Functionalized Nanoparticle Release and Distribution in PEG Hydrogel Delivery Systems*

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ABSTRACT SUMMARY

Nanoparticles have emerged as a promising therapeutic tool due to their unique physicochemical properties and tunable biological interactions. Delivery of therapeutic nanoparticles can be enhanced locally through standard drug-delivery platforms, such as poly(ethylene glycol) (PEG) hydrogels. In this work, functionalized nanoparticles were encapsulated in three-dimensional PEG hydrogels with varying mesh size. Nanoparticle size, surface chemistry, and hydrogel mesh size all influenced the release of particles from the hydrogel matrix, as demonstrated by measurements of particles in solution. Size influenced nanoparticle diffusion as expected, with larger particles diffusing more slowly. However, negatively charged nanoparticles diffused out of the gel at slower rates than neutrally charged nanoparticles of the same size. To further investigate the distribution and transport of gold nanoparticles (AuNPs) and quantum dots (QDs) within the PEG hydrogel matrix, a novel transmission scanning electron microscopy (SEM) technique has been developed. This method provides improved nano-scale resolution of particles embedded within relatively thick hydrogel sections.

INTRODUCTION

Many nanoscale vectors including viruses, liposomes and nanoparticles, are capable of effectively transporting therapeutics to cells. Nanoparticles are a particularly useful delivery tool, as the properties of their core and shell structures can be independently tailored. This allows for versatility in both diagnostics and therapeutics, where particles can be fabricated for controlled loading and release of drugs or genes, as well as enhanced imaging capabilities or targeted delivery.

Both AuNPs and QDs have been pursued for use as therapeutic agents through biological surface modifications. AuNPs have many advantages for biological therapies. They are bioinert, non-toxic, have uniform chemical composition, and can be easily surface-modified. AuNPs have demonstrated potential in many therapeutic applications, notably in cancer therapies.[1,2] QDs have also proven useful in biological systems, as they are intrinsically fluorescent and therefore easy to track. Although their surface chemistry can be modified, their core is toxic, rendering them less desirable for biological applications.

Most therapeutic nanoparticles have been tested for use in systemic delivery. However, some nanoparticle-based medical procedures under development, such as ablation or targeted recognition of cancerous tumors,[3,4] could benefit from a localized particle delivery. We hypothesize that many hydrogel platforms already developed for drug delivery could be translated for use in nanoparticle delivery. PEG has been well studied as a polymeric matrix from which drugs can be released.[5] PEG is bioinert, hydrophilic, and its mesh size can be tuned to yield distinctly different diffusion rates.

To better understand transport of therapeutic nanoparticles within 3D hydrogel matrices, it would be advantageous to visualize the particles during development of new particle-matrix systems. Traditional methods to visualize nanoparticles within cells or a 3D matrix rely primarily on fluorescence which cannot be resolved on a single-particle level. Alternatively, it is possible to section thin samples and augment the features with contrast agents. Here, we investigate a recent technique developed to image nano-scale features in transmission on a standard SEM.[6] This method has practical advantages for sample preparation, and allows for imaging with low energy.

EXPERIMENTAL METHODS

AuNPs with diameters of 10 nm (RM 8011, NIST) or 30 nm (RM 8012, NIST) were modified with methoxy-PEG-thiol (MW 1000) and concentrated. PEGylation was verified through dynamic light scattering (DLS) and SEM. QDs with a cadmium selenide core, zinc sulfide shell and either a PEG outer layer, or a PEG layer capped with carboxyl or amine groups were purchased, and their diameter was verified by DLS to be about 14 nm.

Macromer solutions of 5 wt %, 10 wt %, or 20 wt % PEG dimethacrylate (MW 4600), 0.1 wt % photoinitiator, and water were photopolymerized in molds (365 nm, 4 mW/cm^2). Measurement of wet and dry hydrogel weight were used to calculate the swelling ratio, and subsequently determine the average mesh size for each hydrogel composition.

To encapsulate nanoparticles, AuNP or QD suspensions were substituted for some of the water in the macromer solutions described above. Following photopolymerization, the hydrogel discs were placed in water. AuNP concentrations were determined by absorbance measurements at 522 nm (for 10 nm AuNP), and 529 nm (for 30 nm AuNP), and compared to a standard curve. QD concentrations were similarly determined by fluorescence intensity measurements. Additional measurements were made by atomic absorption spectroscopy (AuNPs) and epi-fluorescence microscopy of the bulk hydrogel (QDs).

To verify uniform particle encapsulation within the hydrogel, and changes in particle concentration over time, 500 nm thick hydrogel sections were prepared by cryo-substitution. Transmission SEM, where the electron beam penetrates the sample, was performed on hydrogel

sections containing encapsulated nanoparticles. Stereo-images were taken to further evaluate the 3D location of particles within the hydrogel matrix.

RESULTS AND DISCUSSION

Hydrogel swelling studies indicated final average mesh sizes of approximately 10 nm (20 wt % PEG), 25 nm (10 wt % PEG), and 140 nm (5 wt % PEG). These mesh sizes constitute a range relevant to diffusion of 10 nm and 30 nm AuNPs, and 14 nm QDs.

AuNPs were PEGylated to eliminate particle agglomeration or interactions with the hydrogel network. PEGylation resulted in a uniform layer of about 5 nm on each particle's surface. Once encapsulated in the hydrogel matrix, AuNP diffusion showed a clear dependence on particle diameter and hydrogel mesh size. After only one hour, the largest mesh gels (5 wt % PEG) allowed release of nearly 35 % of the encapsulated 10 nm AuNPs, whereas the tightest mesh gels (20 wt % PEG) allowed less than 25 % release. After two weeks, over 60 % of the 10 nm AuNPs had been released from the 5 wt % gels, but only 30 % had been released from the 20 wt % gels. When 30 nm AuNPs were encapsulated in the same matrices, release from each hydrogel composition was generally halved for the same time period (Fig. 1).

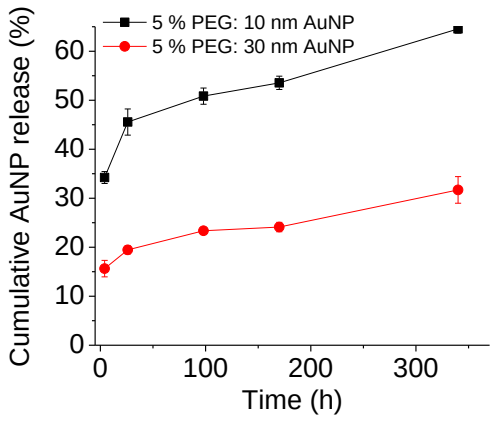

Figure 1. The diameter of PEGylated AuNPs affected diffusion out of the hydrogel, where 10 nm AuNPs (black) diffused out more rapidly than 30 nm AuNPs (red). Error is reported as standard deviation.

Interestingly, carboxylated QDs encapsulated within the same PEG hydrogel system showed very little diffusion after one week in culture, when compared to PEGylated QDs of the same core/shell composition and dimensions (Fig. 2). Fluorescence intensity measurements of the bulk hydrogel decreased over time, suggesting decreases in QD concentration. However, the decrease in fluorescence was greater amongst hydrogels containing QDs with the PEG surface layer. It is hypothesized that entrapment of the nanoparticles is due to either clustering of the particles in the polymer precursor solution, or tethering of the negatively-charged surface groups into the polymerizing hydrogel network.

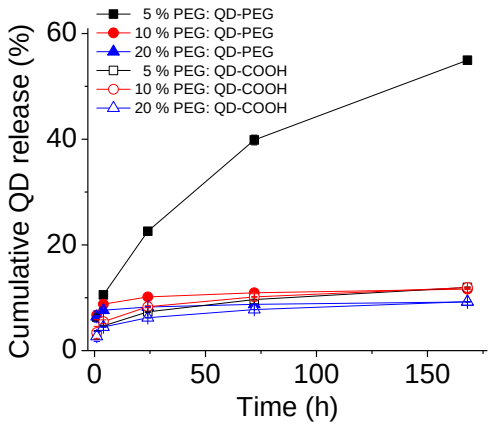

Figure 2. Cumulative release of 14 nm QDs was dependent on whether the particle surface was neutral (filled) or negatively-charged (open). Error is reported as standard deviation.

SEM of hydrogel cross-sections was performed to further investigate particle distribution. Initial encapsulation of dispersed nanoparticles was observed at a low resolution, but a high-resolution method holds promise in resolving individual particle locations. This would allow observation of particle movement out of the hydrogel matrix over time, as well as particle-particle interactions, or particle-matrix interactions.

CONCLUSION

Nanoparticles of varied diameter, core composition, and surface chemistry were successfully encapsulated within 3D PEG hydrogel matrices. Release of nanoparticles from the hydrogels was dependent on size, as well as surface chemistry. The influence of surface chemistry on release was not expected of this system, and will be further investigated with SEM in transmission to determine likely mechanisms behind entrapment. These studies will lay the foundation for future development of localized delivery of functionalized nanoparticles.

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