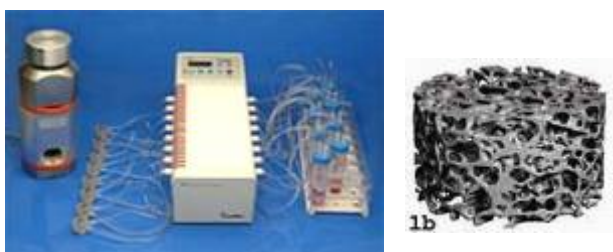


## Mechanically loaded *ex vivo* culture system for cancellous bone biopsies

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**Introduction:** In order to understand mechanical support and mineral homeostasis of bone, one must have both the cells and bone matrix combined in an isolated, 3D culture. A need exists for an *ex vivo* bone culture system, where a controlled biochemical and mechanical environment is created to be able to determine the influence of different parameters. The Zetos<sup>[1]</sup> model with mechanical loading has been validated (Fig.1) with ovine, bovine and human samples to keep cancellous bone tissue viable *ex vivo*. The samples maintain osteocytes, osteoblasts, osteoclasts and bone marrow cells in their natural 3D relationship to each other. Mechanical loading is a known anabolic stimulus for bone that is imperative to its natural development. The Zetos bioreactor may potentially be used for tissue engineering of bone to fill defects caused by tissue trauma or disease. The system could also be used to test possible tissue engineered constructs, reducing the amount of animal experimentation required. The goal of this study was to assess the response of three dimensional human explant cancellous bone to the addition of TGF $\beta_3$  during long term culture with mechanical loading in the *ex vivo* loading bioreactor.

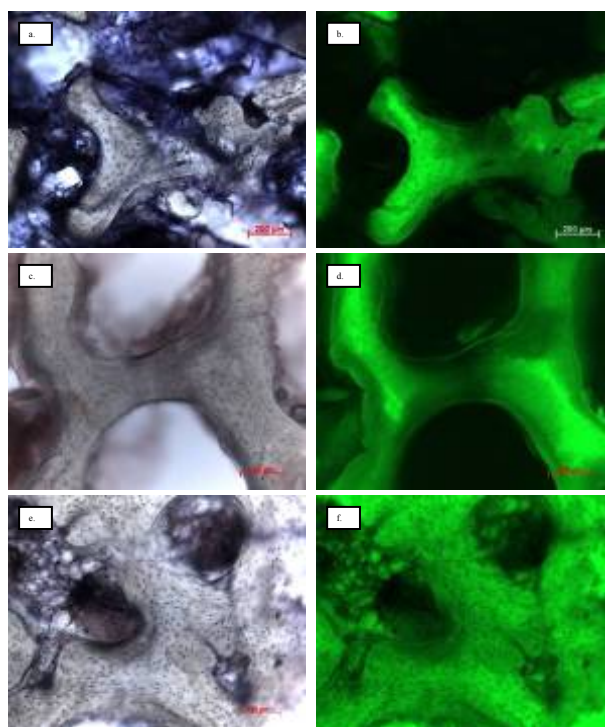


**Figure 1.** *Ex vivo* Zetos culture system. Microprocessor controlled pump allows perfusion of fresh media through the chambers. The bone cores (1b) are stimulated daily.

**Methods:** Human femoral heads (Ethic Commission Graubünden approval (18/02)) were processed into cylindrical cores (5mm height, 9.5mm diameter). The cores were inserted into the culture chambers, randomly assigned to groups and subsequently cultured for up to 14 days. Groups included, with or without TGF $\beta_3$  (15ng/ml) and with or without loading (300 cycles at 1 Hz, giving 4000 microstrain) and heat treated dead cores as a control. As fresh tissue controls bone cores were fixed with 70% ethanol immediately after excision

(T0). Post culture cell viability was assessed by cutting the cores into 250 $\mu$ m thick sections and the LDH assay was performed<sup>[2]</sup>. All remaining cores were fixed in 70% ethanol, dehydrated through an ethanol series and embedded into Technovit 9100 New<sup>[3]</sup> for subsequent histological and immunohistochemical evaluation.

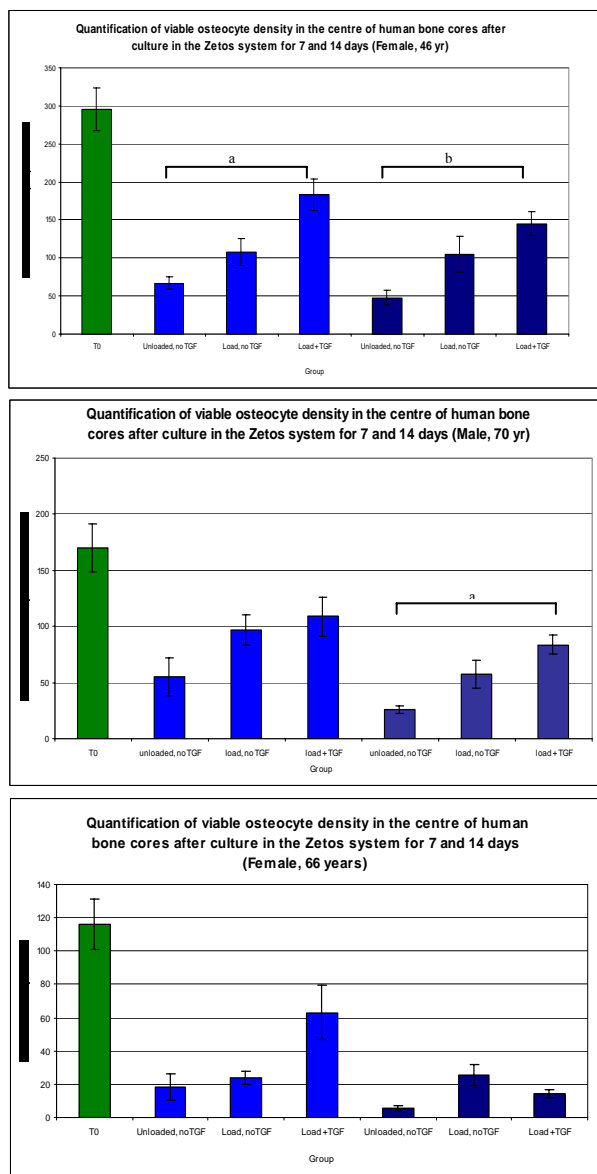
**Results:** Histology of live cultured samples after 14 days in the Zetos system was comparable to fresh bone (T0). Non collagenous proteins such as bone sialoprotein and osteopontin were localised through immunohistochemical labelling of sections. The LDH assay displayed a uniform purple/blue staining (LDH positive) over the entire section on a macroscopic scale of the fresh, live tissue. Many dark stained osteocytes were seen in the bone cores cultured for 14 days in the loaded Zetos culture after administration of TGF $\beta_3$ . The dead sections however did not exhibit any dark, defined osteocytes and only the empty lacunae were visible (Fig. 2)



**Figure 2.** Human bone tissue (male, 54 yr). Mid-sections, stained for LDH-assay. a) and b). T0 section, darkly stained osteocytes and marrow can be seen. c) and d). A dead core taken after 14 days in loaded Zetos system. No darkly stained osteocytes or marrow can be observed. e) and f). Show an image of a core taken after

14 days in loaded Zetos culture with many darkly stained osteocytes and viable marrow can be seen.

The number of viable osteocytes observed in the fresh tissue (T0) was greater than after 7 and 14 days in Zetos culture. However, in all cases there appeared to be a positive effect of loading on the number of viable osteocytes present after 7 and 14 days in Zetos culture compared with the unloaded samples. In most cases the effect of loading plus TGF $\beta_3$  on viable osteocytes was even greater. (Fig. 3)



**Figure 3.** Graphical representation of the quantified osteocytes present in the central area of bone cores at T0 (fresh tissue) and after 7 and 14 days in Zetos culture under different experimental conditions. Light blue depicts 7 day time point, dark blue depicts 14 day time point.

human trabecular bone cores up to 14 days. The outcome of this work shows that this *ex vivo* loading bioreactor is able to maintain a high percentage (over 50%) of viable osteocytes throughout the bone cores after 14 days in *ex vivo* culture. Further to this, the combination of daily loading and TGF $\beta_3$  administration produced superior osteocyte viability at the core centres when compared to loading alone. The bioreactor has potential in pre-testing the integration of human bone with biomaterials, studying basic bone biology including osteoporotic bone.

#### References:

- <sup>1</sup>Jones DB. et al. (2003) *Eur Cell Mater* **5**:48-60.
- <sup>2</sup>Stoddart MJ. et al. (2006) *Eur Cell Mater* **12**:16-25.
- <sup>3</sup>Yang R. et al. (2003) *Eur Cell Mater* **6**:57-71

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**Discussion and Conclusion:** The Zetos bone bioreactor system permits the culture of viable 3D