Electronic supplementary material for Journal of Materials Chemistry B

Nanoscale mineralization of cell-laden methacrylated gelatin hydrogels using calcium carbonate - calcium citrate core-shell microparticles

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1. Preparation of calcium citrate-calcium carbonate nanoneedles

A solution containing $CaCl_2$ (1M) and NaOAc (2M), 25 mL was added to 25 mL of 1M NaHCO₃, mixed, held for 5 min, and stirred for 4h. A 2 mL sample was taken, centrifuged (1,000 X 5 min), washed with 1X10 mL portion of water (2,000 X 10 min), re-dispersed in 5 mL of water. This yielded intermediate particles of CaCO₃.



Then the mixture was split into two equal parts.

<u>Part 1</u> was stirred with 1 mL of 0.02 M of citric acid for 22 h, centrifuged (1,000 X 10 min), washed with 2X20 mL portions of water (1,000 X 5 min), re-dispersed in 10 mL of water. Gravimetric analysis – 53.7 mg/mL. This yielded nanoneedles of $CaCO_3$ – calcium citrate from a lower concentration of citric acid.



<u>Part 2</u> was stirred with 1 mL of 0.468 M of citric acid for 22 h, centrifuged (1,000 X 10 min), washed with 2X20 mL portions of water (1,000 X 5 min), re-dispersed in 10 mL of water. Gravimetric analysis – 45 mg/mL. This yielded nanoneedles of $CaCO_3$ – calcium citrate from a higher concentration of citric acid.



2. Preparation of calcium carbonate – calcium citrate nanoclusters

An aqueous NaHCO₃ (1M, 12.5 mL) was added over 2 min to a vigorously stirred homemade CaOAc₂ (1M, 12.5 mL). The mixture was kept without stirring for 5 min and then stirred for 1h. Citric acid (1 mL, 0.468M) was added. The mixture was stirred for 18h, centrifuged (1,000 X 10 min), washed with water (2X20 mL, 3,000 X 5min), re-dispersed in 10 mL of water. This yielded calcium carbonate – calcium citrate nanoclusters.

