one hand, Augat et al. [60] demonstrated that shear loads delay the healing process, and could result in a non-union. In their experiments they compared the effect of axial and shear loads on the fracture callus of sheep, for interfragmentary movements of similar magnitude. All fractures that healed under axial load resulted in successful union; nevertheless, only partial healing was achieved under shear loads. On the other hand, Park et al. [59] postulated that shear loads are beneficial for bone healing; when small shear strains are permitted the healing process is accelerated, generating different effects on tissues in the interfragmentary space.

(iii) Bending loads

Hente et al. [61] studied the healing process under pure bending loads. Important differences were observed on the side of the callus subjected to compressive and tension loads in both shape and tissue distribution; indeed, the tension side was bigger than the compression one.

(iv) Additional external stimulation

Different external stimuli have been applied to stimulate and accelerate bone healing [62], such as low-intensity ultrasound, electromagnetic fields, electric currents of low potential and, more recently, LMHF mechanical stimulations. Results support the assumption of the beneficial effects of external biophysical stimulations on bone healing. However, there are still only a few commercial devices able to apply external mechanical stimulations [62]. In contrast to biochemical stimulations [63], LMHF mechanical stimulation is safer.

The idea of LMHF mechanical stimulation was originally proposed to mimic muscle activity, which presents a signal of strain lower than 10 με in a range of frequencies of 10–50 Hz [64]. In fact, these researchers assumed that age reduces muscle activity [64] and this effect could be one of the factors causing bone mass...
loss. Rubin et al. [64] determined that the muscle activity in frequencies higher than 20 Hz decreases by a factor of three in elderly people compared with young adults.

LMHF mechanical stimulation with a frequency of 30–35 Hz has been applied to stimulate fracture healing in long bones of healthy and osteoporotic rats [65–67] and sheep [68]. It also has beneficial effects on the healing of non-weight-bearing bone defects in rats [69] and in distraction osteogenesis [70]. A frequency of 45 Hz was used to stimulate scaffolded and non-scaffolded cranial defects in rats, showing a clear beneficial effect with respect to the non-stimulated defects [71]. This effect was even greater when a scaffold was present. Nevertheless, lower frequencies of stimulation (20 Hz) have been demonstrated to have no beneficial effects on bone healing [72].

The idea of applying LMHF to stimulate bone matrix production is not new. It has been previously applied to strengthen healthy and osteoporotic bones [64, 73] and on the growing skeleton [74]. Regarding the beneficial effects on bone mass, higher frequencies of stimulation have been tested (90 Hz), showing a much more beneficial effect than moderate frequencies of stimulation [75].

As it is still not clear which is the most appropriate frequency of stimulation, in this study, we used a mechano-biological model of tissue differentiation [26] to predict the most appropriate frequency of stimulation [6], and we applied it to an animal experiment on sheep to test the reliability of the proposed hypothesis in the mechano-biological model.

3. Mathematical modelling and experiments

The main aim of this study was to design an external fixator that is able to monitor and stimulate fracture healing. A fixator was designed, following biomechanical and mechano-biological principles, and was implanted in sheep in order to evaluate its functionality (see the work scheme summarized in figure 1). In fact, this design was developed using the following protocol:

— **Biomechanical functionality.** The geometrical configuration of the fixator was defined in order to promote natural healing. Finite-element models were used for its design under biomechanical principles.

— **Design of the external device for mechanical stimulation.** After validation of the biomechanical design, we decided to carry out active treatment of the fracture in order to reduce the healing time and improve the quality of bone regeneration. To do so, we incorporated in the fixator a small dimension motor able to stimulate the fracture by LMHF mechanical stimulation.

— **Design of the fixator based on mechano-biological modelling.** As there is some controversy about the most appropriate frequency of stimulation to favour bone healing, we performed a mechano-biological computational analysis to determine the effect of the frequency of stimulation on bone fracture healing [6].

— **Experimental validation.** Experiments were performed to corroborate the findings of the biomechanical and mechano-biological models.
More details of all these steps for both computational and experimental studies are described in order to achieve the main objective of this work: the design of an active fixator from mechano-biological mathematical models.

(a) Biomechanical model: fixator design

First, we designed an external static fixator from a biomechanical perspective [1]. The mechanical environment in the fracture gap was previously analysed at different time points in the healing process by means of static finite-element analyses (figure 2). Different mechanical tests were computationally simulated in the fixator bone system. The same simulated mechanical tests were performed in a mechanical test Instron machine to correctly calibrate the system. An appropriate mechanical environment was used at each time point. In this way, we designed a fixator that ensured a maximum interfragmentary strain of 10 per cent within the fracture gap [1]. Design criteria also included low weight of the fixator, ease of implantation, biocompatibility and comfort for the animal during the surgery and recovery. The fixator incorporated a monitoring device to quantify the fracture-healing process. This specifically designed fixator was mechanized and implanted in a group of sheep (control or static group). These sheep were monitored, and successful healing was achieved in all the animals.

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max. principal strain
- 3.000e–01
- 7.000e–04
- 6.300e–04
- 5.600e–04
- 4.900e–04
- 4.200e–04
- 3.500e–04
- 2.800e–04
- 2.100e–04
- 1.400e–04
- 7.000e–05
- 0.000e+00

Figure 2. Maximum principal strain in the tibia and external fixator 1 day after surgery.

*(b) Mechano-biological models: a computational prediction of the frequency of stimulation*

The influence of the frequency of the external load on the mechanical stimulus in the callus was computationally analysed [6] to determine the effect of LMHF mechanical stimulation on the healing outcome. An axisymmetric simplified model of the fracture callus in the metatarsus of a sheep was simulated [25] at two different time points, one and four weeks after fracture. The one-week callus was assumed to be fully formed by granulation tissue and the four week callus was formed by immature bone, cartilage and granulation tissue (see figure 3). All tissues in the callus were assumed to be poroelastic [76]. The tissues under consideration were: granulation tissue (Young’s modulus ($E$) 0.2 MPa, Poisson’s ratio ($\nu$) 0.167 and permeability ($k$) $9.8 \times 10^{-8}$ mm seg$^{-1}$); cartilage ($E = 10$ MPa, $\nu = 0.167$ and $k = 4.9 \times 10^{-8}$ mm seg$^{-1}$); immature bone tissue ($E = 1000$ MPa, $\nu = 0.3$ and $k = 9.8 \times 10^{-7}$ mm seg$^{-1}$) and cortical bone tissue ($E = 20 000$ MPa, $\nu = 0.3$ and $k = 9.8 \times 10^{-11}$ mm seg$^{-1}$) [6]. A displacement of 0.02 mm was applied at the top of the cortical bone at different frequencies of stimulation (1, 50 and 100 Hz) in order to evaluate their impact on tissue differentiation following the theory proposed by Prendergast et al. [26]. Nevertheless, it is important to note that this displacement stimulus was applied for 15 min, which represents different numbers of load cycles, i.e. 90 000, 45 000 and 900, respectively. The computational model used here is the simplest model to quantify the effect of LMHF mechanical stimulation, although it is not able to represent the complete in vivo situation. To do so, a more realistic three-dimensional geometry should be included as well as more complex loading regimes. Figure 3 shows the evolution of the magnitudes of some mechanical variables when applying an interfragmentary displacement of 0.02 mm at these frequencies and their
Figure 3. (a) Tissue distribution and boundary conditions in the one- and four-week calluses (adapted from Claes & Heigele [25]). (b) Evolution of the mean mechanical stimulus at one point in the gap (see (a)) when stimulating with different frequencies (1, 50 and 100Hz) for 15 min.

variations at two stages after the osteotomy. The fluid flow velocity varies in amplitude when the frequency of the external stimulus changes, whereas the other mechanical variables (octahedral strain and second invariant of the deviatoric strain tensor) are just slightly modified. These changes in the fluid flow velocity may induce movement of waste, nutrients or growth factors, which could also contribute to stimulating cellular activity (differentiation, proliferation, migration) by means of changes in the mechanical environment of the callus. As far as we know, the only differentiation law which takes into account the frequency of stimulation is the one proposed by Prendergast et al. [26], by means of the fluid flow since the other mechanical variables are just slightly modified when the frequency of stimulation is changed. Variations in this mechanical variable are important mainly for frequencies of stimulation higher than 50Hz (figure 4). According to the differentiation rule proposed by Prendergast et al. [26], an increase in the frequency of stimulation may promote chondrogenesis and endochondral ossification. As our aim was to improve the bone-healing process in our experiments, we decided to stimulate the fracture site with a frequency higher than 50Hz. As previous experimental studies have used frequencies up
to 90 Hz [75] to stimulate bone mass formation in osteoporotic conditions, we decided to use this frequency. Thus, we stimulated the fracture with a frequency of 90 Hz for 15 min twice a day. The period of stimulation was chosen based on the computational analysis as the mechanical stimulus stabilizes for a period of stimulation longer than 15 min (figure 3). We further performed an eigenmode analysis of the bone and the external fixator at different time points in the healing process in order to avoid stimulation at the natural frequencies of the bone–fixator system.

(c) Experimental follow-up

A monolateral external fixator, described previously [1], was developed in order to study experimentally the process of fracture healing. This fixator allowed us to monitor telemetrically the repair process in osteotomized long bones, correlating the load transmission through the main body of the system to the increasing stiffness of the regenerated bone. The monolateral external fixator that was developed was further improved to induce an interfragmentary displacement in the osteotomized gap. This displacement was generated by means of vibration produced by a small electronic cylindrical motor (10 mm diameter and 20 mm long) to obtain a LMHF displacement by a controlled stimulus [77]. This motor was designed to always have the same excitation frequency. The interfragmentary movement applied by the motor corresponds to 1 per cent, which is regulated by the input power limitation of the battery, keeping the frequency the same. A three-axis accelerometer was also included inside the fixator. This sensor, after real-time post-processing, could provide the actual displacement of the fixator during the stimulation.

Animals selected for the study were adult ‘Rasa Aragonesa’ female sheep. The gap size was 2 mm and the monolateral external fixator was attached medially to the transversally osteotomized right tibiae [1]. Sheep were divided into two groups of two sheep in each group: a stimulated group and a static or control

Figure 4. Differentiation theory proposed by Prendergast et al. [26]. The area outlined by the dashed line indicates the peak mechanical variables (octahedral shear strain and fluid flow) at all points in the fracture callus for a mechanical stimulation of 0.02 mm and frequency of 50 Hz in the one-week callus. Arrows show the tendency when the frequency \( F \) of stimulation is varied.
group. The former received a daily stimulation of 90 Hz for 15 min twice a day as the mechano-biological model had indicated (stimulated), while the latter did not receive any external mechanical stimulation (static).

The fracture-healing outcome was evaluated by means of the force through the fixator each day. Animals were induced to walk for 10 min each day and the load through the fixator was monitored during this period. The mean maximum axial load through the fixator at each step of walking was evaluated. Results for both groups are shown in figure 5. We should mention that the variations in the load through the fixator during the first days after fracture could be attributed to adaptation to fixator. Even if a sheep walked normally during the first days after fracture there was still no regular step for the sheep. Regarding the evolution of this load until day 15 after osteotomy, most of the load went through the fixator in both the static group and the stimulated one. However, this load decreased quickly from this time point until it reached 20 per cent of the body weight of the animal, a value that did not significantly vary during the following days. Thus, we assumed that, if the stiffness of the tissues does not vary significantly, tissues of the callus are mainly mineralized. This assumption was corroborated by radiographs of the injured leg. This occurred 30 days and 24 days after fracture for the static and stimulated sheep, respectively. The quicker reduction of the load through the fixator in the stimulated sheep compared with the static sheep indicates an acceleration of the healing process in the stimulated sheep. Several image tests (radiographs during healing and micro-computed tomography after the sheep were killed) were performed in the operated tibiae. These tests indicate complete bony union in both groups at the time the sheep were killed. After death, torsion tests were performed in the healed calluses. The control calluses showed a mean torsional stiffness of 0.8 Nm per degree, while the average torsional stiffness of the stimulated group was 2.2 Nm per degree.

All these tests indicate a better quality of regenerated bone for the stimulated sheep. These experimental results corroborate those predicted by the mechano-biological model: stimulation by a LMHF mechanical stimulus with a frequency

Figure 5. Evolution of mean maximum walking axial loads through the external fixator normalized to the animal body weight (BW) in the control and stimulated groups (filled grey circles, control; filled black circles, stimulated).
of 90 Hz improves the healing outcome, probably by means of the promotion of chondrogenesis and endochondral ossification.

4. Concluding remarks

Both experimental and computational studies indicated improvements in the healing process with LMHF mechanically stimulated fractures. On the one hand, the computational mechano-biological simulation showed an improvement in the bone-healing process when LMHF mechanical stimulation was applied to the healing bone at frequencies higher than 50 Hz. This is attributed to changes in the interstitial fluid flow velocity, which would promote endochondral ossification (following the differentiation rule proposed by Prendergast et al. [26]) and may increase cell activity (rates of cell proliferation, migration, differentiation and tissue synthesis) [6]. The LMHF mechanical signal could also be directly sensed by cells and could change its response. On the other hand, the experiments confirmed this improvement in the healing process by an acceleration of one week in the healing process in the group stimulated by LMHF mechanical displacement at a frequency of 90 Hz compared with the non-stimulated control group. The improvement was further confirmed by the better quality of the callus in the stimulated group than that in the control group.

In addition, the results of the mechano-biological model (figure 6) could also help to explain why no improvement was noted in the bone-healing process when stimulating at a frequency of 20 Hz in previous studies [72] and why higher frequencies could result in a better outcome of healing by means of the increase of the fluid flow velocity [65–68]. Nevertheless, we have to keep in mind that the frequency of stimulation is just one of the variables that could have an influence on these experimental results. In fact, many other variables could also have some additional effects.

Nevertheless, further animal experiments are needed to quantitatively determine the effect of LMHF mechanical stimulation on fracture callus and confirm the results of both the computational simulations and the experiments.
Also, different protocols of stimulation should be simulated computationally in order to further improve this treatment. Moreover, although the potential of mathematical models is very promising for the design of clinical treatments once validated against experimental results, it is necessary to incorporate in the models the coupling between mechanical and biochemical factors such as growth factors. Models based just on mechanical factors, such as the one proposed by Prendergast et al. [26], could represent qualitatively the behaviour of the system. Nevertheless, to obtain more realistic results, patient-specific variables should be introduced to the models such as genetics, way of life or previous clinical history.

In this work, the mathematical modelling has provided guidelines for the design of a fracture-healing treatment and an external fixator. In fact, the combination of mechano-biological and biomechanical models has led to the design of an external fixator able to monitor and stimulate fracture healing, reducing animal experimentation. Just a few animals were tested in the experiments because the results have corroborated the outcome of the mechano-biological simulation. Thus, in this study, the mathematical model was used as a tool for the design of the experiments, not in the reverse way as has been done to date. Normally, experiments are used to validate the results of mathematical modelling. Nevertheless, more insight is needed in order to have a complete model that could be used by surgeons to determine patient-specific treatments.

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