Supporting Information for:

Isolation of calcium phosphate crystals from complex biological fluids using bisphosphonate-modified superparamagnetic beads

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Materials:

Chemicals were purchased from either Sigma or Aldrich and were all analytical grade. Chromatography grade water (CHROMASOLV, Aldrich) was used in all the experiments. Synovial fluid samples and synthetic basic calcium phosphate crystals were supplied by Prof. Geraldine McCarthy (Mater Misericordiae University Hospital, Dublin 7, Ireland). Synovial fluid samples were stored at 4 °C directly after aspiration from the affected joint. Dynabeads® My One™ carboxylic acid beads were purchased from Dynal Biotech Ltd., U.K.

General Methods:

Scanning electron micrographs and energy-dispersive x-ray data were acquired on a Hitachi H3000 SEM. Samples (~5 µl in H_2O) were air dried on a carbon pad with no further preparation.

Synthesis of bisphosphonate-modified superparamagnetic beads:

Dynabeads® My One™ carboxylic acid magnetic microspheres were supplied as an aqueous dispersion (10 mg / ml). A magnet was used to pull the beads to the side of the reaction vessel during the washing steps allowing the supernatant to be removed using a pipette. The pellet could then be re-suspended in fresh buffer.

Dynabeads® My One™ carboxylic acid magnetic microspheres (300 µl) were washed twice with conjugation buffer (300 µl, 25 mM MES (2-morpholinoethanesulfonic acid) buffer pH 6) followed by three times with chromatography grade water (300 µl) for 10 minutes with good mixing. Excess liquid was removed. Neridronate in conjugation buffer (225 µl, 10 mg / ml) was then added to the washed Dynabeads, mixed and incubated with slow mixing movement in ice for 30 minutes. EDC·HCl (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide Hydrochloride) was dissolved immediately before use in cold conjugation buffer to a concentration of 10 mg / ml. EDC·HCl solution (75 µl, 0.75 mg) was then added to the Dynabeads/bisphosphonate suspension and mixed well. The reaction was incubated for 18 hours at 4 ºC with occasional mixing. Un-reacted activated carboxylic acid groups were quenched by washing the beads once with quenching buffer (300 µl, 50 mM Tris-HCl buffer pH 7.5), resuspending the beads in the same volume of buffer and leaving to stand at room temperature for 15 minutes. Finally, the beads were washed three times with chromatography grade water (300 µl) and stored at 4 ºC. Final concentration was 10 mg / ml (supplier recommends final concentration of magnetic beads after coating to be 10-30 mg / ml).

Isolation of calcium phosphate crystals from synovial fluid using bisphosphonate-modified superparamagnetic beads:

Spiked samples were prepared by dispersing calcium phosphate crystals (HA, BCP or CPPD) in RA synovial fluid (1 mg / ml). An aliquot of the spiked sample or patient sample (100 µl) was diluted with water (400 µl) and mixed with bisphosphonate-modifed magnetic beads (10 µl) in an eppendorf microtube (1.5 ml). The samples were then incubated at room temperature under rotary mixing for one hour. A magnet was used to pull the beads to the side of the reaction vessel, allowing the supernatant to be removed using a pipette. The beads were then resuspended using vortexing in chromatography grade water (300 µl), transferred into a clean eppendorf and washed five times with chromatography grade water (5 x 300 µl) in the same manner. Patient samples with suspected OA were processed in the same way.

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SEM images of reference crystals used in the spiked samples:

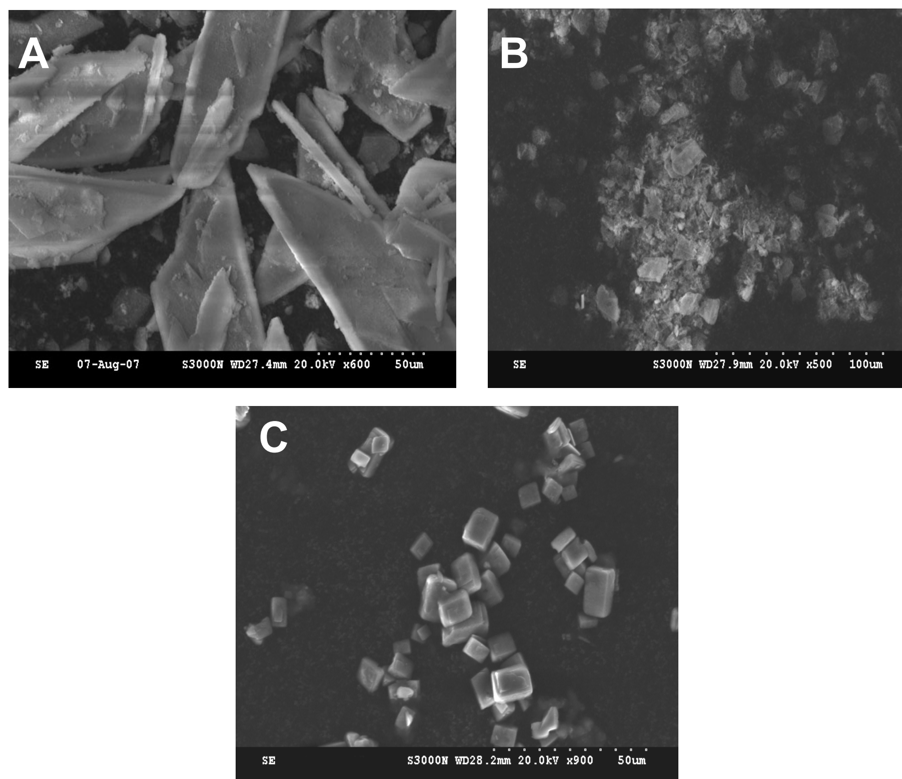

Figure S1. Scanning electron micrographs of (A) HA, (B) BCP and (C) CPPD reference crystals.

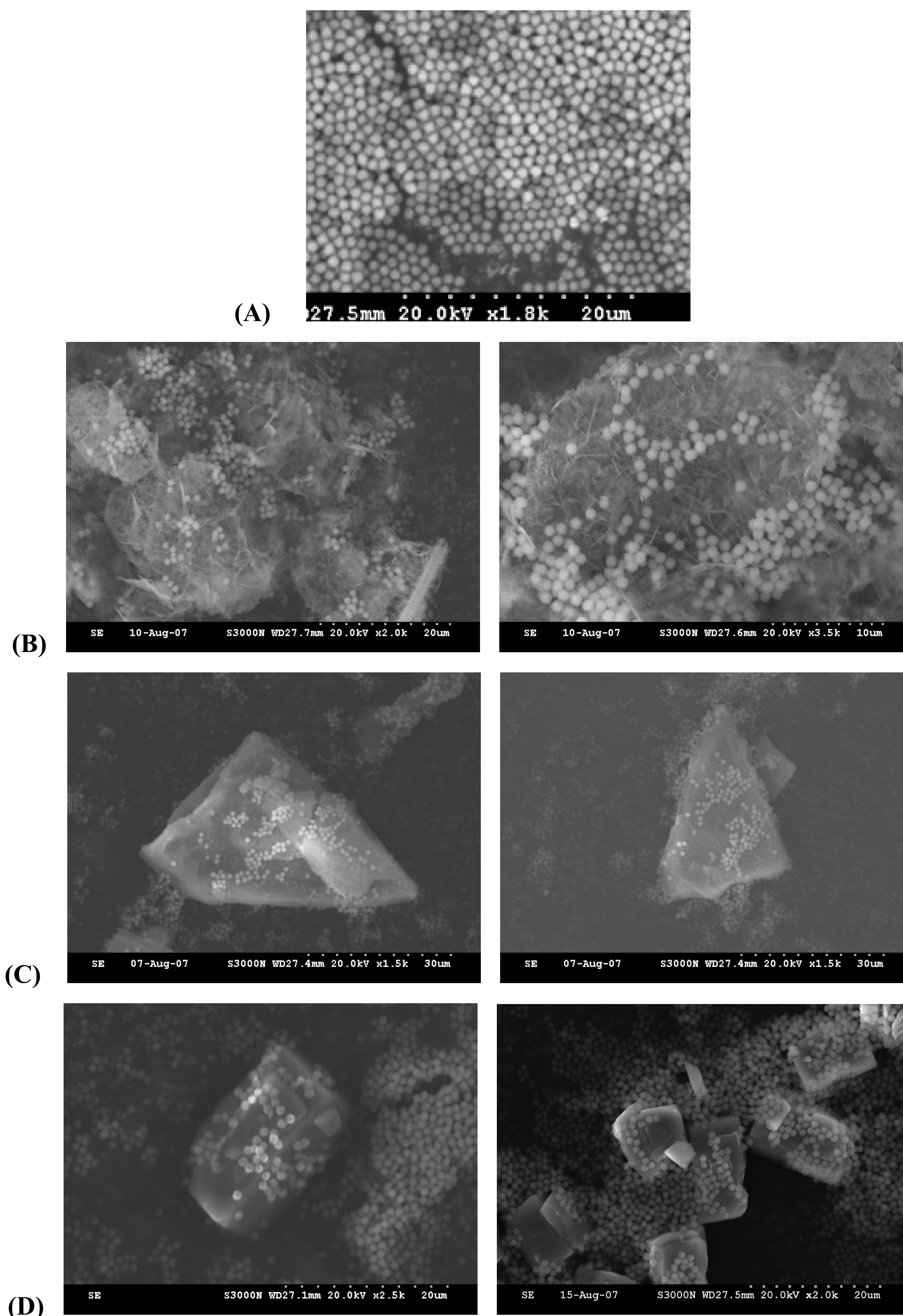

Figure S2. Scanning electron micrograph (SEM) images of (A) BPSM beads, and captured (B) BCP, (C) HA and (D) CPPD synthetic crystals.

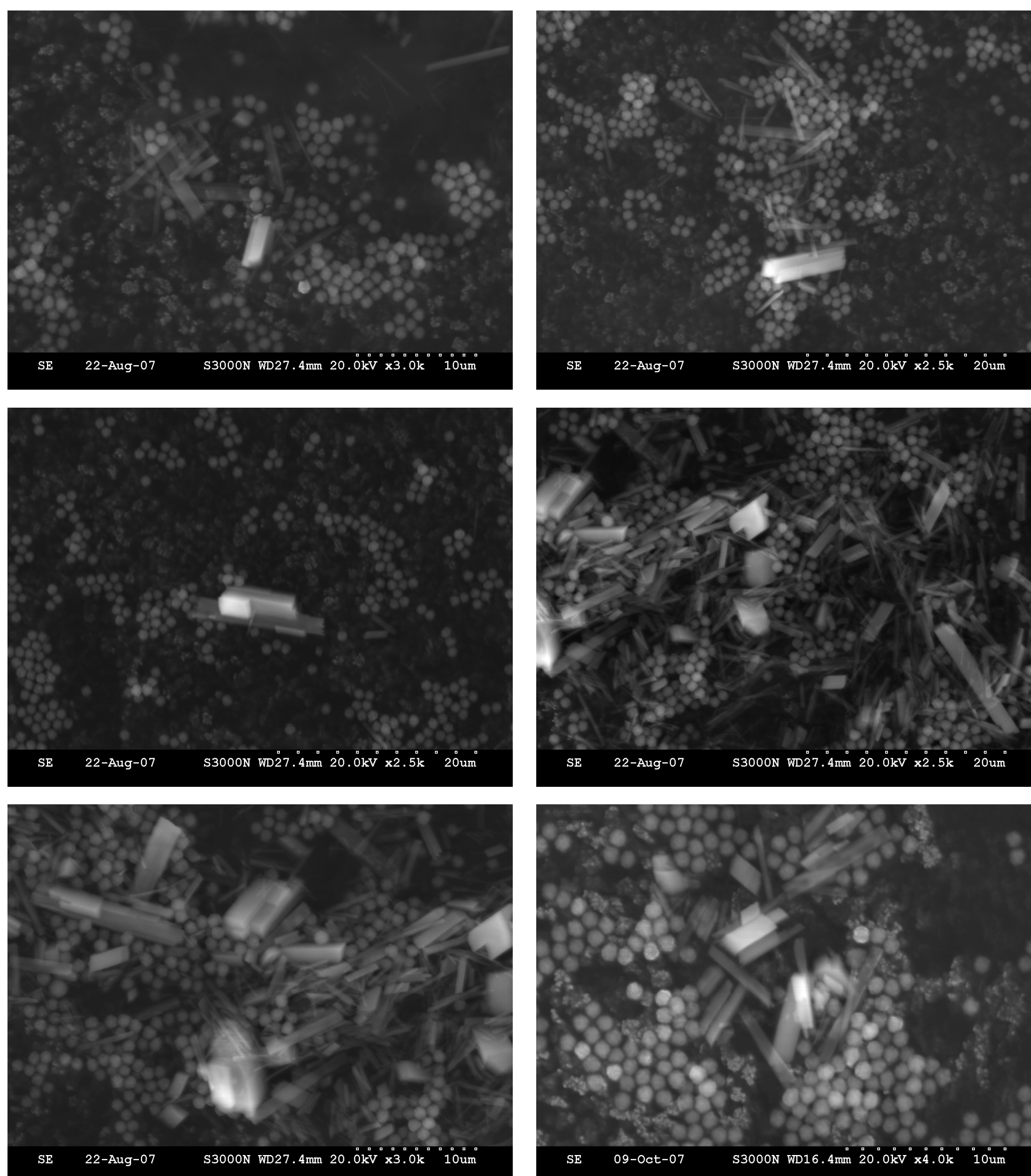

Figure S3. Scanning electron micrograph (SEM) images of calcium phosphate crystals extracted from patient samples using BPSM beads.