Thermally triggered release of a pro-osteogenic peptide from a functionalized collagen-based scaffold using thermosensitive liposomes

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ABSTRACT SUMMARY
A novel thermoresponsive pro-osteogenic peptide-elu ting collagen- hydroxyapatite (CHA) scaffold has been developed based on the attachment of thermosensitive liposomes to the surface of the CHA scaffold. In these constructs, release kinetics of bioactive peptide can be altered by external heat pulses, allowing on demand triggered release of the pro-osteogenic molecule thus enhancing the regenerative capacity of the scaffold.

INTRODUCTION
Research in bone regeneration is increasingly turning to the developing field of tissue engineering (TE). TE typically aims to regenerate damaged tissue by using cells from the patient combined with highly porous scaffolds which act as templates for the growth of new tissue. While significant advances have been made developing scaffolds which mimic the native extracellular matrix, the effective and safe utilization of molecules which enhance bone healing, essential for the regeneration of large fractures, still remains a challenge. Bone healing is a very complex process which involves the sequential utilization of different growth factors secreted by the organism. Ideally, a scaffold for bone TE should replicate this cascade of external chemical stimuli for achieving an enhanced regeneration. One way for achieving this would be the development of a responsive scaffold which delivers pro-osteogenic factors in response to external stimuli which could be applied as required on demand. In the present work we describe, for the first time, a thermoresponsive drug eluting collagen-based scaffold. Drug-loaded biocompatible thermoresponsive liposomes were attached to the chemically modified surface of highly porous CHA scaffolds, which are well accepted osteoinductive materials designed specifically for bone repair in our laboratory¹. The therapeutic molecule of interest in this study is a pentapeptide of the Parathyroid Hormone related Protein (PTHrP 107-111), which is well known for its pro-osteogenic and anti-osteoclastic properties². The external heat pulse-responsive release kinetics variations shown by these materials demonstrates their potential for further development of multifactor eluting systems.

EXPERIMENTAL METHODS
Liposomes fabrication: Maleimide modified thermosensitive liposomes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-n-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000). Maleimide modified thermosensitive liposomes composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1-monostearoyl phosphatidylcholine (MSPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-n-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) and maleimide with molar ratio 86:10:2:2 were prepared. Fluorescently labeled liposomes were also prepared by co-dissolving 0.6% mol of L-α-phosphatidylethanolamine-n-(lissamine rhodamine B sulfonyl) (ammonium salt). Liposomes were loaded by lipid film hydration in the presence of PTHrP 107-111.

Scaffold fabrication: CHA scaffolds were initially fabricated using a well established freeze-drying procedure developed in our lab¹, and cut into 6.5mm diameter pieces. In order to functionalize the surface of the scaffolds with sulphydryl (SH) groups, they were soaked for 45 min in a solution of 500μL of dimethyl sulfoxide with 0, 40, 160 or 800μg of N-Succinimidyl S-Acetylthioacetate (SATA) and then gently washed with phosphate buffer solution (PBS). Scaffolds were then deacetylated by immersing them during 2 hours in 500μL of a solution at pH 7.4 of 25 mM ethylenediaminetetraacetic acid (EDTA) and 17.4mg of hydroxylamine and soaked for 2 hours at 4ºC in suspensions containing the maleimide functionalized liposomes, allowing the formation of a thioester bond between the scaffolds and the liposomes, as shown in Figure 1. Eventually, the materials were crosslinked with 1-Ethyl-3-(3-Dimethylaminopropyl)-Carbodiimide (EDAC) following a well established protocol³.

Material characterization and PTHrP 107-111 delivery test: The final degree of functionalization with SH groups was quantified by Ellman’s reaction⁴.

Figure 1. Scheme of the chemical interaction between the liposomes and the CHA scaffolds.
Confocal microscopy was used for assessing the distribution of fluorescently labeled liposomes and the retention of the liposomes onto the scaffolds by taking daily images from a scaffold immersed in PBS during 4 days. Release studies were carried out by soaking the liposomes-attached scaffolds in PBS at 37°C. Some of the materials were introduced in waterbaths at 42°C for 20 min after 3 or 8 days. Release media were removed and replaced by fresh PBS at different time points. Samples were ultracentrifuged at 45,000 rpm during 10 min and then the concentration of PTHrP 107-111 was determined by High Performance Liquid Chromatography (HPLC).

Assessment of the bioactivity of the released PTHrP 107-111: Pre-osteoblastic MC3T3 cells were grown on liposomes-attached-scaffolds and were kept at 37°C for the whole study or underwent 42°C pulse for 20 min after 3 days. ALP activity was measured and the expression of OPN and OCN genes were analyzed by PCR, as indicators of osteogenic activity at different timepoints. In addition, RANKL gene expression was studied as an indicator of antiosteoclastic action.

RESULTS AND DISCUSSION

An extensive characterization has been carried out in this study to demonstrate the feasibility of attaching modified liposomes to collagen-based scaffolds by a covalent bond. The amount of SH groups on the surface of the scaffold can be tuned by changing the concentration of SATA, the functionalizing agent, during the fabrication process. Confocal images from fluorescently labeled liposomes-scaffold constructs demonstrate that the liposomes are homogeneously distributed through the scaffolds and that they are attached to its surface after 4 days in PBS.

The results from the release studies show the thermoresponsive behavior of the system described in the present work. Figure 2 shows the release profile of PTHrP 107-111 from liposomes-attached scaffolds under different conditions. While all the materials show a similar initial trend (burst followed by a more controlled release), it can be observed that the release kinetics can be effectively tuned after 20 minutes pulse at 42°C, given either at day 3 or 8.

Figure 2. Peptide release from scaffolds at 37°C and pulsed for 20 minutes at 42°C at 3 and 8 days.

Figure 3. ALP activity (A) and OCN, OPN and RANKL genes expression (B) of MC3T3 cells grown on liposomes-scaffolds in ... and therapeutics release systems from different responsive liposomes attached to the surface of the scaffolds.

CONCLUSION

A thermoresponsive release system was developed by attaching peptide loaded thermosensitive liposomes to the chemically modified surface of collagen-hydroxyapatite scaffolds. These materials showed, for the first time, a heat responsive release of a therapeutic thus enhancing the regenerative capacity of the scaffold.

REFERENCES


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