P149

Enhancement of bone Ultra structure preservation using high pressure freezing and microwave assisted fixation

D.S. Daghma[1], C. Heiss[1,2] , T. El Khassawna[1]

[1]Institute for Experimental Trauma Surgery, Justus-Liebig University, Giessen, Germany

[2]Department of Trauma Surgery, University Hospital of Giessen-Marburg, Giessen, Germany

SFB/Transregio 79

1 Bone complexity hinders high quality preservation of cellular components

Tissue fixation is the pillar of histological and electron microscopy investigation. Inferior fixation of bone tissue affects ultrastructure quality (Fig. 1). However, alternative methods depending on enhancing fixative penetration (i.e. microwave radiation) or ultra-rapid immobilization of biological events by cryo preservation can improve hard tissue fixation.

This study achieved staggering improvement in ultrastructure preservation of bone tissue using microwave assisted fixation and high pressure freezing techniques.

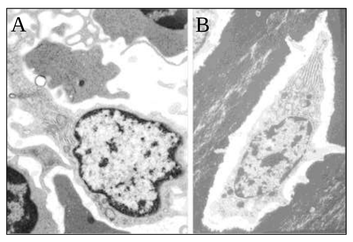


Fig. 1: Conventional chemical bone fixation reflects on structural preservation and visualization in TEM. A) Bone marrow. B) Osteocyte in adult bone (1).

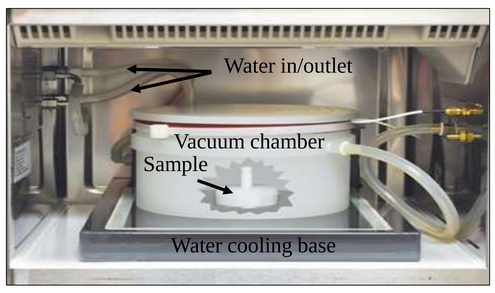


Fig. 2: Microwave Assisted Chemical Fixation (MWCF) is enhanced due to radiation, cooling and vacuum. MWCF was used to fix whole bone samples (Femur, tibia and vertebrae).

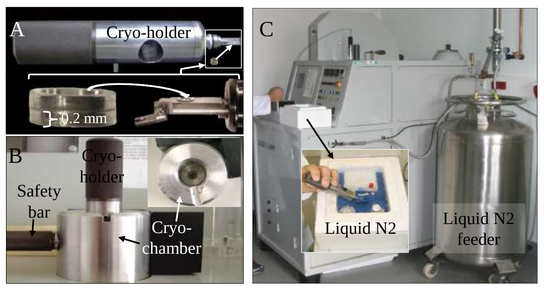
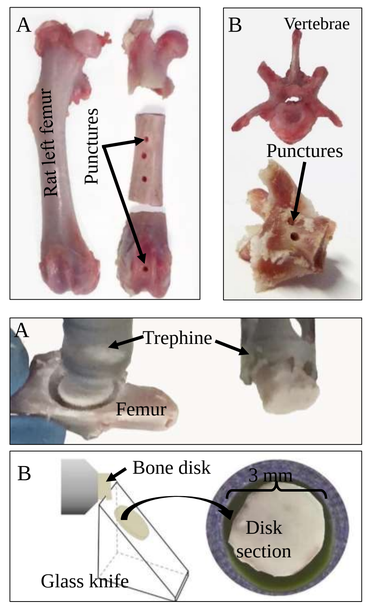


Fig. 3: High Pressure Freezing (HPF) suitable for micro-sized samples. Rat bone samples, human bone reaming debris and marrow aspirates were smaller than 3mm dimeter and 0.2 mm thick.

2 Bone fixation – The Trifecta Large, Micro-sized and Liquid



MWCF enhanced sample fixation and notably reduced the processing time.

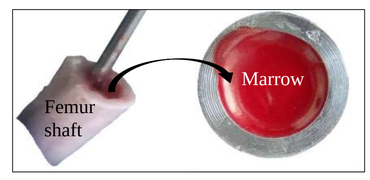
Fig.4: Whole bone regions of small animal models are challenging large samples to fix for TEM investigation. (A&B) Directly after euthanasia femur and lumbar vertebrae fixed using MWCF technique, the regions were perforated by drill-holes and segmentation.

Beside the use of HPF for reaming debris. The method is valid for small bone samples or pathologic biopsies acquired from larger areas.

Fig. 5 (Left): Alternatively small sized bone samples can be prepared for HPF. Beside. (A) Diameter-fit collection of bone disk is ensured using surgical trephine under cooling. (B) The disk thickness is achieved in ultramicrotom with a glass knife, before placing in aluminum platelets with biological filler.

HPF is very useful in fixation of liquid samples such as bone marrow aspirate without stressful preparation.

Fig. 6: Bone marrow is very important cell-rich tissue that is hard to investigate from clinical aspirates. HPF assists the assessment of cellular composition of bone marrow with high preservation of cellular structure. This enables the differentiation of stem cells ultrastructure.



3 Bone structure integrity depends on sample processing methodology

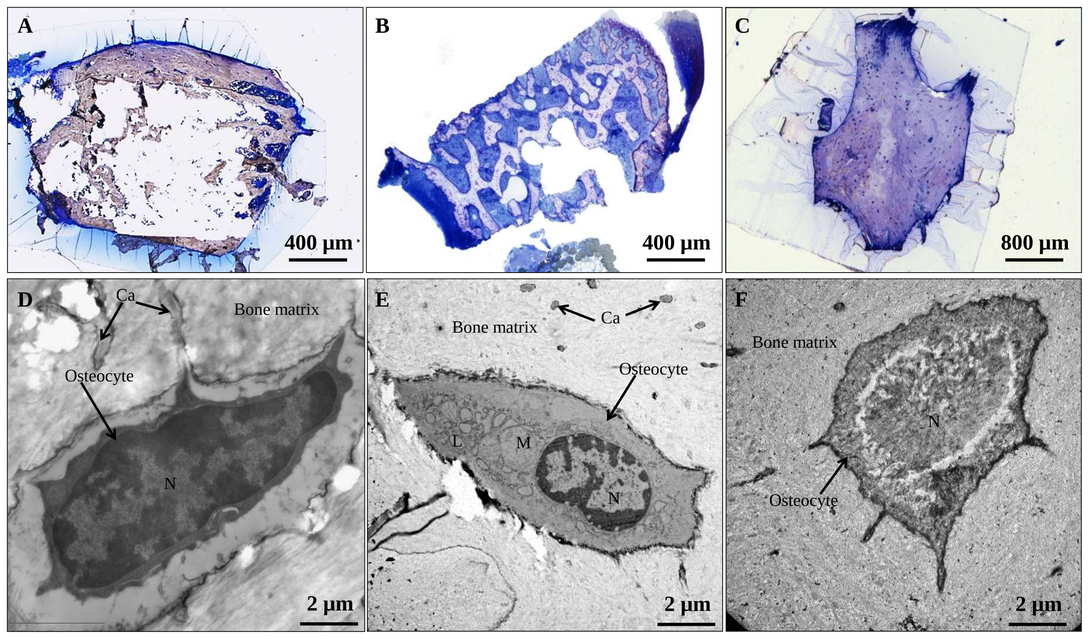


Fig. 7: Inferior subcellular structure of bone samples in chemical fixation. A - C) Histological stain reflects issue integrity after processing in conventional, MWCF and HPF, respectively. Osteocyte reflects cellular and matrix preservation. . D) Osteocyte with less recognizable subcellular structure. D) MWCF fixation enhances mitochondrial visualization in rat femur. C) Reaming debris after HPF fixation shows high matrix conservation. Ca; canaliculi, L; lysosome, M, mitochondria, N; nucleus.

4 HPF facilitates processing of reaming debris and bone marrow aspirate

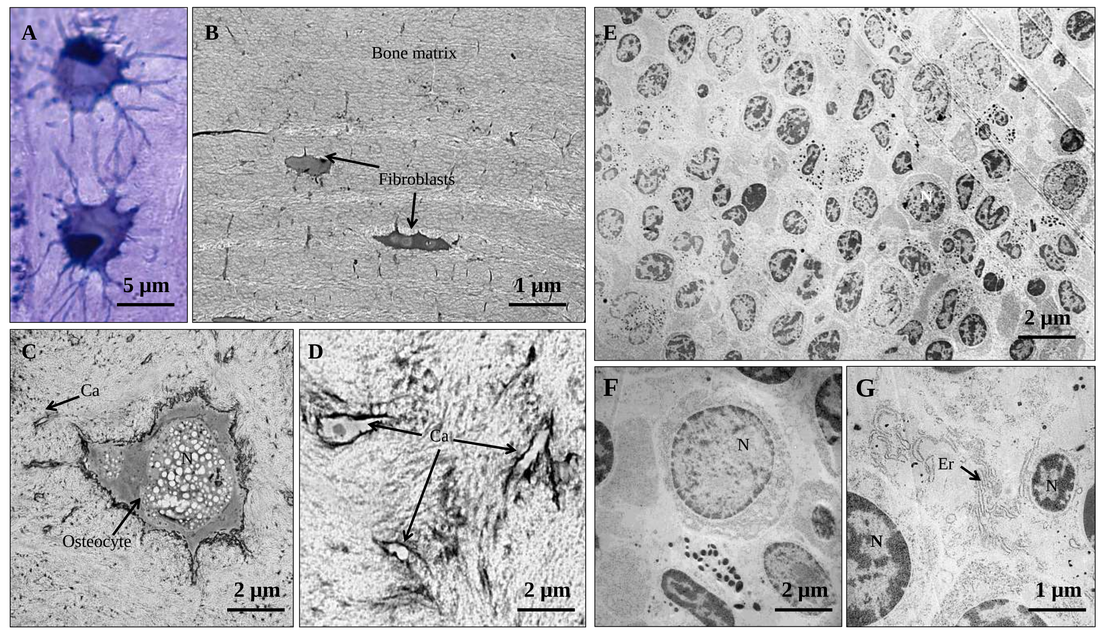


Fig. 8: HPF requires minimal preprocessing to preserve cellular ultrastructure and bone matrix. A) Methylene blue stain is able to visualize osteocyte caniculi in simple histograph. B) Fibroblasts in artifact free bone matrix. C-D) Osteocyte and caniculi well preserved within the matrix. E) Overview of bone marrow cellular composition. F-G) Ultrastructure of bone marrow. Ca, canaliculi, Er: endoplasmic reticulum, L; lysosome, M, mitochondria, N; nucleus.

5 Microwave chemical fixation of large samples enhances preservation

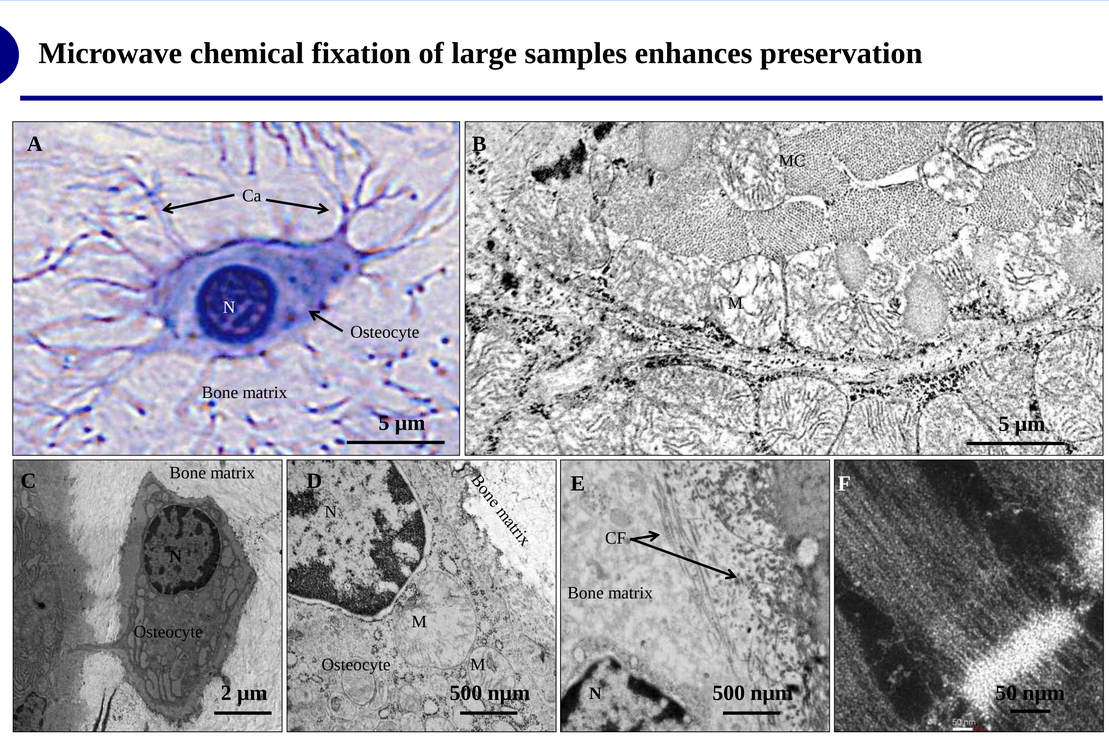
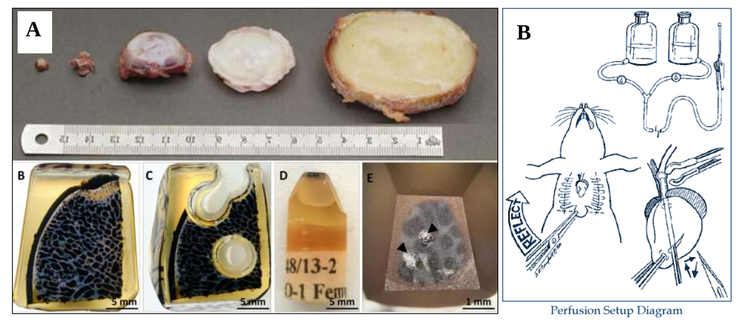


Fig. 9: Ultrastructure is crucial in studying systemic bone diseases or aging phenomenon in preclinical studies.. A) Osteocyte visualization is possible without Rhodamine staining or confocal microscopy. B) Muscle tissue around the bone intact. C) Osteocyte cell bridged to the marrow. D) subcellular components of osteocyte. E) Highly preserve cartilage fibers in bone vacinity. F) High resolution TEM micrograph of fibrillar structure of bone muscles. Ca; canaliculi, CF; cartilage fibers, M, mitochondria, MC, bone muscle cell, N; nucleus.

6 Never change a running system is outdated ….. A REMINDER

Fig. 8: Size, tissue complexity and preprocessing are the main challenge in bone histology and TEM. A) variable sample size (up), orientation is crucial before TEM. (B) Perfusion is time consuming not applicable for clinical samples.



Check list

- Enhanced fixation of wide variety of bone sample sizes ☑
- Avoidance of artifacts related poor resin infiltration ☑
- Investigating distinct region of interest in TEM based on the histology overview ☑
- High quality of ultrastructure preservation ☑
- Shortening processing time ☑

(1) Baron, R., *Growth and Remodeling*, in *Endotext*, L.J. De Groot, et al., Editors. 2000.

DFG gefördert : SFB/TRR 79 Teilprojekt T1

The authors claim no conflict of interest