Development of biocompatible hydroxyapatite–poly(ethylene glycol) core–shell nanoparticles as an improved drug carrier: structural and electrical characterizations

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S1. *In vitro* drug release kinetic assay

Briefly, the AF drug loaded carrier materials were dispersed in the 1.2 ml phosphate buffered saline (PBS) of pH 7.45 at 37.4 °C in a shaking incubator (LSI-3016, Labtech) with a speed 50 rpm for 1 h. The concentration of released drug in the solution was evaluated at time points 0, 20, 30, 40, 50, and 60 min using ultraviolet-visible (UV-Vis) spectrophotometer (Cary 50 Probe, Varian). The drug loaded carrier solution was then measured being in quartz cuvette between the wavelength (λ) 200 and 800 nm.

S2. *In vitro* human fibroblast cell compatibility assay

S2.1. Cell culture

In brief, the harvested HDF cells, isolated from the unused skin by outgrowth method, of concentration 2×10^4 cells/ml were prepared for cell proliferation tests of the HA and HA-PEG scaffolds (*Φ* 10 mm × 2 mm). Here, following cosmetic-plastic surgery of a young female, the unused disposal dermal tissues were collected from the Medical Hospital, University of Malaya after receiving ethical approval (PPUM/MDU/300/04/03, 22 April 2011) by University of Malaya Medical Centre (UMMC)[1, 2]. Both the samples including control (special Thermanox™) were properly sterilized and then soaked quickly in 80 µl freshly prepared chondrogenic Dulbecco's modification of Eagle's medium (DMEM) (Cellgro Mediatech) in 24-well plate. Then, they were incubated for 24 h at 37 °C, 5% CO_2 and 95% relative humidity (RH) environments. Here, the thermanox was used as positive control culture (with cell) and a scaffold of each sample was used as blank or negative control (without cell). The 20 µl HDF cells of made concentration were successively seeded on the soaked surface of each scaffold and placed in the incubator for 3 h. To assess the proliferated cellular activity, 200 µl freshly prepared DMEM was then dropped to the cell adhered scaffolds and kept in the incubator for 7 days at the time points day-1, day-3 and day-7. The cultured medium was changed regularly at every even day.

S2.2. Alamar Blue assay

In AB assay, a significant change in dye colour (purple blue to pink) indicated about cytotoxic status of the scaffolds owing to the redox reactions of resazurin solutions with the mitochondria of cells. The chondrogenic media of cell cultured scaffolds were replaced with 1 ml pre-heated (at 38°C) PBS from 24-well plate for washing pellets on respective time points. Each scaffold was added with 1 ml of blue coloured AB dye solution (10 vol% resazurin in culture medium at concentration of 0.14 mg/ml in PBS) and incubated for 4 h. Then, 300 µl supernatant of the incubated AB treated cellular suspension was replaced from 24-well plate and was triplicated in 96-well plate (100 µl/well for three 96-well plate). The triplicated 96-well plate was inserted in microplate reader (FLUOstar OPTIMA BMG LABTECH, Germany) to record the resorufin dye absorbance at the excitation (λ_{ex}~570 nm) and emission (λ_{em}~595 nm) wavelengths. The cell metabolic activities, such as cell viability and cell proliferation, as well as toxic properties of the composites on HDF cells with indirect reaction would be demonstrated from the absorption values owing to drastic change in solution colour from purple blue (oxidation) to pink (reduction).

S2.3. Deoxyribonucleic acid (DNA) assay

In DNA assay, the cell seeded pellets (n=5) were treated with 1 ml papain digest buffer (0.01 M L-cysteine, 0.125 mg/ml papain in 0.1 M sodium phosphate buffer, and 0.01M Na_2EDTA) solution for deproteinizing cells to explore DNA and incubated at 65°C for 16 h. The 1 ml digested supernatant was withdrawn from each pellet and centrifuged at 10,000 rpm for 5 min at 25°C for separating digest solution from undigested debris. The resulting digest solution of each sample was triplicated in 96-black well-plate with Hoechst 33258 dye (0.1 µl in 1ml TEN buffer) using calf thymus DNA (Sigma-Aldrich) as the standard and the well-plate was placed in a microplate reader at λ_{ex}~355 nm and λ_{em}~460 nm.

S2.4. Confocal microscopy – live/dead cell assay

For this study, two dyes such as calcein AM (live strain, 1 µl) and ethidium homodimer-1 (dead strain, 2.5 µl) were mixed with 1 ml pre-heated PBS (at 38 °C for 16 h). The calcein-AM (4 mM in anhydrous dimethyl sulfoxide (DMSO)) and ethidium homodimer-1 (2 mM DMSO/H_2O of 1:4 (v/v)) were present in a LIVE/DEAD® Viability/Cytotoxicity Kit (Invitrogen, UK). The cell seeded scaffolds were soaked shortly in 1 ml dye solution, which was diluted with calcein-AM and ethidium homodimer-1 strains, and incubated for 45 min at 37°C in CO_2 incubator. The stained samples were then used for CLSM. The live cells were distinctly found under CLSM via green fluorescence owing to staining of cytoplasm of live cells with calcein-AM, which attributes intracellular esterase activity.

Ethical statement: Authors declare that the study received appropriate approval from an ethics committee.

References

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