

Supplementary Materials

Early Stages of Biomineral Formation—A Solid-State NMR Investigation of the Mandibles of Minipigs

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Implants

The screw type implants used for the present study, mimicking the shape of common dental implants (Xive S, Dentsply Sirona Implants, Mannheim, Germany), were manufactured of poly-ether-ether-ketone (PEEK) (Figure 1). Each implant had a diameter of 4.5 mm, a length of 11.5 mm, and a cavity of 1.35 × 0.35 mm along the length axis. A layer of titanium (Ti) measuring 100 nm in thickness was deposited onto the implants by physical vapor deposition (GeSiM mbH, Großerkmannsdorf, Germany). Commercially available titanium implants would hinder both CT and NMR imaging and would result in drastic image artefacts. The coating of polymer implants using Ti provides the same biochemical environment as the commonly used Ti implants.

Animal Model

The study protocol was approved by the Commission for Animal Studies at the District Government Office, Dresden, Germany (file reference: 24-9168.11-1/2010-19). Three female miniature pigs of the breed "Mini-Lewe" (average weight of 81 ± 13 kg and aged 3 years) were used for the experiment. All surgical procedures were performed under general anaesthesia. Prior, 1 mg/kg body weight Dormicum® (Ratiopharm GmbH Ulm, Germany) and 10 mg/kg body weight ketamine (Riemser Arzneimittel AG, Greifswald, Germany) were injected intramuscularly. To reduce salivation, 0.05 mg/kg body weight atropine (Eifelfango Chem. Pharm. Werke, Bad Neuenahr-Ahrweiler, Germany) were added. Maintenance of sedation was achieved via intravenous injection of the mixture. For local anaesthesia, Ultracain® D-S forte (Sanofi-aventis Deutschland GmbH, Frankfurt/Main, Germany) was used. Antibiosis was performed by intramuscular administration of 15 mg/kg body weight Duphamox® LA (Fort Dodge Veterinär GmbH, Würselen, Germany). For postoperative analgesia, 3 mg/kg body weight Rimadyl® (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was injected subcutaneously.

Before implant insertion, the premolar teeth in the mandible were removed carefully to preserve the bony walls of the extraction socket. Re-entry was performed after 8 weeks of healing. After a vestibular incision, a muco-periosteal flap was elevated. Subsequently, the sharp edges of the alveolar ridge were flattened using a round burr. Three implant sites on each side of the mandible were prepared under permanent irrigation with saline solution. Implant burrs with an increasing diameter up to 5 mm were used. The implants were inserted manually with a torque-calibrated instrument. After reposition of the muco-periosteal flap, wound closure was performed using resorbable sutures (Vicryl 3×0, Ethicon, Norderstedt, Germany). Postoperatively, wound healing, food ingestion, and behavior were monitored daily.

Control Experiments on Sample Preparation and Storage

In order to demonstrate that the sample preparation grinding in a cryogenic mill and storage in phosphate buffer at 8°C have not altered the mineral phase or composition (on the length scale of our experiment), an additional control experiment was performed.

Therefore, bone blocks of the mandible of an adult minipig were measured by solid-state NMR immediately after resection and after storage for 4 weeks in a phosphate solution. In parallel, pieces of the bone blocks were ground in a cryomill at both stages (fresh and after storage in the solution) and their solid-state NMR spectra were measured in order to observe the effects of milling on dehydration and mineral transformation. The results of the control experiments for four samples mentioned above (fresh bone blocks, fresh ground bone, stored bone blocks, and stored bone ground) are presented in Figure S1 (2D HETCOR spectra) and Figure S2 (1D ^1H and ^{31}P MAS NMR spectra).

The data in Figure S1 show similarities in the ^{31}P dimension proving preservation of the mineral structure after storage in a buffer solution and grinding. This fact has been confirmed by very similar 1D ^{31}P MAS spectra in Figure S2 in respect to their line positions, line shape, and line widths. The only slight difference observed along the ^1H dimension for the samples before and after storage in the solution but not between bone blocks and ground bone samples. The 1D ^1H MAS spectra (Figure S2) demonstrated significant differences at each storage and preparation step, which results from dehydration of the ground bone and from the presence of solvent. As the signal from the latter is not visible in the 2D spectra, we can conclude that the buffer molecules are remotely located from the phosphate groups (that is, not through space heteronuclear dipolar interactions) and, thus, do not affect the mineral structure in bone.

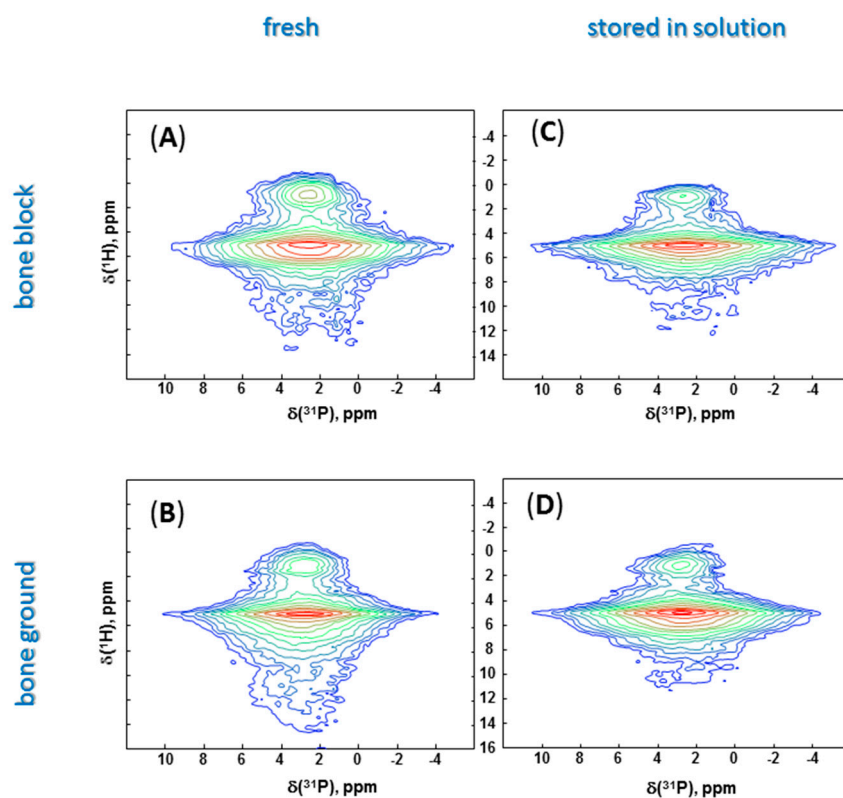


Figure S1. ^1H - ^{31}P heteronuclear correlation (HETCOR) spectra of the bone (A) in block after extraction, (B) cryogenically ground after extraction, (C) in block after storage for 4 weeks in phosphate solution in a fridge and (D) cryogenically ground after storage for 4 weeks in phosphate solution in a fridge.

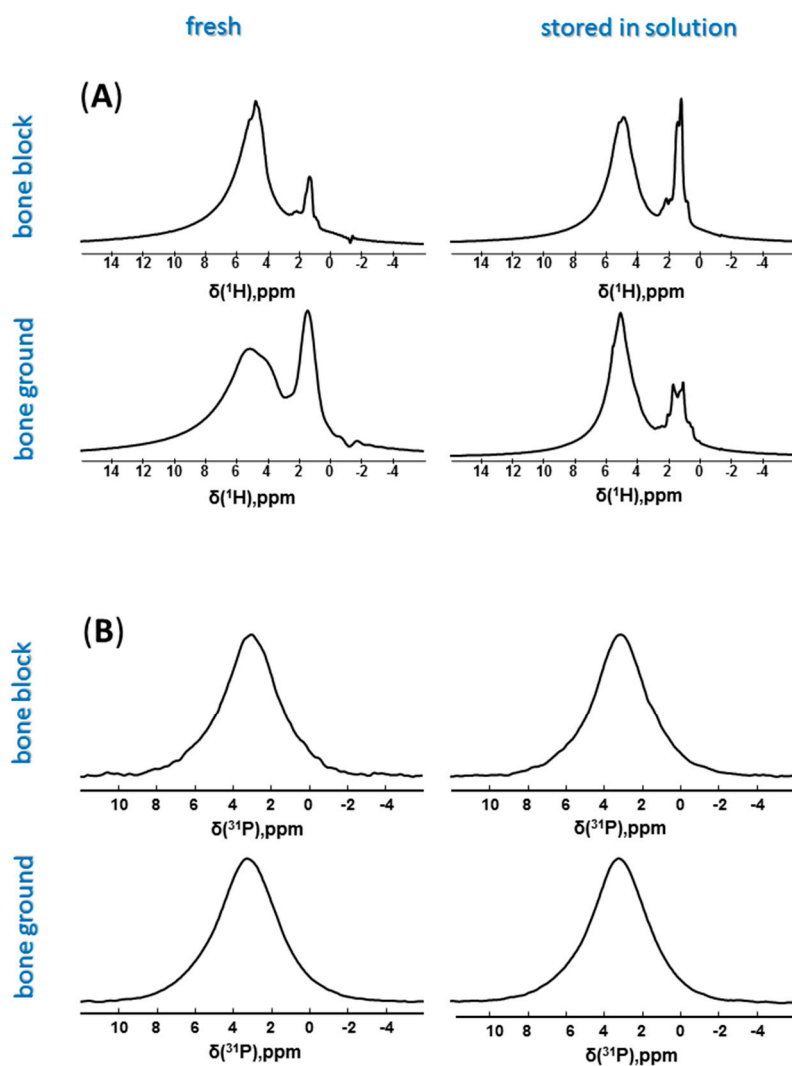


Figure S2. (A) ^1H magic angle spinning nuclear magnetic resonance (MAS NMR) spectra and (B) ^{31}P MAS NMR spectra of the bone in block after extraction, cryogenically ground after extraction, in block after storage for 4 weeks in phosphate solution in a fridge and cryogenically ground after storage for 4 weeks in phosphate solution in a fridge.

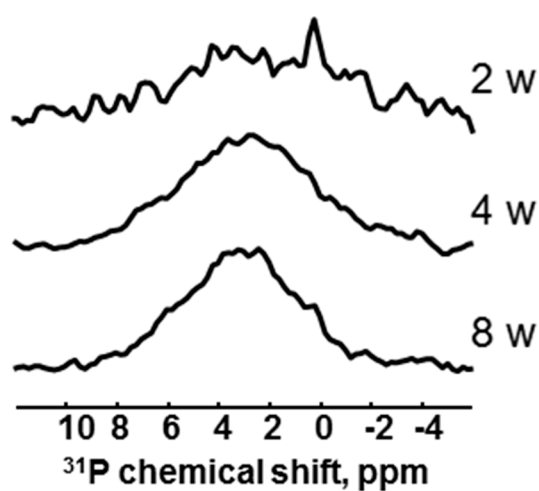
^{31}P CP MAS spectroscopy

Figure S3. $^{31}\text{P}\{^1\text{H}\}$ CP MAS (10 kHz) spectra of newly formed tissues in the second animal (after two (2w), four (4w), and eight weeks (8w) healing time). The spectra were measured at a contact time of 0.1 ms. Normalization on the sample weight and no line broadening were applied here to demonstrate the sensitivity of the spectra.

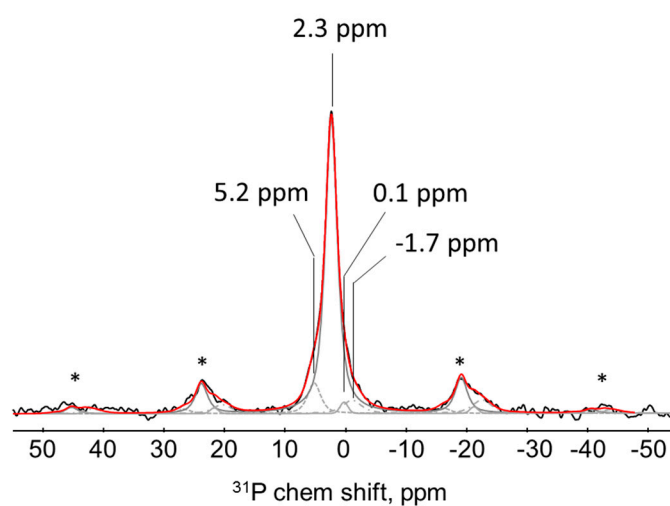


Figure S4. $^{31}\text{P}\{^1\text{H}\}$ CP MAS (2.6 kHz) spectrum of the 2w sample measured at -20°C .