Liposome-Hydroxyapatite Core-Shell Structure as Drug Carriers

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Statement of Purpose: Hydroxyapatite (HA) is widely used as a bone substitute material due to its biocompatibility and osteoconductivity. Coatings, particles, porous granules, beads of HA have been investigated as carriers of various drugs, such as antibiotics, proteins, etc.[1] However, there are many disadvantages for these HA drug delivery systems, such as their low encapsulation efficiency and high drug release rate. Liposomes have been extensively investigated for improved delivery of various active agents. Liposome-cored calcium phosphate nanoparticles have been prepared by the liposome templated self-assembly of calcium phosphate[2] and was believed to have the potential for drug delivery.[3] Here we propose the liposome-HA core-shell structure prepared by constant composition method as drug carriers for treatment of bone diseases.

Methods: Indomethacin (IMC) was chosen as the model drug. Drug-loaded liposomes were prepared from the lipid film of dimyristoyl phosphocholine (DMPC), dimyristoyl phosphatidic acid (DMPA) and cholesterol (1/1/2 in molar), followed by sonication, dialysis, and finally filtering twice through a 0.45μm membrane. The constant composition experiment was performed following the work of Nancollas.[4] A stable supersaturated solution with respect to HA was prepared by mixing $CaCl_2$ and KH_2PO_4 at the molar ratio of 1.67, the pH was adjusted to 7.4, and temperature was 25℃. IMC-loaded liposomes were added to induce surface precipitation of HA. The drop in pH of the reaction solution triggered the simultaneous addition of titrants from autoburettes (ABU Triburrete, Radiometer Copenhagen). The titrants: (1) $[CaCl_2] = 1.17\times10^{-2}$M, (2) $[KH_2PO_4] = 7.02\times10^{-3}$M + KOH = 1.3×10^{-2}M. The titrants and the supersatured solution all contained 0.1M KCl. IMC-loaded liposomes and the liposome-HA composite were analyzed by dynamic light scattering (DLS) (Malvern Autosizer Lo-C) and by zeta-potential measurement (Zetamater, Malvern Instruments Ltd.). The liposome-HA particles were separated by centrifugation at 10,000*g* and then dispersed in PBS. The formation of HA is confirmed by fourier-transform infrared (FTIR) spectroscopy (PerkinElmer 2000 Explorer) and transmission electron microscopy (JEOL 2000FX) was used to characterize the morphology of the core-shell structure. *In-vitro* drug release experiments of IMC-loaded liposomes, IMC-absorbed HA and liposome-HA were carried out separately in 10 ml of acetic acid buffer solution (pH 4.0) or PBS (pH 7.4) at 70 rpm and 37℃. The amount of IMC released at certain time was determined by UV-Vis spectroscopy (Jasco V-530) at 320nm.

Results and Discussion: DMPA in the liposome walls provides a negatively charged phosphatidic acid headgroup, which aids in the localization of ions around the liposome. In the case of negatively charged liposomes, the Ca^{2+} is electrostatically attracted by the exterior charge. The formation of an electric double layer effectively increases the local concentration of the calcium and phosphate above the saturation point and causes crystallization to preferentially occur near the surface of the liposomes.[3] The growth of HA in solution with the constant composition method created a surface layer on the liposomes resulting in the formation of a core-shell structure. DLS measured an average liposome size of 163.8nm (poly index 0.229), which increased to 585.4nm (poly index 0.394) after HA precipitation. Liposomes held a zeta-potential of -56.5±2.6mv and decreased to -4.9 ±1.9mv for the liposome-HA core-shell structure after 6 hours of reaction. The negative charge of liposomes is reduced by a layer of HA around the liposomes. TEM images show a core-shell structure of the liposome-HA with particle sizes ranging from 200-400nm. The bands at 961, 1045 and 1092 cm^{-1} (stretching vibration of PO_4^{3-}) and 570, 602 cm^{-1} (deformation vibration of PO_4^{3-}) derived from HA are apparent in FTIR spectrum. The bands at 3570 and 633 cm^{-1} derived from the stretching and liberational modes of OH^- are very week, indicating the HA coating in liposome-HA was poorly crystalline.

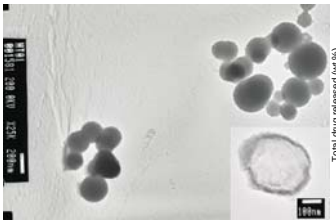

Fig1 TEM image of liposome-HA;

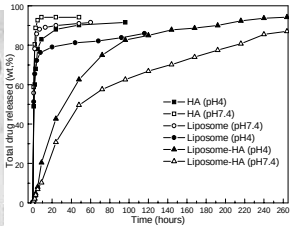

Fig2 Drug release profile.

The drug encapsulation efficiency of liposomes is 16.8%, and the drug leakage during the constant composition precipitation of HA is 7.5%. 92.6% of IMC is released from HA in 5 hours at pH7.4. Meanwhile drug release from the IMC-loaded liposome is much slower, and there are 81.2% and 90.2% IMC released at day3 in pH4.0 and pH7.4, respectively. A sustained drug release is achieved for the liposome-HA. 93.7% and 85.5% of IMC was released at day10 for pH4.0 and pH7.4, respectively. HA is more easily dissolved at acidic condition, and drug release from liposome-HA can be controlled by dissolution of HA.

Conclusions: Liposome-HA core-shell structure was prepared by constant composition method using liposome as template. The HA in liposome-HA was poorly crystalline, and slightly negative charged. A sustained drug release from the liposome-HA can be achieved. This core-shell structure of liposome-HA has the potential to be used as drug carriers for the treatment of diseased bones. Further experiments regarding the effect of HA dissolution on drug release are under investigation in our laboratory. The evaluation of liposome-HA as drug carriers by loading antibiotics and growth factors along with the release profiles is underway.

References:

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