Development of a new magnetically responsive device for localized cancer theranostics

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Cancer is one of the main causes of death worldwide and its occurrence has been increasing over the years. Due to the therapeutic inefficiency and severe side effects of conventional cancer treatments, the development of new cancer treatment options has been one of the most studied research areas. The development of new multifunctional systems that deliver therapeutic agents locally to the tumor site allows a more personalized and effective treatment. The present work focuses on the development a dual-stimuli responsive device composed of Fe_3O_4 magnetic nanoparticles (mNPs) and poly(N-isopropyl acrylamide) (PNIPAAm) microgels (MG) embedded in electrospun polymeric fibers capable of providing a simultaneous and local combinatory cancer treatment: chemotherapy and magnetic hyperthermia. mNPs with an average diameter of 8 nm were synthesized by chemical co-precipitation technique and stabilized with dimercaptosuccinic acid (DMSA) and oleic acid (OA) [1]. Thermoresponsive PNIPAAm MG were obtained by surfactant-free emulsion polymerization [2]. At room temperature, MGs are in a swollen state with a hydrodynamic diameter of around 1 μm. Above 32 ºC, their hydrodynamic diameter decreases, confirming their negative temperature response and Lower Critical Solution Temperature [3,4]. Poly(vinyl alcohol) (PVA) was used as fiber template and 10 wt.% of PNIPAAm MG and 10 wt.% of mNPs were incorporated in the fibers through colloidal electrospinning technique to produce dual-stimuli nanofibrous membranes with a bead-on-a-string morphology. The presence of MG and mNPs in the electrospun fibers decreases the membrane swelling ratio and increases its stiffness, raising its Young's modulus when compared to the plain PVA membrane. DMSA-mNPs appear to have a slight impact in the rise of rigidity of the membrane when compared to the OA-mNPs. Magnetic hyperthermia assays show that a higher concentration of mNPs leads to a higher heating ability, as expected. The composite membrane with 10 wt.% DMSA-mNPs shows the highest temperature variation, 5.1 ºC. Cytotoxicity assays were performed demonstrating that PVA membranes incorporated with PNIPAAm microgels and mNPs do not present any type of cytotoxicity and therefore could be used in biomedical applications. To assess adhesion, immunostaining analysis of focal adhesion (FA) protein vinculin and filamentous actin in different tumor cells lines (melanoma cells, WM983b, osteosarcoma, SaOs) was performed and compared with non-malignant cells (adult dermal fibroblasts). To assess proliferation, resazurin and 3D laser scanning confocal microscopy was used. Cell death was quantified by combining resazurin measurements and imaging of fluorescently probes, namely live/dead dyes (calcein-AM and Ethidium homodimer-1) and immunostaining with apoptosis markers (cleaved caspase-3). PVA/MG/NP displayed similar rate of cellular adhesion and the FA staining did not change. FA were more elongated in cell seeded onto PVA fibers when compared to collagen type I coated glass. These demonstrates that neither NP nor MG altered the affinity of cells to the PVA fibers. Proliferation was analysed within a period of 14 days, being the most dramatic changes observed amongst controls. This data suggests that the incorporation of MG and/or NP do not alter cellular responses in comparison with PVA alone. Currently, we are investigating the full potential of PVA/MG/NP to deliver chemotherapeutic agents in 3D cultures of tumor cells. Preliminary data shows that doxorubicin loaded NP/MG promote cell death. In the early future, we will incorporate doxorubicin loaded NP/MG into PVA fibers and analyse their ability to promote cell death in response to HFAFM.

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