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Part-1

Effects of Non-Invasive Pulsed Low-intensity Ultrasound on Rat Femoral Fracture

-Time course study-

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Effects of Non-Invasive Pulsed Low-intensity Ultrasound on Rat Femoral Fracture

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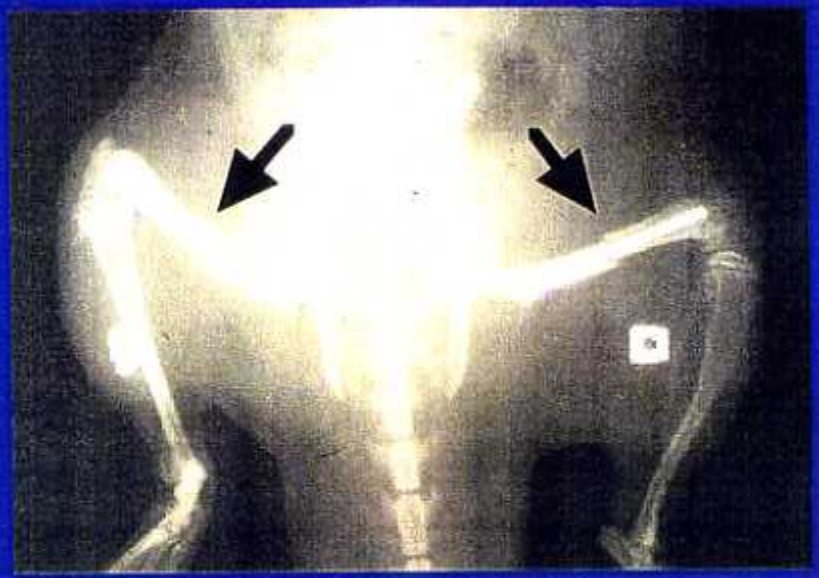
Purpose

In order to confirm effects of non-invasive pulsed low-intensity ultrasound on rat femoral fracture

Rat Fracture Model

- Male Long Evans Rats (10 weeks)
- Closed Femoral Fracture (Einhorn 1992)
  - √ intramedullary pinning with Kirschner wire
  - √ fracture using three point bending device



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Ultrasound Treatment

- **Ultrasound Signal:**
  - Intensity(SATA); 30 mW/cm^2
  - Fundamental Frequency; 1.5MHz
  - Pulse Width; 200 μsec
  - Pulse Repetition; 1.0 KHz
- **Transducer: PZT-4**
- **Using Sonic Accelerated Fracture Healing System (SAFHS®, Exogen) for 20 minutes every day under anesthesia with ketamin (50mg/kg) and xyladine (2 mg/kg) for 14, 17, 21 days after the operation**

Transducer (PZT-4)

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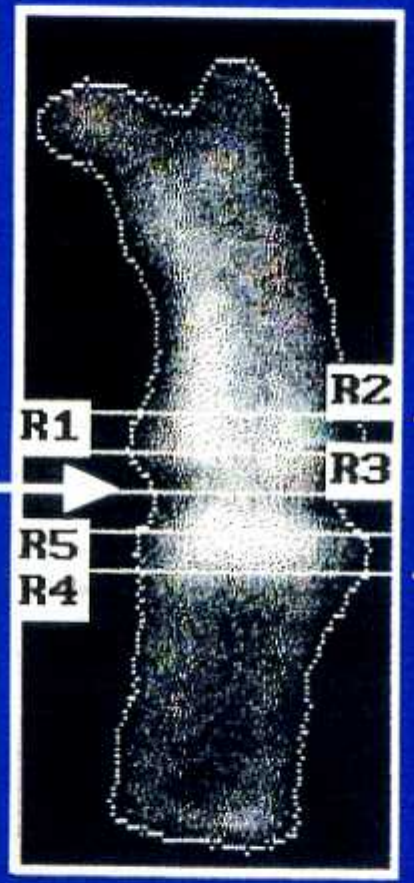
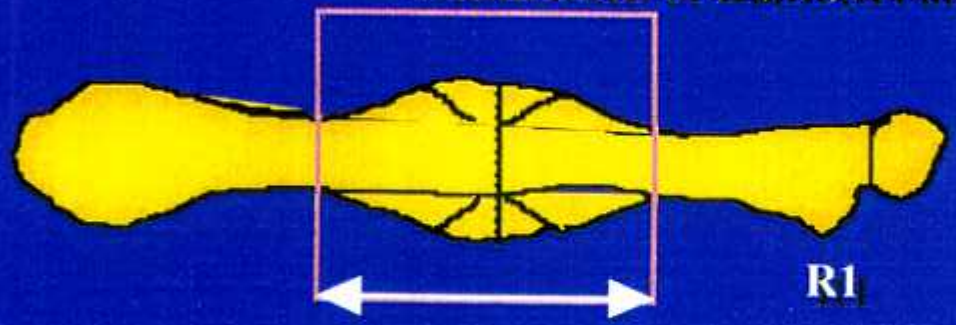
Evaluation



1. Bone Mineral Density (BMD) and Bone Mineral Content (BMC) at Fracture site
   - √ on days 14, 17 and 21 after fracture
   - √ using DEXA (Hologic; QDR-2000)

BMD at Fracture site =
Mean BMD of R2,R3,R4 and R5



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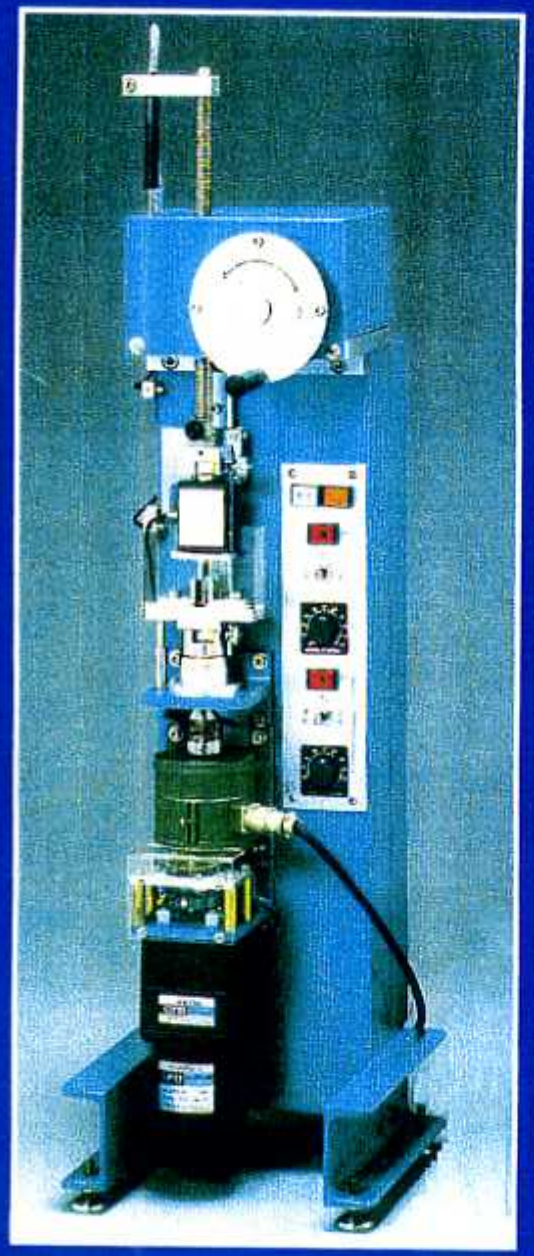
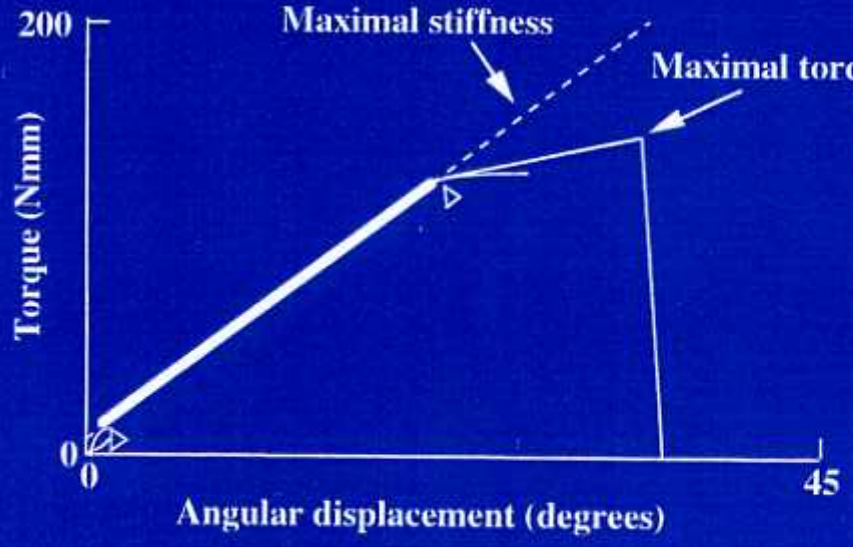


Evaluation

2. Mechanical Testing (Torsion)

- √ on days 14, 17 and 21 after fracture
- √ maximal torque (N.mm)
- √ maximal stiffness (N.mm/deg.)



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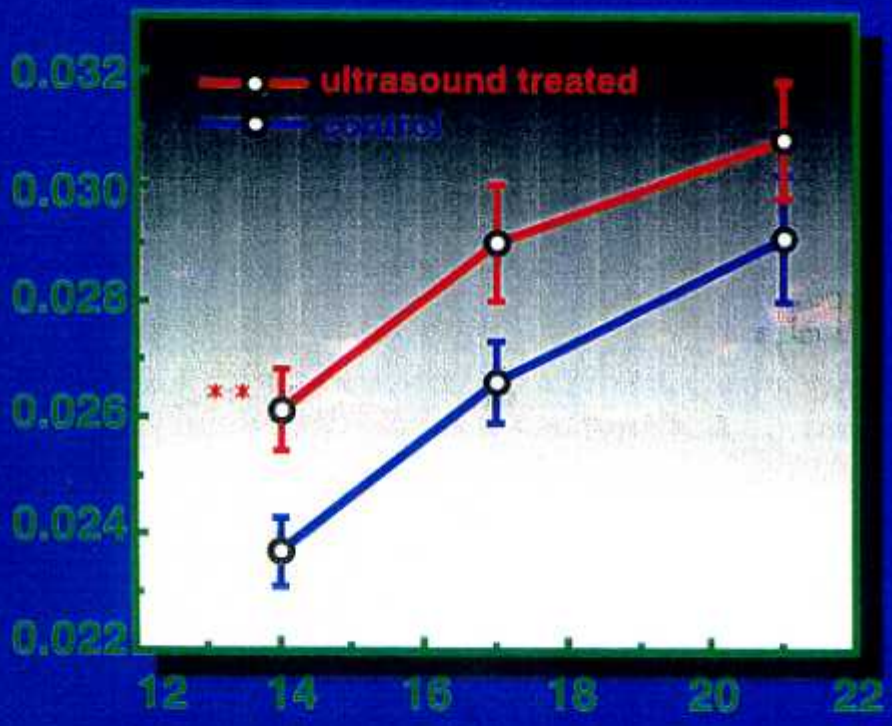
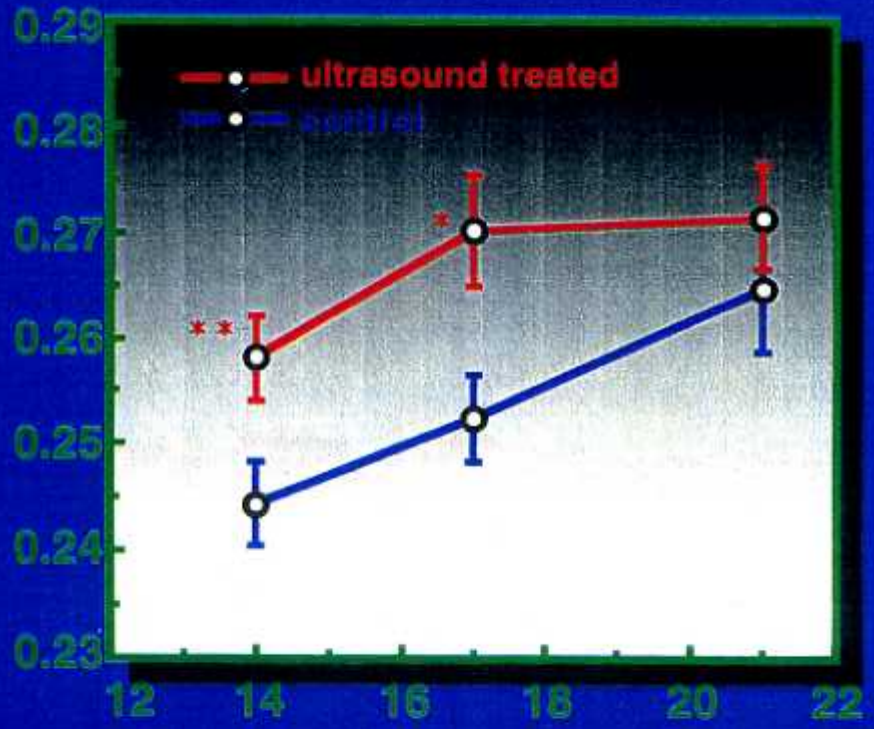
Bone Mineral Content (g)



days after fracture

Bone Mineral Density (g/cm²)



days after fracture

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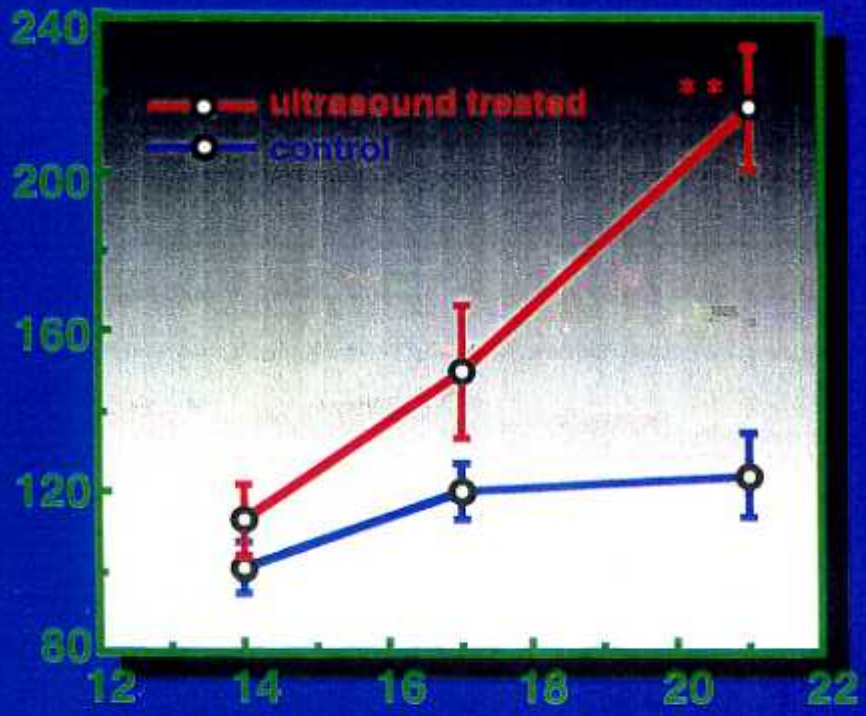
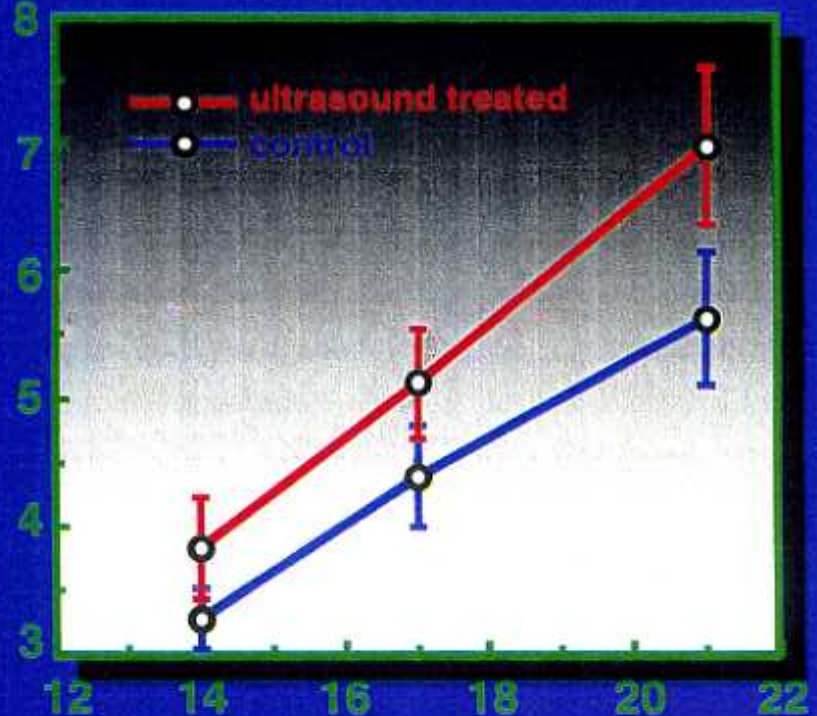
Torsional Torque (N-mm)



days after fracture

Torsional Stiffness (N-mm/deg)



days after fracture

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Summary
- Part-I -

- Hard Callus Area — no differences
- BMC/BMD — treated > control
- Peak Torque — treated > control
- Endochondral Ossification (Histology)
  earlier in treated than in control

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Conclusion
- Part-1 -

- Ultrasound treatment on rat femoral fracture resulted in better mechanical property of the callus than in controls on day 21 after fracture.

- It may be due to accelerate fracture healing processes including intramembranous and endochondral ossification, additionally bone remodeling.

Part-2

Effect of Pulsed Ultrasound on Fracture Healing Depends on the Signal Conditions of Ultrasound in Rat Femoral Fracture Model

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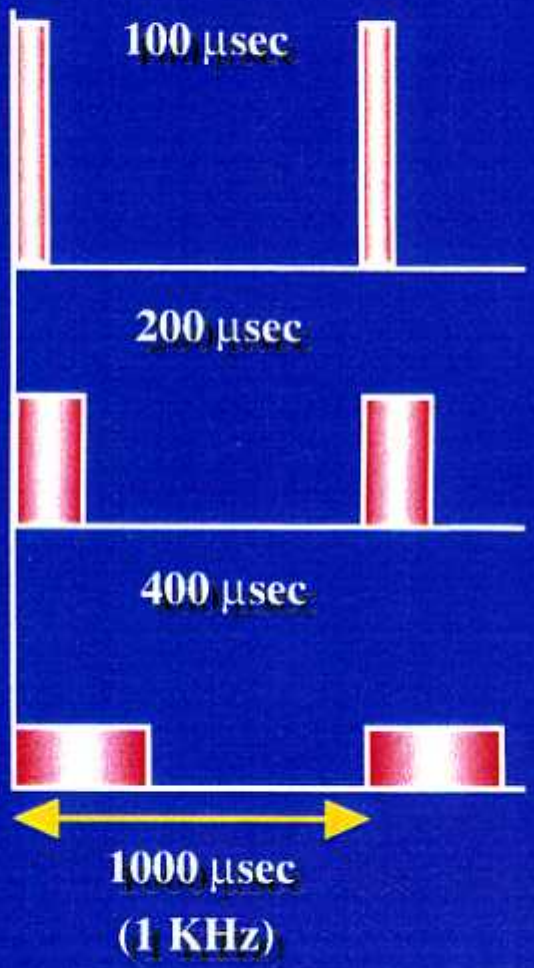
Purpose

In order to evaluate the ultrasound signal conditions on rat femoral fracture

- √ **Pulse Width**
- √ **Pulsed Burst Repetition Frequency**

Ultrasound Treatment
- Pulse Width -

- **Ultrasound Signal:**
  - Intensity(SATA); 30 mW/cm²
  - Fundamental Frequency; 1.5MHz
  - Pulse Repetition; 1.0 KHz
    - Pulse width-1; 100 μsec
    - Pulse width-2; 200 μsec
    - Pulse width-3; 400 μsec



- **Ultrasound treatment: 20 minutes every day under anesthesia with ketamin (50mg/kg) and xyladine (2 mg/kg) for 24 days**

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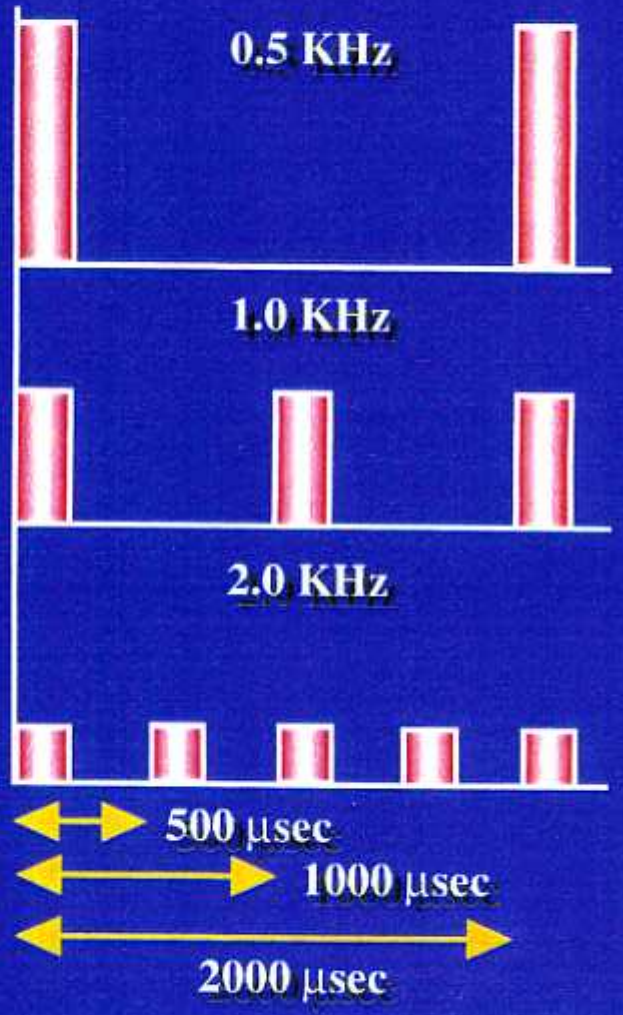
Ultrasound Treatment
- Repetition Frequency -

- **Ultrasound Signal:**
  - Intensity(SATA); 30 mW/cm²
  - Fundamental Frequency; 1.5MHz
  - Pulse Width; 200 μsec
    - Repetition Frequency-1; 0.5 KHz
    - Repetition Frequency-2; 1.0 KHz
    - Repetition Frequency-3; 2.0 KHz

- **Ultrasound treatment: 20 minutes every day under anesthesia with ketamin (50mg/kg) and xyladine (2 mg/kg) for 24 days**



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Evaluation

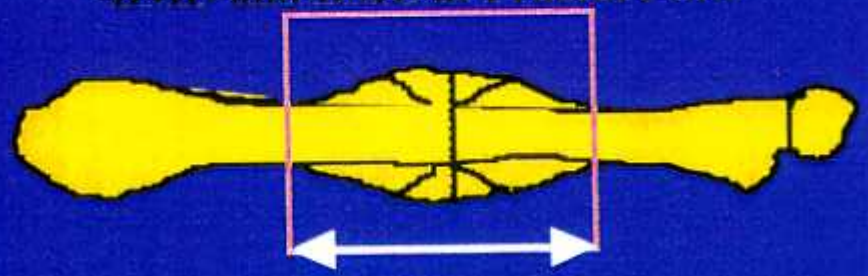
1. Bone Mineral Density (BMD) and Bone Mineral Content (BMC) at Fracture Site

- √ on day 24 after fracture
- √ using DEXA (Hologic; QDR-2000)

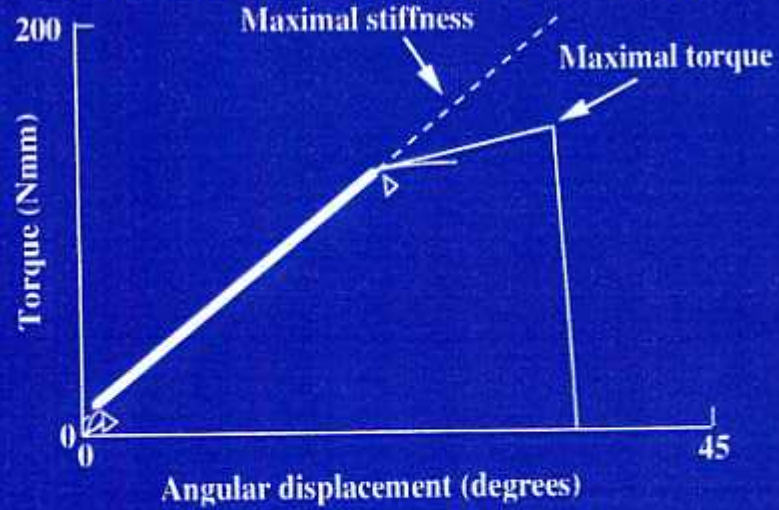
2. Mechanical Testing (Torsion)

- √ on day 24 after fracture
- √ maximal torque (N.mm)

BMD and BMC at Fracture Site



Torsional Strength at Fracture Site



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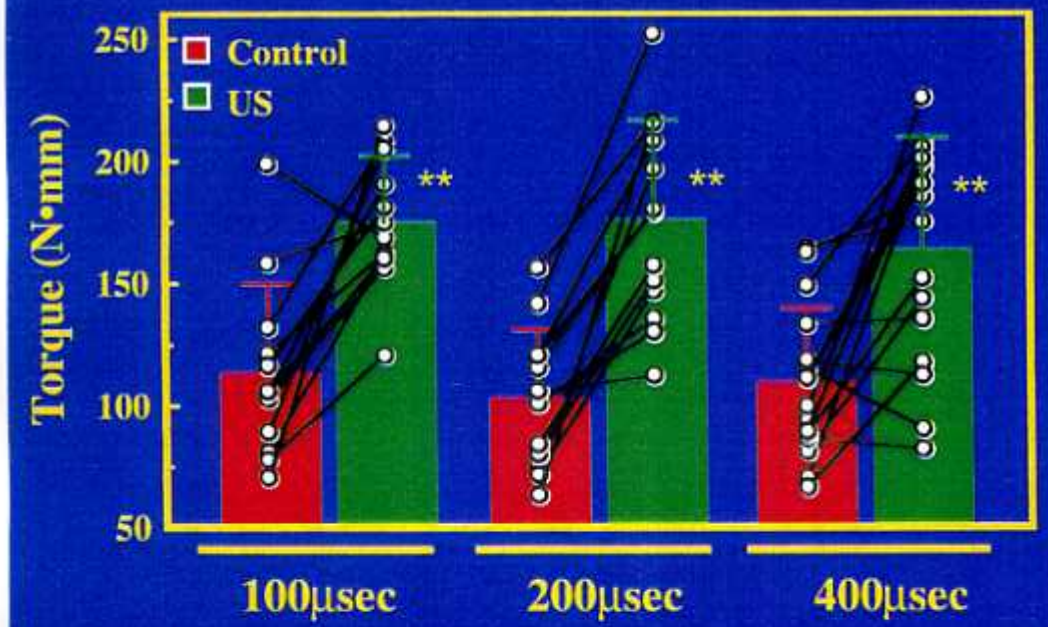
Pulse Width

- Torsional Torque on day 24 -

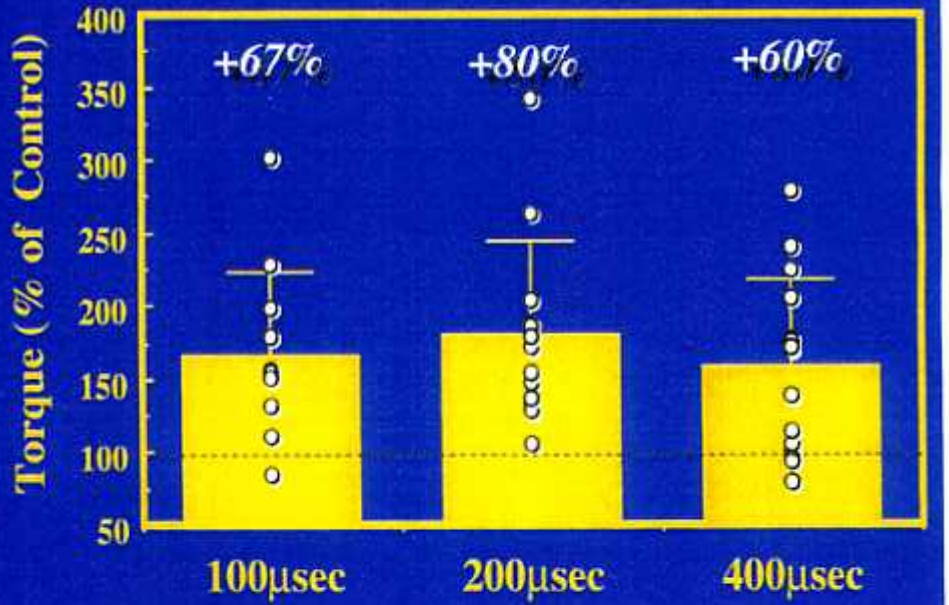
Torsional Torque (N-mm)



Mean ± SD

*** ; p<0.01 vs. control by paired t-test*

(% of control)



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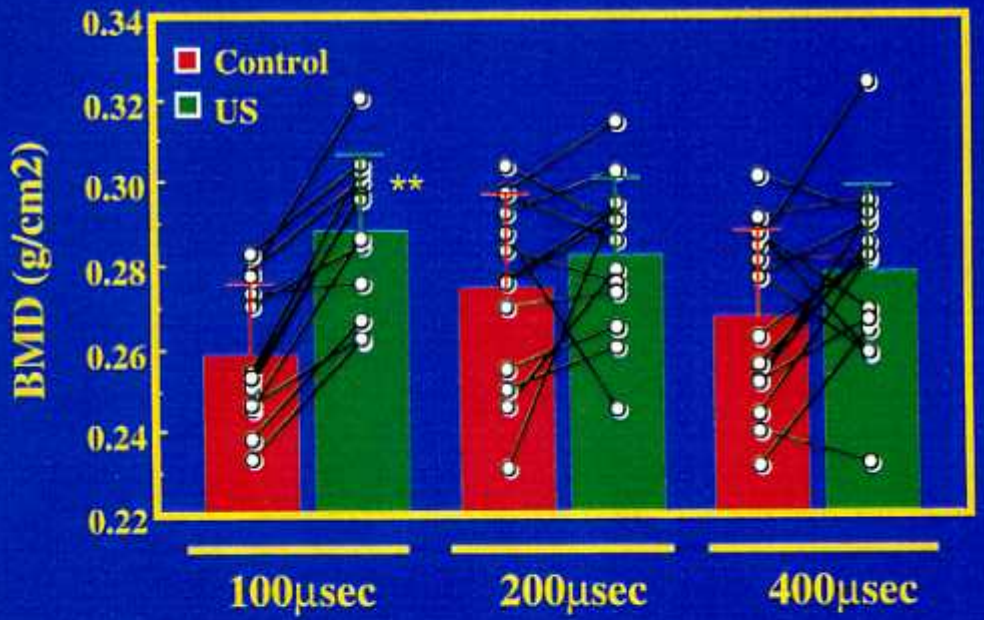
Pulse Width

- BMD on day 24 -

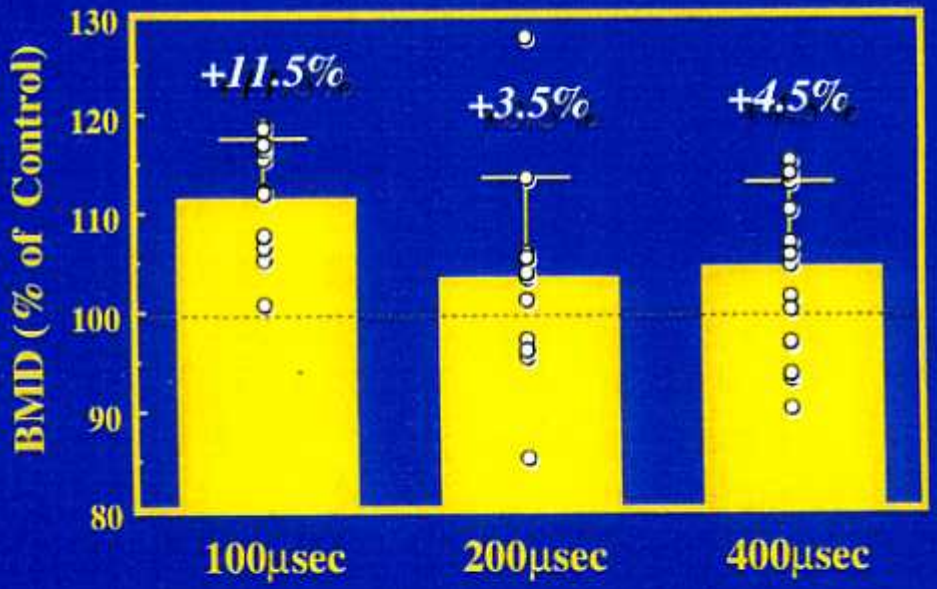
BMD (g/cm²)



Mean ± SD

*** ; p<0.01 vs. control by paired t-test*

(% of control)



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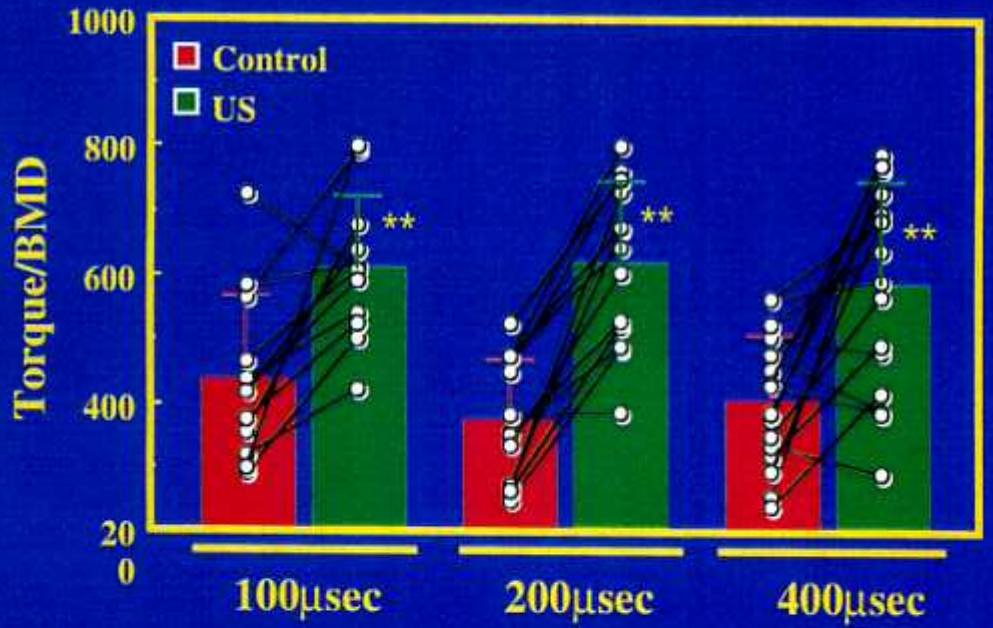
Pulse Width

- Torque/BMD on day 24 -

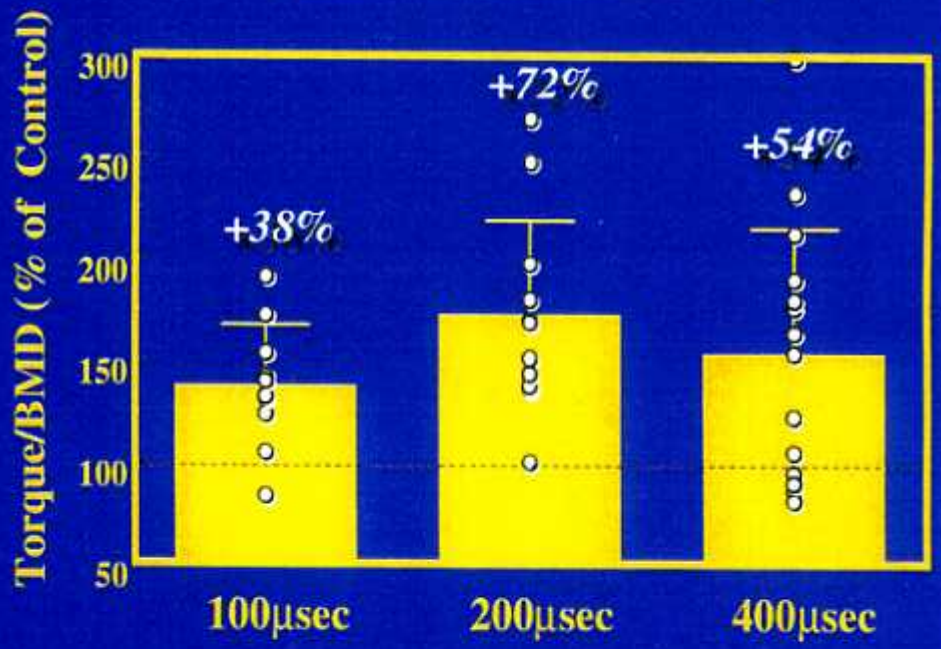
Torque/BMD (N-mm/g/cm²)



Mean ± SD

*** ; p<0.01 vs. control by paired t-test*

(% of control)



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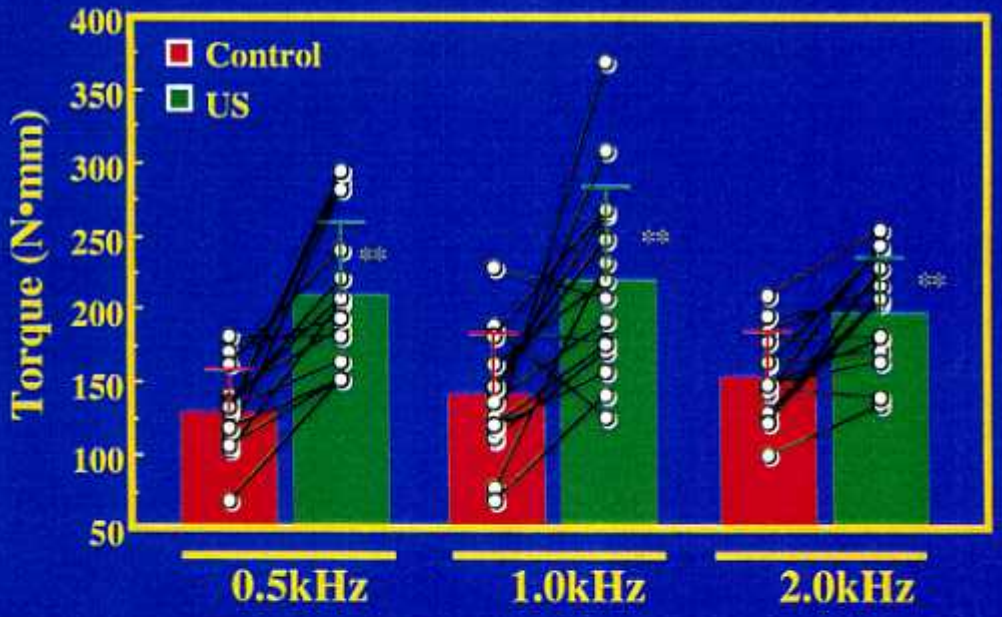
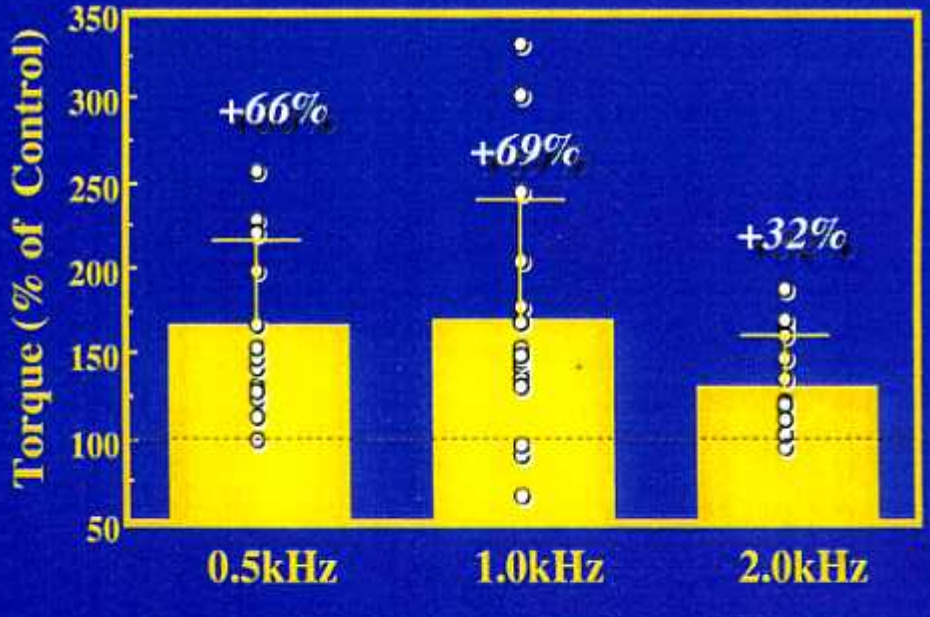
Repetition Frequency
- Torsional Torque on day 24 -

Torsional Torque (N-mm)



Mean ± SD
*** ; p<0.01 vs. control by paired t-test*

(% of control)



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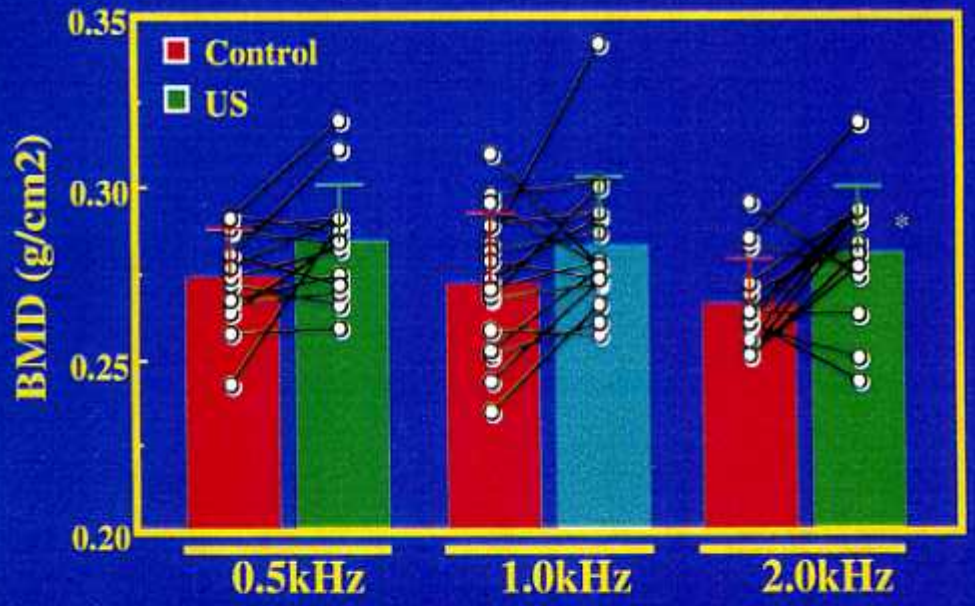
Repetition Frequency
- BMD on day 24 -

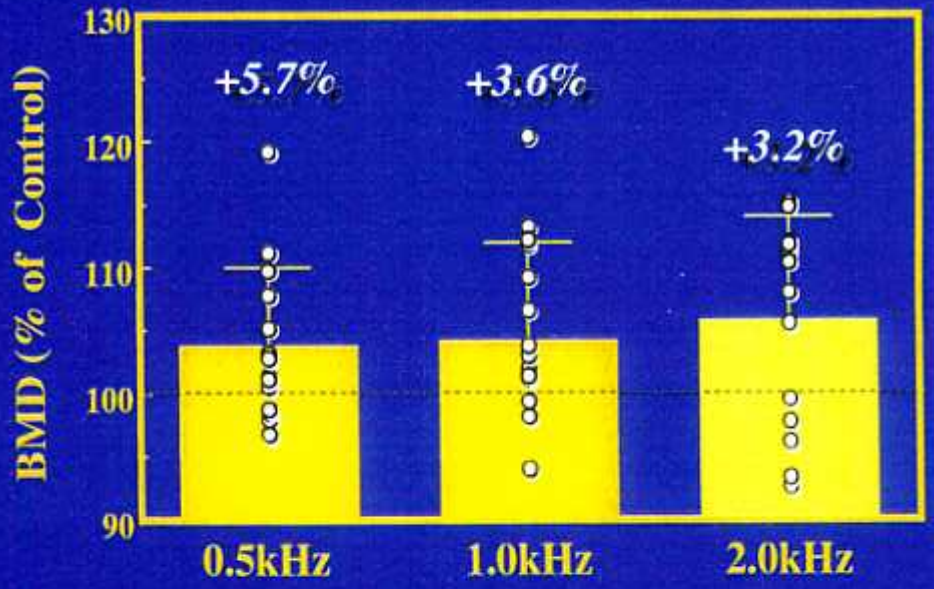
BMD (g/cm^2)



Mean ± SD

** ; p<0.05 vs. control by paired t-test*

(% of control)



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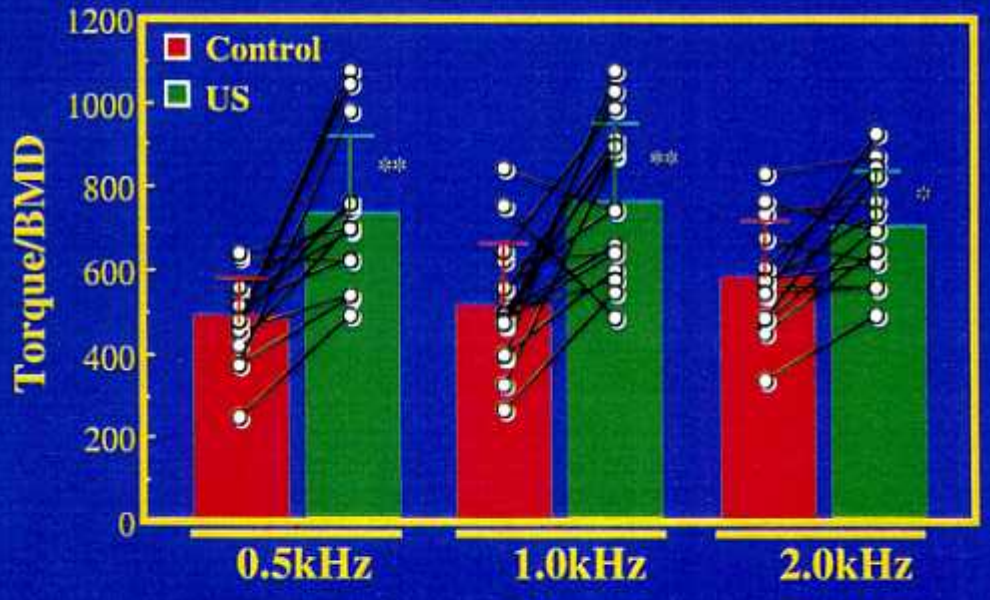
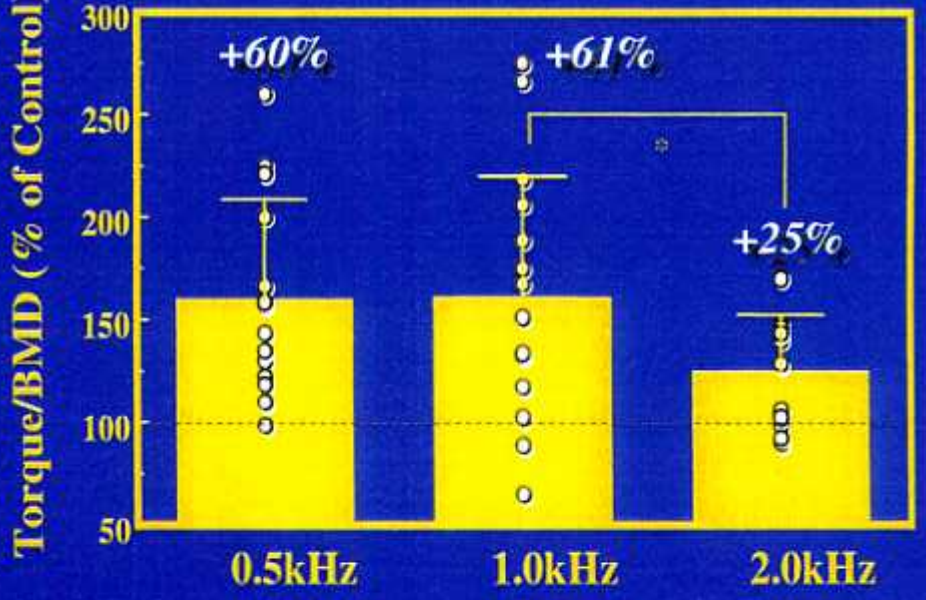
Repetition Frequency
- Torque/BMD on day 24 -

Torque/BMD (N-mm/g/cm^2)



Mean ± SD
** ; p<0.05, ** ; p<0.01 vs. control by paired t-test*

(% of control)



** ; p<0.05*
vs. 1.0kHz group by Dunnet's test

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Summary
- Part-2 -

- Torque (Torque/BMD) — treated > control (in all groups)

√ Pulse Width: 200μsec > 100μsec = 400μsec

√ Repetition Frequency: 1.0kHz = 0.5kHz > 2.0kHz

Conclusion

- Part-2 -

- The animal group stimulated with the 200μsec pulse presented better mechanical property as compared to the other group.
- The maximum healing was reached at 1.0kHz with no increase at a higher frequency of 2.0kHz.
- These results suggest that 200μsec pulse width and 1.0 kHz repetition frequency are optimum for fracture healing in rat femoral fracture model.

Feature

We should do further experiments to be clear the accelerating mechanisms of Ultrasound on fracture healing *in vivo* and/or *in vitro*.

- Osteogenesis
- Chondrogenesis
- Angiogenesis

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