Rationally Designed Discoidal PLGA/PEG Nanoconstructs for Cancer Theranostics

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ABSTRACT SUMMARY

Non-spherical nanoconstructs have been shown to accumulate within the tumor vasculature more efficiently than spherical ones. Here, discoidal nanoconstructs with a diameter of 1,000 nm and a height of 500 nm were precisely fabricated via a modified hydrogel template strategy. The discoidal nanoconstructs were composed of poly(lactic acid-co-glycolic acid) (PLGA), polyethylene glycol (PEG) dimethacrylate and loaded with ultra-small super-paramagnetic iron oxide nanoparticles (USPIOs) and doxorubicin. These theranostic nanoconstructs were characterized for their geometrical and physicochemical properties using different microscopy techniques and magnetic assays. The controlled release of doxorubicin was evaluated under different pH conditions. The in vivo performance was analyzed in tumor-bearing mice.

INTRODUCTION

In the rational design of nanoconstructs, the importance of size, shape, and surface charge (3S parameters) has been highlighted by several authors, including.[1-3] While bottom-up approaches based on self-assembly are very useful for making spherical nanoparticles, they cannot precisely control the 3S parameters over multiple scales. A few groups have only recently developed novel, top-down fabrication methods for the synthesis of non-spherical nanoconstructs, showing excellent NP geometry control at the nano- and sub-micron scales.[2, 4-6] Thin discoidal particles have revealed excellent vascular behavior in terms of lateral-drifting and adhesion, demonstrating enhanced accumulation within the tumor vasculature and reduced sequestration by the RES organ. [1] Here, we modified the hydrogel-template strategy [6] to synthesize discoidal PLGA/PEG theranostic nanoconstructs with improved yielding and quality.

EXPERIMENTAL METHODS

The wells with a diameter of 1,000 nm and a depth of 500 nm) on a single silicon (Si) master template were engraved by using e-beam lithography. 5 mL of polydimethylsiloxane (PDMS) layer was deposited over the Si wafer to produce a second PDMS template presenting cylindrical pillars. After the deposition, PDMS solution with the Si template was stored at room temperature for 30 min and completely polymerized at 60 °C for 3-4 h. Next, over the PDMS template, 10 mL of polyvinyl alcohol (PVA) solution was deposited and fast dried at 60 °C for 2-3 h and further dried at room temperature for overnight. The fabrication procedure of the PVA templates was repeatedly performed to produce numerous PVA templates. These templates were utilized to yield a number of discoidal nanoconstructs at a time. The mixture for yielding the discoidal nanoconstructs was prepared by the co-solvent of dichloromethane and dimethylformamide before putting over the PVA templates. The mixture contained PLGA, PEG dimethacrylate, USPIO, and Dox. The deposition of the mixture was executed on the PVA templates and dried under the UV-exposure to induce cross-linking of PEG dimethacrylate. The harvest was achieved by dissolving the PVA templates in distilled water. The discoidal nanoconstructs were characterized by various microscopy techniques, including scanning electron microscopy (SEM), atomic force microscopy (AFM) and fluorescent optical microscopy (FOM) considering the 3S parameters of them.

RESULTS AND DISCUSSION

Discoidal nanoconstructs fabricated by the sacrificial PVA templates presented homogenous morphology corresponding to the size and shape in the Si master template. The morphology of the Si master template was confirmed in SEM images displaying numerous wells with the 1,000 nm of diameter and 500 nm of height. The size and shape were identical. (Figure. 1A) AFM images of the PDMS template indicated the pillars presenting the opposite shapes to the wells in the Si master template. (Figure. 1B) One of the dried PVA templates was observed in optical microscopy and AFM, which showed the uniform wells with the 1,000 nm diameter and 500 nm in height. (Figure. 1C) The PVA templates were collected and the mixture was deposited for the fabrication of discoidal nanoconstructs. Completely dried PVA templates were dissolved in water and the pellet of the solution was collected by centrifugation. Small fragments from the PVA templates were removed by filtering. The nanoconstructs were observed by AFM and SEM. They maintained uniform 1,000×500 nm size and discoidal shape. (Figure. 1D)
The USPIO and Doxorubicin-loaded discoidal nanoconstructs were further tested with HeLa and J774 cells. We cultured the cells at 37°C in MEM Alpha, GlutaMAX (Life Technologies, Carlsbad, CA, USA) with 10% fetal bovine serum and 1% penicillin-streptomycin.

The discoidal nanoconstructs were prepared in the water phantom performed with a 3T MRI clinical scanner. (Figure. 3) Interestingly, the $r_2$ relaxivity in MRI of the discoidal nanoconstructs showed 4-5 times higher than commercially available USPIO/SPIO contrast agents, similarly to what observed previously by the authors for a different nanoplatform. [7]

CONCLUSION

We demonstrated the synthesis of discoidal polymeric nanoconstructs with a precise geometry at the submicron scale. The nanoconstructs were loaded with USPIOs for MR imaging and doxorubicin for cancer treatment. The rationally selected geometry of the nanoconstructs and the multifunctional payload enhance the percentage of injected dose reaching the diseased vasculature; imaging contrast for the early detection of small tumor masses; and therapeutic efficacy.

REFERENCES


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