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# Mechanical Stimulation of Fracture Healing – Stimulation of Callus by Improved Recovery

## Mechanická stimulace hojení zlomenin – stimulace svalku prodloužením fáze zotavení

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## ABSTRACT

PURPOSE OF THE STUDY

Mechanical stimulation plays an uncontested role in the surgical treatment of bone fractures. The effect of frequency, shape, amplitude and rise time of usually symmetrical mechanical stimuli is well known. The recovery period immediately after single pulses is potentially a critical period but has attracted little attention so far. The present study addresses the effect of extending the recovery period which may conceivably increase the efficacy of postoperative fracture management and physiotherapeutic intervention.

MATERIAL AND METHODS

The research method consisted of moving an isolated fragment of bone at a fixed amplitude followed by different recovery periods of between ten seconds and two hours between repeated single pulses. The amount of callus produced was observed radiologically and micro-radiologically and the material property of the healing unit was assessed by measuring the stiffness of the unit.

RESULTS

The result was that pulses applied after a recovery period of up to two hours produced abundant callus and a large increase of stiffness. Pulses applied after a recovery period of ten seconds produced only traces of callus and no increase of stiffness. The significant differences of mean values at 5 weeks were 74 $mm^2$ vs. 13 $mm^2$, and 31.5 MPa vs. 5.5 MPa, respectively.

CONCLUSIONS

The working hypothesis postulates a time-dependent change in tissue reaction to deformation in relation to the time allowed for recovery. Damage and consequent irritation inducing callus does not occur below the critical interval. The observed, unexpected lack of reaction to recovery intervals below 10 seconds may trigger the expectation that in postoperative physiotherapy loading at longer intervals would be preferable to activation at frequencies of locomotion.

**Key words:** fracture healing, mechanical stimulation, callus.

## INTRODUCTION

The effect of physical stimulation on fracture healing is uncontested. The question is whether and how the conditions of stimulation, e.g. in today's postoperative treatment and physiotherapy can be improved. Bone fracture produces a local discontinuity of stiffness, which disables function of the affected limb. Restoration of function requires solid stabilization of the fracture, early on achieved by bridging technique using splinting implants like plates or nails and later by bony callus. Immediate recovery of function using compressing or splinting implants is achieved at the expense of no or only diminished mechanical stimulation of callus (26). Less rigid internal fixation as a way to induce callus formation has been investigated (4, 22, 28). Nowadays flexible fixation aims to stimulate callus formation while maintaining function (23, 25). Recent development of fracture treatment using so called dynamic plating is intended to the recovery of function of the injured limb by creating sufficient periosteal callus through increased interfragmentary motion (1). However, the optimal amount and numbers of cyclic displacement that is optimal to promote fracture healing remains unclear until today.

Relationships between load and structure of intact bone has been made to improve fracture healing by subjecting bones and fractures to a variety of mechanical inputs (11, 16, 20, 31). Attempts to transfer this relationship to improve fracture healing have been undertaken by submitting bone and fracture to a variety mechanical input. In the last decade the topic of improving fracture healing by cyclic application of a relatively low load has been repeatedly addressed (12, 15, 17, 30). Several aspects of mechano-transduction processes have been postulated (24) and the developmental processes involved in bone healing at the tissue, cellular and molecular level have been studied (8). A crucial aspect in fracture healing has been identified to be the mechanical environment together with various biological factors (13). The search for optimal fixation flexibility has triggered many mechano-biological studies, which often lean to-

wards an expectation that intensifying mechanical input improves bone formation. However, the influence of a timely adapted flexibility of the stabilization system has been shown to create a higher bending stiffness of the fracture if a stiff fixation system is used (2). It can be assumed that increasing flexibility and the amount of callus formation is not the only important factor to promote fracture healing.

Interfragmentary cyclic compression has been shown to stimulate best fracture healing compared to other types of loading (7). Earlier experiments studied the effect of amplitude and frequency based mostly on periodic, that is, symmetrical movement amplitudes (6, 14).

A further important aspect that has not been mentioned so far is the period between each single cycle. During this time processes inside the fracture will occur that create the developmental changes to heal the fracture. The effect of modifying the recovery period between each cycle therefore needs to be addressed. The open question for the present study was: *What is the biological effect of changing the duration of the recovery interval between regularly repeating mechanical stimuli?*

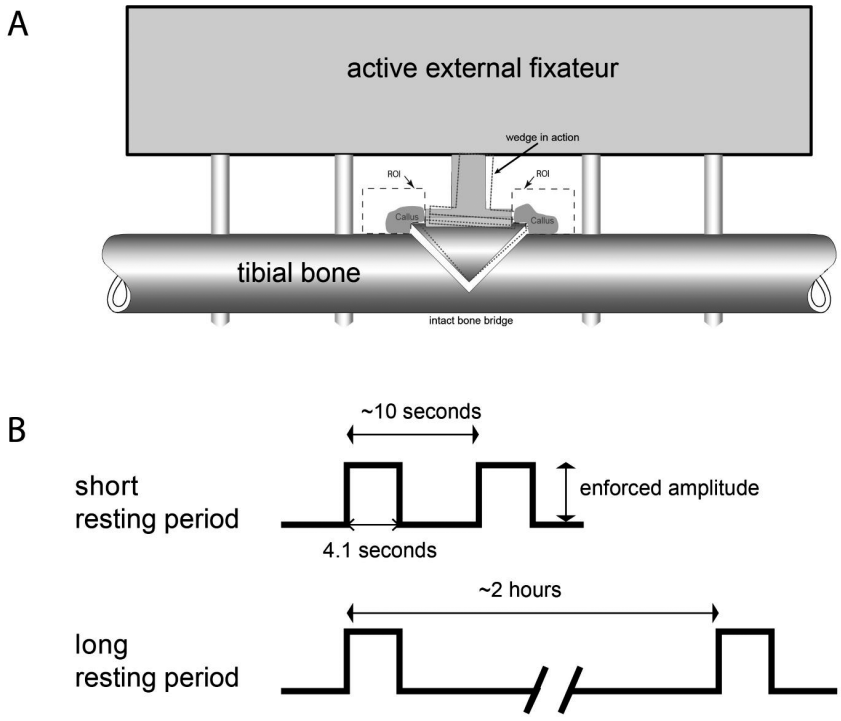


*Fig. 1. Experimental setup. A wedge of bone was cut out of the tibial diaphysis and connected to an activator that allowed controlled tilting of the wedge at enforced constant amplitude (A). The dotted line shows the extent of the movement of the wedge. An active external fixator was applied to bridge the active area of the intervention. Opposed to the wedge, the bony cortex remains intact to stabilize the system as a rigid frame. The wedge was moved once at different intervals, creating resting periods of 10 seconds in the one group and two hours in the other (B). Radiological evaluation of new bone formation after 5 weeks was performed at both sides of the wedge (ROI) on the compression and the distraction side.*

## MATERIAL AND METHODS

An experimental *in-vivo* model was created that allowed programmed deformation of repair tissue. In 19 sheep mechanical stimuli of identical, enforced amplitude were applied to a bone fragment that was cut out of a sheep tibia and moved at different recovery intervals against the wall of the cut-out. The amount of callus formation and resulting resistance to enforced movement, indicating fracture healing, was measured.

The mechanical construction consisted of a so-called "external fixator" containing an activator that served to tilt a wedge fragment (Fig. 1A) around a fulcrum at the tip of the wedge. The gap between the two faces of the wedge and the corresponding cut-out of the bone was 2mm wide in the resting position. During activation the gap closed to 0.2mm at the top of the compression side and opened to 3.8mm on the distraction side. The amplitude of movement was held constant by a mechanical stop. The force applied and the resulting movement of the activator were monitored allowing for calculation of the stiffness of the repair tissue.

Surgery was performed under sterile conditions and in full anaesthesia. Guided application of two cuts at right angles produced the wedge. The two cuts met at a sharp angle (Fig 2). To avoid producing a stress riser a small drill hole was made where the two cuts met near the tip of the wedge. The cut-out left an intact bone bridge which, together with the rigid external fixator, produced a solid full frame, thus preventing uncontrolled deformation at the osteotomy site. To minimize functional loading of the bone the patellar and Achilles tendons were cut, and a sling prevented peak loading associated with any uncontrolled attempts to change posture but allowed unrestricted stance and supported resting. The tenotomies prevented the sheep from bearing weight on the operated leg for four weeks after surgery. In the daytime the sheep stood on three legs; at night they rested supported by a sling harness. This study was carried out in strict accordance with the recommendations set out in the Guide for the Care and Use of Laboratory Animals of the Swiss veterinary office. All protocols were approved by the animal experiment committee of Canton Graubünden-Switzerland.

Experimental groups: 19 Swiss Mountain sheep were divided randomly into two main groups of six for statistical evaluation. Two smaller groups provided additional information.

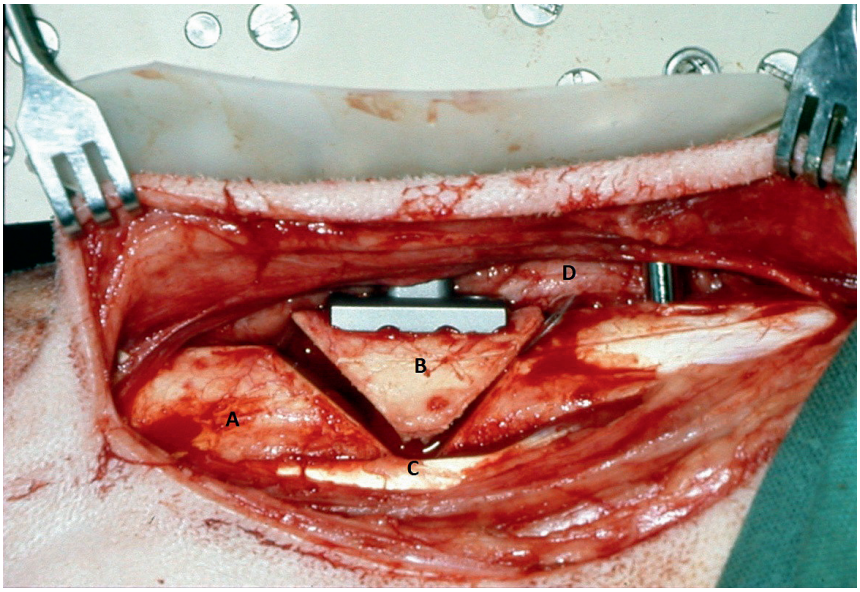


*Fig. 2. Overview of surgical intervention. Through an anteromedial skin incision, the approach to the tibial diaphysis (A) was created. A small connector was fixed to the bone and a standardized cut was performed from proximal and distal to create a uniform bony wedge fragment (B). Prior to the cuts, a drill hole was created at the tip of the fragment using a special guide to prevent uncontrolled breakage of the intact cortical bone (C) opposed to the wedge. The active external fixator bridges the side of the tibial diaphysis where the wedge was cut out of the bone. The wedge was elevated 2mm to create a uniform rectangular gap. In the operative view, the fragment is tilted to the proximal side down to 0.2 mm (D), representing the cyclic displacement that was used.*

Movement of the wedge was started immediately postoperatively. In the main groups either 10 cycles or 10 000 cycles per day were applied resulting in recovery periods of 2 hours and 10 seconds, respectively (Fig. 1B). In this demanding experiment the absence of uncontrolled effects was monitored in a group of three with a fixed wedge. An additional group of three provided information on the critical recovery interval by application of 1000 cycles per day. The 10 cycles per day produced a recovery interval of 2.4 hours (called the 'two hour' group). The 10 000 cycles per day produced a recovery interval of 8.6 seconds (called the 'ten second' group). The additional 1000 cycles group produced a recovery interval of 1.4 minutes (called the 'one minute' group).

The evaluation of periosteal callus formation was performed using routine X-rays at fixed intensity and were taken immediately after the surgical procedure and at weekly intervals during the experimental period. The tibiae were oriented such that the external fixation device was in the plane of the film. This orientation was kept constant by using a special bracket which engaged with the fixation device. At 5 weeks high resolution radiographs of the bone specimens were taken (AGFA D4-film; Faxitron Model 43855A, Faxitron X-ray corporation, Illinois, USA). The radiographs were digitized with a high-resolution transmitted light scanner (CanoScan D2400U, Canon, Tokyo, Japan) and read into an image analysis system for further evaluation of new bone formation (Image Pro Plus, Media Cybernatics, Silver Spring, MD, U.S.A.). The amount of callus seen on the X-ray images in close relation to the gaps was statistically evaluated at the end of the five week observation period for the two main groups (Fig. 1A).

The area of new bone formation was evaluated separately for each gap area of compression and distraction. Due to the consistent geometry as well as the compression and the distraction side and between each sheep, a standardized region of interest (ROI) was used to evaluate new bone formation, similar to the method of Lujan 2010 (21).

Semi-automatic segmentation was performed starting by selecting the boundary of the fixation plate, the tip of the wedge fragment and the edge of the remaining cortex. From this point, the region of interest was extended so that a total width of 20 mm of the ROI was created. The height of the ROI outside the cortex was limited to a height of 15 mm. Using this ROI, the cortex could be excluded from the evaluation. Segmentation of the callus was performed by noise reduction and thresholding. The total area of the discriminated pixels was calculated and used to describe the total amount of new bone formation in any gap.

Stiffness was measured at weekly intervals. Quasi-static measurements were taken with the sheep lying in lateral recumbence. The first measurements were made just after the operation. The pneumatic actuator was replaced by a manually operated crank with an integral load cell (FMDZ, Wazzau, Berlin, Germany) and an LVDT displacement transducer (LP-10F, Greenpot, Putzbrunn, Germany). The crank was manually advanced to the maximally displaced position over a period of 5–8 seconds three times while the load-displacement data was recorded on a portable computer at a frequency of 20 Hz using data acquisition software (Labtech Notebook, LABTECH, Andover, MA, USA). In the group without activation, the crank was advanced only 1 mm, corresponding to 14 % of the maximal possible displacement to maintain quasi rigid immobilization.

The maximal force exerted by the actuator was 180 N, which corresponds to a maximal torque moment applied to the wedge fragment of 11.9 Nm. This was enough torque both to displace the wedge and to regain the neutral position during the five weeks observation period.

**Calibration:** The in vivo stiffness measurement was not strictly conclusive in determining the elastic modulus

because of an unknown frictional component within the external fixator housing and because maintaining the wedge apex as the centre of rotation created bimodal deformation consisting of both bending and shear. To circumvent these difficulties, an in vitro calibration was performed using neoprene discs of known modulus. The modulus range of the selected specimens was 0.1 to 6 MPa based on load-displacement analysis using a material testing system. The neoprene discs were then cemented into the gaps of an ovine tibia with an identical osteotomy gap and external fixator. Correlation of the measured stiffness with the material modulus in this calibration was good, ($y = 22.4x + 10.3$, $R = 0.97$).

Statistical analysis: Results are reported as mean ± standard deviation. A t-test was used to compare the amount of callus on X-rays at 5 weeks. For comparison of the "in-vivo" callus stiffness multiple independent t-tests with Bonferroni correction (t2–t5) were used;equal variances not assumed, normality assumed (Shapiro Wilk test).

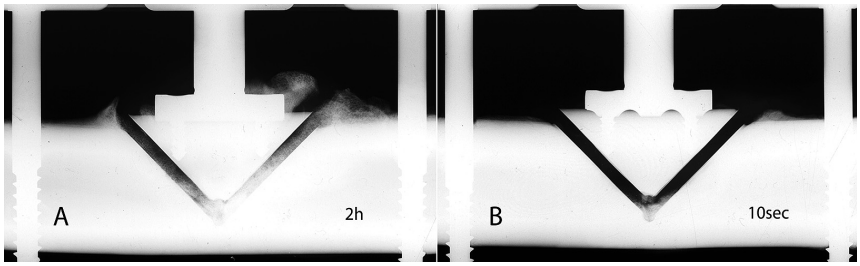


*Fig. 3. Callus formation main groups. Callus formation under identical movement but at different intervals between repeated deformation in the two main groups at 5 weeks. Two hours intervals with large callus formation on the compression side (A). Ten seconds intervals (same movement) with no callus formation in the active gap (B). There are traces of callus near the drill hole at the fulcrum and at the upper surface.*

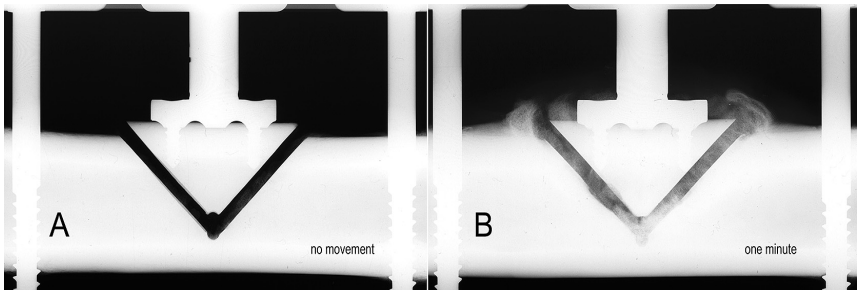


*Fig. 4. Callus formation in the two additional groups at 5 weeks. Checking for uncontrolled deformations, no movement was imposed (A). No callus formation in the gap indicates the absence of uncontrolled deformation. Callus formation in the additional one-minute group (B) similar to the two-hour group (see Fig. 2A).*

## RESULTS

Clinically, no relevant complications such as infections or implant failures were observed. In one case in the zero-movement group the bone bridge of the cut-out, a critical element providing stability, was fractured. This case was excluded from evaluation.

Callus started to appear on X-rays at the third week. The amount of callus as measured on the X-rays at five weeks showed a large amount of callus in the two hour group while in the group with the short interval of 10 seconds practically no callus was formed (mean 74 $mm^2$ vs. 13 $mm^2$, $p = 0.011$, (Fig. 3 and Fig. 5).

In a smaller group that served to exclude artefacts from functional loading of the tibia the absence of relevant periosteal callus allowed the exclusion of non-controlled deformation (Fig. 4A).

To narrow the critical interval in a further small group (Fig. 4B), the recovery period was 1 minute. In

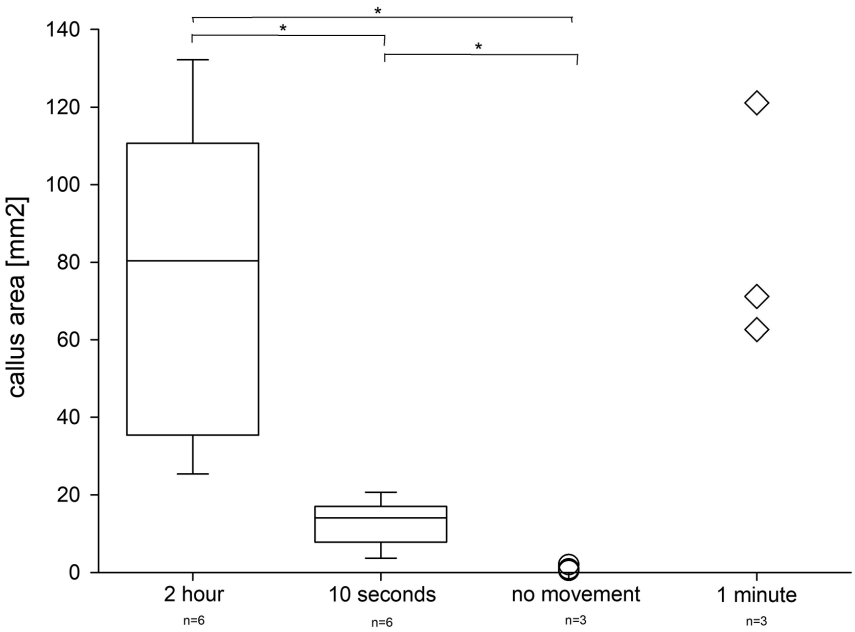


*Fig. 5. Radiological evaluation of callus formation. The large difference between the two hour group, ten seconds group and no movement group are significant (all $p < 0.016$). The group with no imposed movement practically shows no callus formation. The small group with the one-minute interval reveals callus similar to the 2 hour group and indicates that the critical interval of resting period is less than one minute.*

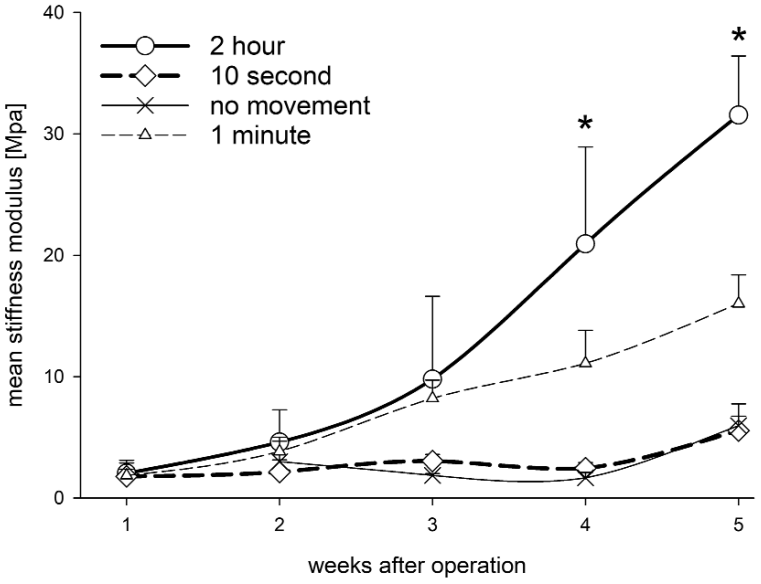


*Fig. 6. Callus stiffness in-vivo. The stiffness is plotted against time of observation. The resistance to wedge movement increases with ongoing differentiation of repair tissue. The stiffness increases in the two-hour group but not in the ten-second group. The difference is statistically significant from the fourth week on. The small group with a one-minute interval seems to indicate that the critical interval is between one minute and ten seconds. The group "no movement" did not reveal any stiffness at all times, similar to the ten second group.*

this group the scatter of radiological data and the lower stiffness indicate approximation to the critical interval. In this group, a large amount of callus formation was still observed that appeared on average like the callus formation in the two hour group (Fig. 5).

Callus stiffness measurements: Measuring the force required to effect movement in the two hour group revealed continuously increasing levels of stiffness from the second week onwards (Fig. 6). In the ten second group and the group without movement no relevant callus stiffness was observed. The difference between the two main groups was significant at four ($p = 0.012$) and at five weeks ($p < 0.001$). In the one-minute group increasing stiffness was observed.

## DISCUSSION

The problem addressed concerns the surgical treatment of fractures which aims to restore function of the affected limb while stimulating solid healing. The search for improved stimulation of healing has generally focused, for example, on amplitude, frequency and shape of the applied cyclic movement. The present study addressed the effect of the critical recovery interval between single mechanical pulses where the time dependent reactivity of tissue differentiation obviously plays a determining role.

Of the two aspects of bone formation, i.e. bone homeostasis and fracture repair, this study only deals with fracture repair. Furthermore, the method used was not intended to simulate the entire complex process of fracture healing but addressed single time dependent elements thereof. Our observation of tissue reaction relates to what Elliot calls the 'Bone Healing Unit': "…the concept that the tissue that forms in and around the fracture should be considered as a specific functional entity. The 'bone healing unit' produces a physiological response to its biological and mechanical environment…" (9). The cortical bone of the fragments contributed minimally to repair during the observation time of 5 weeks and did not play a role here.

Standardized osteotomies were used as a simplified fracture model, avoiding the large variance of shape of experimental fractures. This simplification was considered acceptable because in respect to the goal of the study the surfaces of the fragment ends served mainly to transmit displacement to the repair tissues within and around the gap.

The mechanical movement was enforced, meaning that the same amplitude of movement was imposed even as stiffness progressed whereas in spontaneous fracture healing the amplitude decreases with increasing stiffening of the repair tissue. The enforced movement was selected to create a maintained, well-controlled condition over the whole observation time.

The stiffness of fracture healing was measured to assess the stabilizing effect of the differentiating soft tissue of the 'Bone healing unit' (9). The amount of callus plays an important role as with increasing diameter of the callus the leverage of the soft tissue improved with increasing diameter of the callus. In respect to judging fracture healing the amount of callus is a prerequisite while the stiffness is directly related to tissue differentiation in healing. As Fig. 4B shows, the fracture gap may appear to be solidly bridged by bone while the stiffness measurement disproves this.

The additional group with zero movement consistently showed no relevant callus except for one case where the bone bridge of the cut-out was fractured. This case was excluded from evaluation as the condition of a stable fixator H-frame was not met. The remaining cases consistently showed no relevant callus formation, which indicated that the stability of the experimental setup reliably prevented uncontrolled displacement, thus proving that the rigidity of the external fixation and its coupling to bone were ample. The absence of relevant callus formation in the group with zero movement proves that a single initial trauma causing structural discontinuity (osteotomy or fracture) alone is not enough to induce ongoing fracture healing. The additional group with one-minute intervals showed a similar amount of callus as the two hour group and a moderate increase of stiffness, thus indicating that this was the critical level

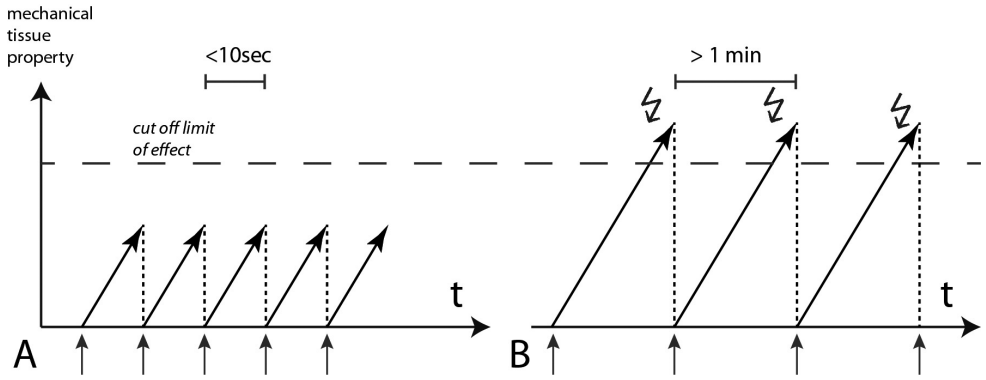


*Fig. 7. Hypothesis. Short resting interval has no effect (A). After a pulse the sensitivity to deformation recovers gradually. If the next impulse occurs after a short interval the sensitivity is still below cut off, and no effect occurs. Long interval has a positive effect (B). After a pulse the sensitivity to deformation recovers gradually. If the next impulse occurs after a long interval the sensitivity is now restored and a positive effect results.*

of recovery time. The strong reaction of repeated trauma at 2 hours recovery and no reaction with singular initial trauma in the zero-movement group gives rise to the expectation that there is also an upper critical level of recovery time. Stimulation of intact bone approaching daily intervals was studied regarding homeostasis (27). They showed that in intact bone long recovery intervals maintained bone homeostasis.

Stiffness starts to increase two weeks after surgery, a week earlier than the radiological data. This is due to the contribution of increasing soft tissue stiffness and increased diameter of the soft callus. The stiffness data showed no discontinuous increase with time, which confirms that the callus did not solidly bridge the fracture gap under the imposed conditions of enforced movement. From clinical experience one would expect that when discontinuing movement at 5 weeks quick solid healing would occur (2, 10).

### Hypothesis

The observed total lack of callus formation as a response to mechanical pulses spaced less than 10 seconds apart begs an explanation that is difficult to provide. As timing plays a critical role, a gradual recovery of reactivity to deformation may occur, assuming that the mechanical pulse resets the reactivity (Fig. 7A). A longer interval seems to be required for the reactivity to recover that is, become effective before the next pulse occurs (Fig. 7B).

As a possible mechanism of recovering of reactivity, one may consider the time dependent modification of structural "brittleness" of the bone healing unit: While the deformation at rupture remains greater than the deformation imposed by the next pulse nothing happens (Fig. 7A). With time the elongation at rupture would become smaller. If the imposed deformation exceeds the elongation at rupture of the bone healing unit, the temporary structure would be disrupted (Fig. 7B) stimulating differentiation.

One could speculate that deformation induced fluid displacement (3, 19, 29) in the tiny intraosseous channels with high resistance to flow represents a time-consuming mechanism with a similar time evolution as the above-mentioned pseudo fluid modifications.

Concerning the mechano-sensor activity of bone cells the osteocyte attracts increasing interest (3) whereby the timing of such reactions needs further study.

Many chemical processes are time-dependent, for instance, the timing of cyclic Ca activity of MLO-Y4 (5). It might be of interest to study the timing of the observed loading intervals in relation to Ca oscillations (18).

## CONCLUSIONS

The question was: What is the biological effect of changing the duration of the recovery interval between regularly repeating mechanical stimuli? In respect to the mechanical stimulation of fracture healing the recovery time between single displacements plays a critical role. Repeated single cycles with a long recover period create relevant callus. A more frequent stimulation with shorter intervals can stop callus formation. Functional rehabilitation after fracture surgery needs to consider the impeding effect of too short a recovery time and avoid intensified activity with its converse effect on repair.

The observed lack of effect at short recovery intervals needs to gain clinical attention given that physiological function, like gait, occurs in the range of the observed critical recovery interval and the healing process might be improved by adapting the interval.

The findings of this study should stimulate further research as unexpected observations are often a strong incentive to break new ground.

***Conflict of interest statement***

*The authors have no conflict of interest.*

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