Chitosan-Based Photozymes Switching on Bone-Like Apatite Deposition
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Introduction
Photozymes are copolymers consisting of both hydrophobic chromophoric and hydrophilic comonomers [1]. Photozymes are known to perform: (i) light-harvesting by the antenna effect in combination with (ii) solar energy transduction into chemical energy, accompanied with singlet oxygen production; these two processes are the first two stages of photosynthesis. In this work, chitosan-xanthene photozymes were investigated and their ability towards a photo-activated deposition of hydroxyapatite was studied for applications in the field of bone tissue engineering. The incorporation of photozymes into scaffolds as blend components or coating layers are promising tools for carrying out efficient electron transfer process, responsible for the osteointegration ability of the devices.

Materials and Methods
The following photozymes were supplied from University of Sofia: chitosan-fluorescein (CHFL; 0.5 mol.% FL) and chitosan-rose bengal (CHRB; 0.1 mol.% RB). The photosensitizing activity of photozymes was measured by standard tests using probe molecules [2]. In vitro biocompatibility of photozymes was studied using MG63 osteoblasts. The photo-bioactivity of photozyme cast films was evaluated by tests in simulated body fluid (SBF) under proper irradiation and in the dark (control). Photozymes were incorporated inside porous scaffolds for bone repair obtained by wet spinning or freeze-drying. Alternatively, scaffolds were coated with alternately anionic polyelectrolytes and cationic photozymes by layer-by-layer (LbL) method. Physicochemical characterisation (SEM-EDS, FTIR, UV-vis, contact angle analysis) of photozymes and scaffolds was performed.

Results
The work was focused on the synthesis and characterisation of CHFL and CHRB conjugate polymers, as biocompatible amphiphilic watersoluble photosensitizers, able to stimulate hydroxyapatite (HA) deposition upon visible light irradiation. Continuous exposure of CHRB and CHFL cast films to visible light for 7 days was found to activate the deposition of calcium phosphate crystals from a conventional simulated body fluid (SBF 1.0X) (Fig. 1).

EDS and FTIR-ATR analyses confirmed the apatite nature of the deposited calcium phosphate crystals. Interestingly, the combination of CHRB and CHFL with not-bioactive materials by LbL coating or blending strategies made the resulting scaffolds photo-bioactive. The mineralization ability of MG63 osteoblast-like cells was significantly improved on CHFL and CHRB films preincubated in SBF under visible light exposure (alkaline phosphatase activity (ALP) test for 1, 3, 7 and 14 days).

Discussion and Conclusions
Photo-excitation of the chromophoric groups induced variations in the local charges of the photozymes inducing HA precipitation. The use of photoactive biocompatible conjugate polymer, such as CHFL and CHRB may lead to new therapeutic options in the field of bone/dental repair, exploiting the photo-excitation mechanism as a tool for biomineralisation.

References

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