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Supplementary data

PI based live/dead assay using flow cytometer

MG-63 cells retrieved from the beads were stained with 50 µg/ml PI solution for 20 min in the dark and analyzed using flow cytometer (FACS Accuri C6, BD Biosciences) [1].

Alkaline phosphatase assay

For ALP activity, p-nitrophenol phosphate based colorimetric assay was performed [2]. After 10 days of cell encapsulation, the beads were dissolved using 55 mM sodium citrate buffer and then centrifuged. The collected cells were then permeabilized with 0.1 % PBST (0.1 % Triton X-100 in PBS) for 20 min. Later, the cell lysate was incubated with the p-nitrophenol phosphate for 2 h (37 °C) and the absorbance was measured at 405 nm using double beam UV-visible spectrophotometer (Systronics, India) [3].

Alizarin Red assay

In vitro bone mineralization by the encapsulated cells were studied both qualitatively and quantitatively by Alizarin red assay [4]. After 10 days of cell encapsulation, the cells were fixed with 4 % paraformaldehyde for 20 min. Post fixing, the beads were washed with DPBS and stained with 0.2 % Alizarin red for 2 h at 37 °C. After staining, the beads were washed thoroughly with distilled water to remove non-specific stains of Alizarin and visualized under phase contrast microscope. To quantify the mineral synthesis by the encapsulated cells, cetylpyridinium chloride based colorimetric assay was performed. The stained beads were dissolved with 55 mM sodium citrate buffer and the cells stained with Alizarin red was spun down. Bound alizarin red was eluted using 10 % aqueous cetylpyridinium chloride solution, and absorbance was measured at 562 nm using double beam UV-visible spectrophotometer (Systronics, India) [5].

HIF-1α expression analysis by Western Blot

HIF-1α expression profile of the cells was analyzed by western blotting. For this study, the cells encapsulated in the beads were cultured at 37 °C, 5 % CO_2 and 95 % relative humidity for 5 days. After 5 days, spent media of the beads was withdrawn and stored at -20 °C for analyzing VEGF expression. The cells inside the beads were harvested by treating the beads with 55 mM sodium citrate buffer, washed twice with DPBS, and subsequently lysed with lysis buffer supplemented with a protease inhibitor. The Bradford assay was employed to measure the protein concentration in the lysate. The protein (20 µg) of each sample was fractioned through 7 % SDS-PAGE and electro-transferred onto a nitro-cellulose membrane. The membrane was then washed with PBS thrice and blocked with

5 % BSA in PBS. Post-blocking, the membrane was immuno-stained against HIF-1α antibody (ab1, Mouse monoclonal antibody, Abcam, UK). The bands were then visualized by a digital camera, and the intensity analysis was performed using ImageJ software [6].

Analysis of VEGF expression by ELISA

For this study, the cells encapsulated in the beads were cultured at 37 °C, 5 % CO_2 and 95 % relative humidity for 5 days. After 5 days, spent media of the beads was withdrawn and stored at -20 °C for analyzing VEGF expression. The VEGF quantification was done using human VEGF ELISA kit (abcam 100662, Abcam, UK) following standard protocol mentioned by the manufacturers [6].

SEM analysis of the microstructure of the cryo-fractured lyophilized beads

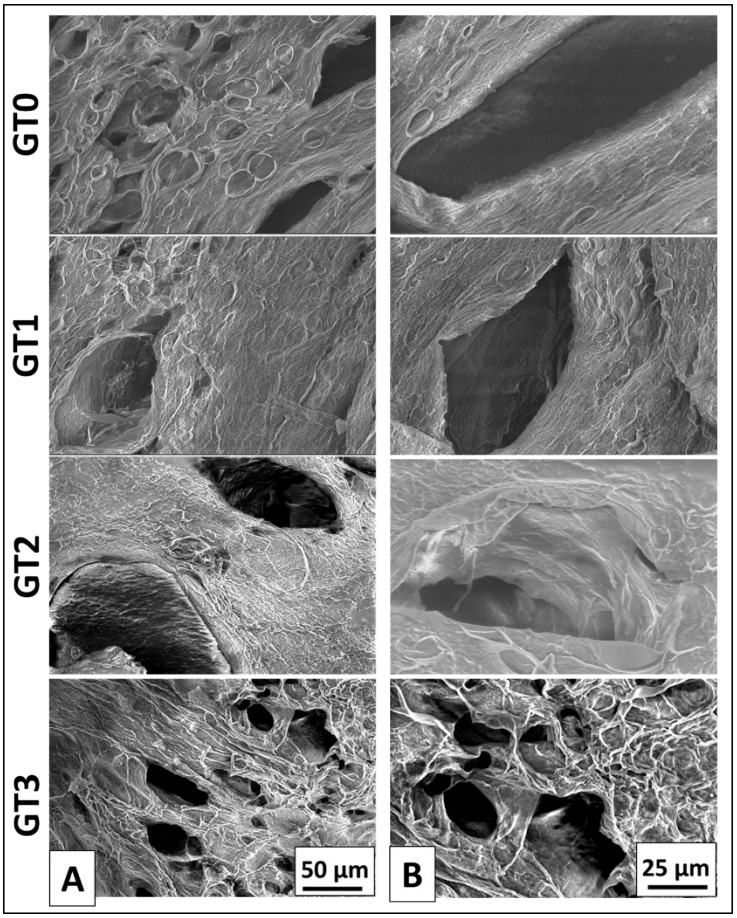

Figure S1. A & B) Microstructure analysis: ESEM micrographs of the cross sections of the freeze fractured lyophilized CA-GT beads of different magnifications

Study of the live cell population of the encapsulated cells by flow cytometry

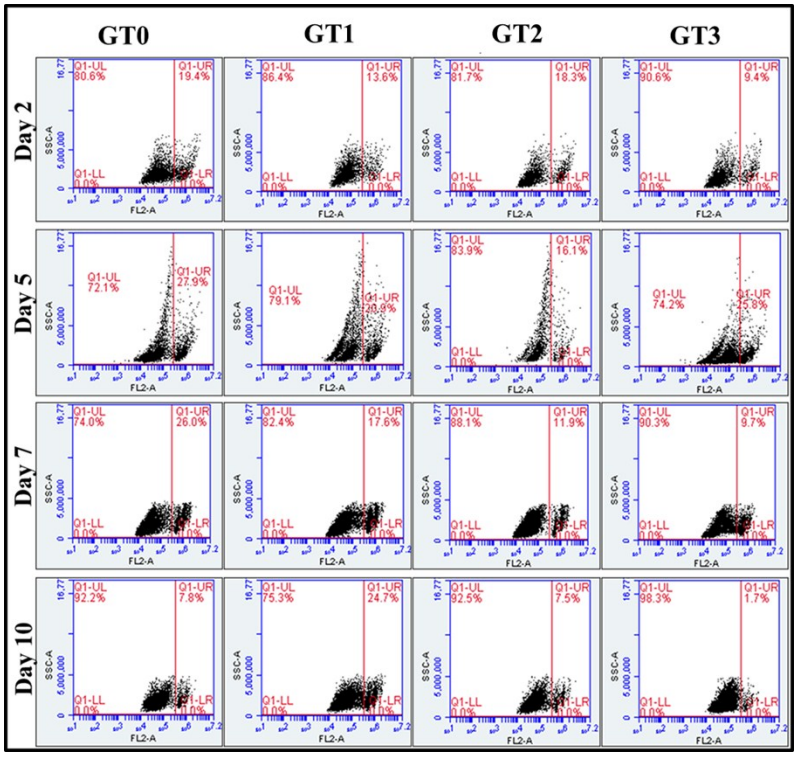

Figure S2. Flow cytometry based live/dead assay of the cells encapsulated in the beads.

Study of the distribution of the live cell population inside the beads

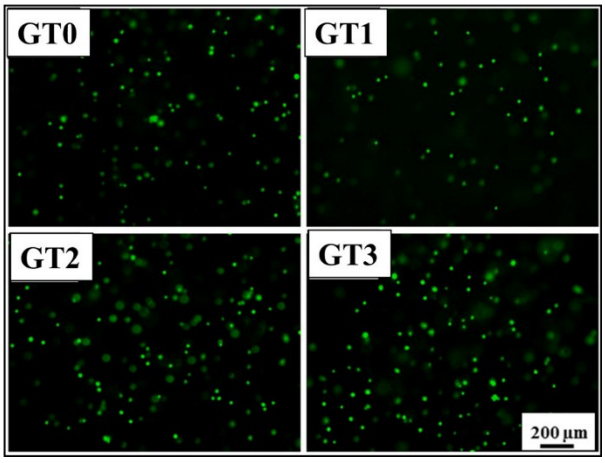

Figure S3. Calcein AM based fluorescent microscopic image of live cells inside the beads on day 5.

Qualitative analysis of the bone mineralization by the encapsulated cells through alizarin red assay

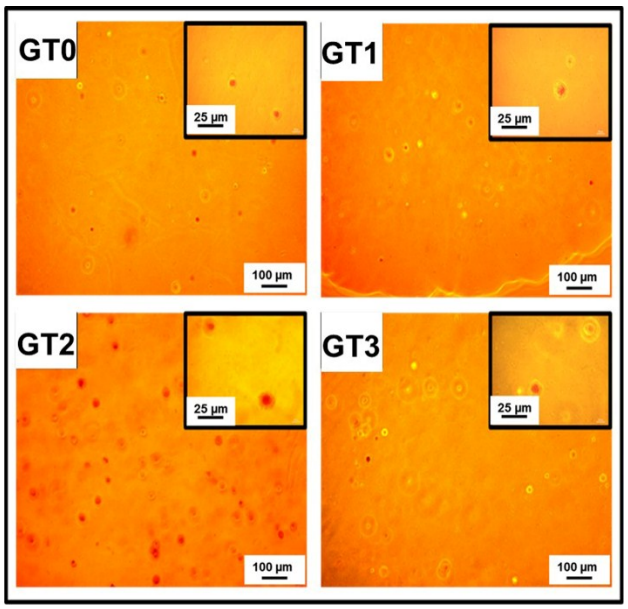

Figure S4. Phase contrast images of the minerals (calcium phosphate) synthesized by the encapsulated cells. The deposited minerals were stained by alizarin red.

References

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