Electronic Supplementary Information

Enzymatically regulated demineralisation of pathological bone using sodium hexametaphosphate

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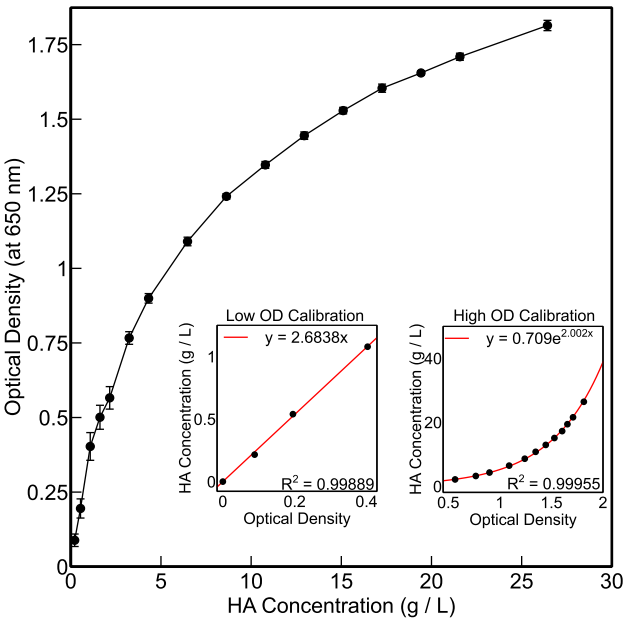
Supplementary Material

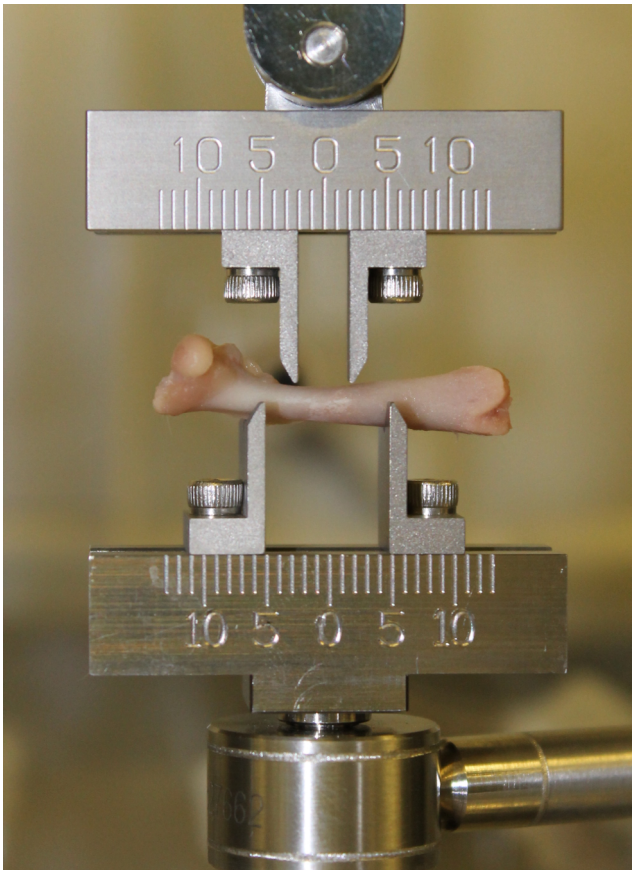
Note: Numbering refers to associated sections in main article

2.1.3 and 2.1.4 Hydroxyapatite Sol Optical Density Calibration. In order to demonstrate that the transmission of light through hydroxyapatite (HA) sol may be used to quantify the concentration of available HA in sol form, the following calibration process was performed. HA sol was synthesised as described by Ahshar et al.[1] and the concentration of available HA per ml of sol was determined. Triplicate volumes of 3ml of pure sol were centrifuged at 3900 rpm for 10 minutes, the supernatant decanted, and the tubes containing the residual pellets dried at 80°C overnight. The mean dried pellet mass was 79.3 g (standard deviation 0.265 g). Thus each ml of pure HA sol yielded 26.43 mg of dried HA. Subsequently, a volume of the same batch of HA sol underwent serial dilution with deionised water. Triplicate repeat volumes of 245 μL of each dilution were transferred to a 96-well plate and optical density (OD) was measured at 650 nm using a Glomax 9301-010 plate reading spectrophotometer (Promega, Wisconsin, USA). The resulting calibration curve is shown in supplementary figure 1. By using the chart trendline fitting function on Microsoft Excel (Microsoft Corporation, Redmond, Washington, United States), equations relating high (>0.5) and low (<0.5) OD to available HA in sol form were generated (see insets in supplementary figure 1). These equations were used to convert OD data to g / L values in figures 6 and 7 in the main article.



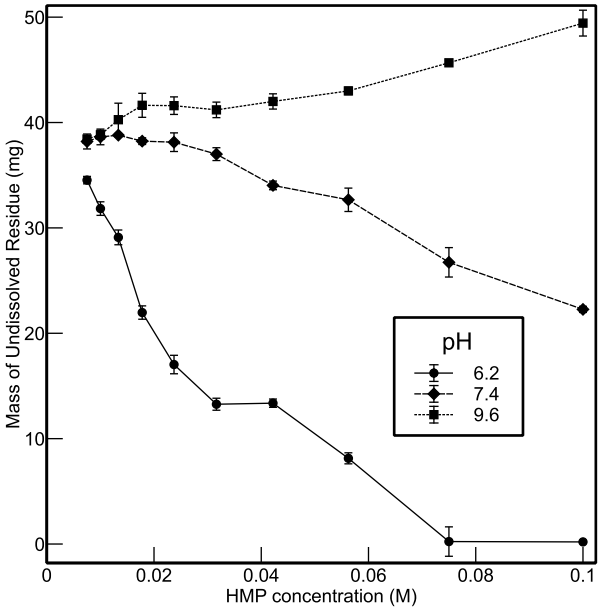
Supplementary Figure 1. Calibration curve showing relationship between optical density (at 650nm) and concentration of HA Sol. Error bars are +/- standard deviation. HA = hydroxyapatite. Insets: Reversing the axes dividing the data into points > or < and OD value of 0.5 allows best-fit lines to be drawn and equations formulated to describe the relationship between optical density and HA Concentration. Note the exceptionally high R^2 values. HA = hydroxyapatite. OD = Optical Density.

2.1.5 Mechanical Testing. In order to clarify the experimental set-up for the mechanical testing, a photograph of the 4-point mechanical testing equipment is included in this supplementary information (Supplementary Figure 2).

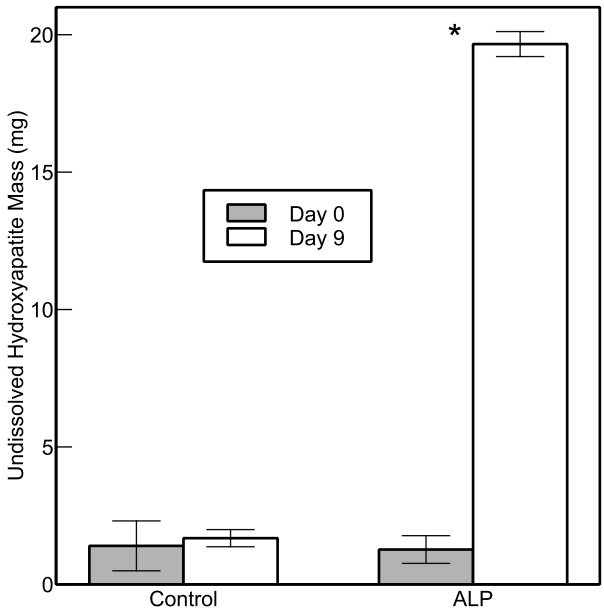


Supplementary Figure 2. Photograph of the 4-point mechanical testing setup. The image demonstrates the arrangement of the contact points. The mechanical driver is attached to the upper assembly and the force transducer is visible supporting the lower assembly.

2.1.4, 2.1.5, and 3.3 Hydroxyapatite Residual Mass Methods and Results. In the main article, sample OD at 650nm was used to determine the concentration of HA sol in order to investigate the effect of pH and alkaline phosphatase (ALP) on the HA-dissolving ability of hexametaphosphate (HMP). The calibration data presented above is reassuring that this methodology is robust. However, to support the conclusions drawn from the data, these experiments were repeated. However, instead of determining the concentration of HA sol through light transmission, direct determination of the mass of HA undissolved in sol form was performed. For the pH experiment, triplicate volumes of 1.5 ml of HA sol was added to 10 ml HMP at varying concentrations and pH (6.2, 7.4, and 9.6). These samples were agitated for 4 hours, centrifuged at 3900 rpm, supernatants decanted, dried overnight at 80^{o}C, and then weighed to determine the HA pellet mass. The ALP experiment was repeated exactly as in the main text but instead of measuring the OD of the samples the above direct method was used to determine the mass of undissolved HA per sample. The results of these experiments are presented here as supplementary figures 3 and 4. These data are strikingly similar to the OD-derived data and therefore support the conclusions in the main article.



Supplementary Figure 3. The effect of pH and concentration of HMP on the mass of undissolved HA. Increasing pH is associated with greater mass of undissolved HA suggesting that HMP is less effective at dissolving HA at higher pH. Error bars are +/- SEM.



Supplementary Figure 4 Change amount (in g / L) of available HA in sol form in a nearly-saturated solution of HA dissolved in 0.1 M hexametaphosphate after incubation with either alkaline phosphatase (ALP) or control (diH_2O) for 9 days. Error bars are +/- SEM. (* p = 0.00)

Reference:

1 A. Afshar, M. Ghorbani, N. Ehsani, M. Saeri and C. Sorrell, *Materials & Design*, 2003, **24**, 197-202.