Sucrose acetate isobutyrate-based nanogels as liquid fiducial tissue markers with potential use in image guided radiotherapy

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Within the field of radiotherapy, modern radiation oncology relies on high precision imaging techniques like Computed Tomography (CT), Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) in order to deliver high radiation doses to precisely defined targets [1-3]. Tumors undergoing fractionated radiotherapy rarely display a fixed position during irradiation or within treatment, due to changes in organ filling, breathing motion and tumor size. This usually entails that the planning target volume has to be increased to ensure radiation dose coverage during treatment [4].

Background

Image-guided Radiotherapy (IGRT) is a recently developed technique that has contributed to decreasing in planning target volume, as 2D- and 3D images are frequently recorded before and during treatment [1,5]. This protocol has reduced treatment toxicity and improved relapse-free survival [6]. However, a remaining challenge within IGRT is fixation and correlation of the anatomical positions of several tumors to internal fixation points such as the skeleton. Radiopaque fiducial injectable markers are therefore of high interest.

Stable and uniform PEG- and PNIPAM-coated AuNPs were successfully synthesized, conferring high biological utility [8]. Figure 2 shows the dithiolethiol-functionalized AuNPs were dispersed due to poor imaging of the nanoparticle. In-vitro stability test of the PEG-AuNP-SAIB gel (Figure 3) shows that 98% release of PEGylated AuNPs. This burst release was completely avoided (<1%) with the PNPAM-AuNP-SAIB gel, even with concentrations up to 100mg·mL⁻¹.

Results

Experimental methods

The AuNPs were synthesized by a three step seedling protocol using cholic acid and trisodium citrate (Scheme 1). Three different coating options were tested: 1) Thiol-terminated PEG₃₅₀₀ polymers, 2) thiol-terminated PNIPAM₃₅₀₀ polymers and 3) a dithiol functionalized SAIB derivative that was synthesized in 4 steps from sucrose.

Scheme 1. Synthesis of PEG-, PNIPAM- and SAIB-coated AuNPs

The AuNP-SAIB gels were made by dispersing the coated AuNPs in ETOH followed by mixing with SAIB and PLA. In vitro stability studies of AuNP-SAIB gels were conducted in PBS-buffer at 37°C. In vivo contrast and stability of the gels were monitored by micro-CT after injection of 200μL of SAIB/ETOH/PLA (7/50:25) + 30mg·mL⁻¹ PNPAM-AuNPs or 10mg·mL⁻¹ PEG-AuNPs at the upper left flank of immunocompetent NMRI-mice. The X-ray contrast level, gel volume and gel homogeneity were evaluated over time by active contour model.

In vivo contrast and stability of the PEG- and PNPAM-AuNP-SAIB gels were likewise analyzed. The PEG-AuNP-SAIB gel suffered from PEG-AuNP migration towards the gel-borders over time [8]. Figure 4 and 5 show micro-CT scans of mice after injection of the PNPAM-AuNP-SAIB gel. The PNPAM-AuNP-SAIB gel provided high stability in vivo, as well as a higher X-ray contrast level than the PEGylated AuNP-SAIB gel (as expected due to higher AuNP loading being possible with the PNPAM coating).

The contrast level and homogeneity of the gels were analyzed manually in each CT scanning slice. First, a bounding box was drawn around each gel, and the gels were segmented by active contour model [9]. The median values of the two gel types were compared using a Wilcoxon rank-sum test [10]. The homogeneity of the gels was then analyzed by first applying a Box-Cox transformation [11] to the two datasets for variance stabilization, followed by a Wilcoxon rank-sum test for comparison of variance.

The PNPAM-AuNP-SAIB gel had a significantly higher median (P-value: 0.0006) as expected due to higher loading of AuNPs. Despite a much better contrast, surprisingly no significant difference was found in the variance of the gels (P-value: 0.0734), thereby indicating the same extent of inhomogeneity in the PNPAM-AuNP-SAIB gel and the PEG-AuNP-SAIB gel. This is due to the much lesser resolution in clinical imaging systems, the slight inhomogeneity of the PNPAM-AuNP-SAIB gel will not be visible and will therefore not be an issue in clinical applications.

Handling of PNPAM-coated AuNPs was furthermore superior to the PEGylated AuNPs, as they can be lyophilized and stored as a stable powder, that is easily dispersible in ETOH. Based on the presented in vitro and in vivo results, the PNPAM-AuNP-SAIB gel was evaluated to be suitable as tissue marker for IGRT.

References

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