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Implantable chemothermal brachytherapy seeds: A synergistic approach to brachytherapy using polymeric dual drug delivery and hyperthermia for malignant solid tumor ablation

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ABSTRACT

Chemothermal brachytherapy seeds have been developed using a combination of polymeric dual drug chemotherapy and alternating magnetic field induced hyperthermia. The synergistic effect of chemotherapy and hyperthermia brachytherapy has been investigated in a way that has never been performed before, with an in-depth analysis of the cancer cell inhibition property of the new system. A comprehensive *in vivo* study on athymic mice model with SCC7 tumor has been conducted to determine optimal arrays and specifications of the chemothermal seeds. Dual drug chemotherapy has been achieved via surface deposition of polydopamine that carries bortezomib, and also via loading an acidic pH soluble hydrogel that contains 5-Fluorouracil inside the chemothermal seed; this increases the drug loading capacity of the chemothermal seed, and creates dual drug synergism. An external alternating magnetic field has been utilized to induce hyperthermia conditions, using the inherent ferromagnetic property of the nitinol alloy used as the seed casing. The materials used in this study were fully characterized using FESEM, H^1 NMR, FT-IR, and XPS to validate their properties. This new approach to experimental cancer treatment is a pilot study that exhibits the potential of thermal brachytherapy and chemotherapy as a combined treatment modality.

1. Introduction

Several types of cancer (CA) that exhibit as solid tumor malignancies have always been a challenge to treat, especially due to their rapid and aggressive growth rate. Examples of these are mammary, prostate, and hepatic tumors. These types of solid malignancies are treated with aggressive Radiation Therapy (RT) and an adjuvant of Chemotherapy (CT). In the United States alone, breast and prostate CA constitute (27 and 29)% of all solid malignancy cases, respectively [1]. In the clinical setting, multimodal therapies are required to enhance the prognosis of the patient. Radio- and Chemo-therapies are staple treatments, and surgery is also known to be an effective form of intervention. A multimodal non-surgical approach has been the mode of treatment for patients with advanced stages of CA, to reduce the size of the tumor, and halt the spread of malignant cells. RT and CT are also known to be used as adjuvant therapies to enhance the effect of one another [2–4].

Another modality that has been known to effectively treat solid malignancies is brachytherapy (BT), where radioactive seeds are implanted in and around the tumor, and give off high and low doses of radiation over time [5–8]. This is known to improve the prognosis of prostate and breast CA patients, and other forms of solid malignancies [9–11]. We borrow the idea of implanting seeds, but use nitinol and its inherent ferromagnetic property to induce heat around and inside the tumor site, while also releasing chemotherapeutics over time to treat cancer. The combined effects of hyperthermia therapy (HT) via alternating magnetic field induced heating and dual drug delivery via pH responsive polymers were studied in this work to assess the effectiveness of these innovative chemothermal seeds (CTS) as a new form of CA treatment. However, one setback of HT is the inappropriate dosage of thermal energy transfer into the tumor tissue. Overheating and under heating is still a major issue of HT; in order to maximize this treatment, we needed an exact analysis of the thermal capability of the CTS. The exact amount and array of CTS can be determined via analysis of the isotherm using *in vivo* tumor model. In the treatment modality of HT, where the surrounding site of the tumor and the tumor itself are heated

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to (43–45) °C to create a cytotoxic environment [12–14], HT is known to enhance the effect of RT and CT by delaying or impeding DNA recombination of damaged cells, leading to higher cell death [15–18]. There are multiple approaches to creating hyperthermia conditions in the body; local and systemic HT are utilized in clinical settings, and these are conducted using microwave implants and heat baths [19,20]. In contemporary research, magnetic nanoparticles are introduced and utilized for HT, examples of which are Iron II magnetite and III maghemite nanoparticles functionalized with ligands and chemotherapeutic drugs for the active targeting and treatment of cancer, and promising results have been reported by such studies [21–28]. However, recent studies have drawn attention to the side effects of magnetic nanoparticles (SPIONS), like liver and kidney toxicities, fluctuations in anti-oxidant and tissue nitrite levels, and phenotypic gene alteration, leading us to rethink our strategy of creating hyperthermia conditions in the body [29–31].

In this research, we utilized polymeric vehicles for drug delivery, especially stimuli responsive polymers. We exploited the catechol-boronic link that is pH cleavable to deliver the drug payload to malignant tissue. We covered the surface of the nitinol tubes with electropolymerized dopamine to act as a pH sensitive drug delivery platform, and conjugated it with a boron-containing drug bortezomib (BTZ) via catechol-boronic bonding. In order to enhance the effect of the chemotherapy, we partnered it with 5-Fluorouracil (5-FU), and loaded it into an acidic pH soluble poly (dopamine-co-acrylamide)-compl-poly (boronic acid-co-acrylamide) hydrogel. The hydrogel is injected into the nitinol tube, and can deliver its drug payload under low pH. This method can provide a dual drug delivery platform in adjunct to hyperthermia therapy, using stimuli responsive macromolecules and bulk ferrogmagnetic material.

2. Experimental

2.1. Materials

Nitinol tubes of 0.7 mm OD and 0.58 ID were purchased from Alfa Aesar (South Korea); bortezomib from LC Laboratories (USA); Tris, NaCl, and KCl were all obtained from Samchun Chemicals (South, Korea); and dopamine hydrochloride, 5-Fluorouracil, 4-vinylphenylboronic acid, dimethyl formamide, and dimethyl sulfoxide were all purchased from Sigma-Aldrich. All aqueous solutions were prepared with ultrapure water purified with a Milli-Q UV-Plus water purification system (Millipore, Bedford, MA). The water had a resistivity of $> 1018\ M\Omega\ cm^{-1}$.

2.2. Electropolymerization process

Multiple scanning cyclic voltammetry was used for the electrochemical polymerization of dopamine. Fig. S1 of the Supplementary Information (SI) shows the typical CV of dopamine polymerized at a concentration of 1 mg/mL Tris buffer saline solution (TBS, pH 7.4) on a nitinol tube at (1.5 to −1.5) A, with the scanning rate set at 50 mV/s. After each cycle, the area under the curve in the voltammogram decreases as a result of the deposition of polydopamine onto the working electrode. After 10 cycles (20 segments), the amount of polymer that had been deposited led to almost complete insulation of the working electrode.

2.3. Loading of bortezomib to the electropolymerized dopamine coated nitinol tubes

First, the drug was weighed at 3.0 mg, and dissolved in a 10 ml DW/DMSO mixture (V/V = 10:1), with a resulting drug concentration of 300 μg/ml. The pH of the drug solution was then adjusted to 9.0 with drops of 1.0 M NaOH. The coated tubes were then fully submerged in the BTZ solution for 24 h at room temperature. The drug loading efficiency was measured via High-performance liquid chromatography (HPLC) (retention time of 6.633; wavelength 263 nm) (JASCO, USA), by measuring the amount of BTZ left in the supernatant after the loading process, and comparing it to the amount of BTZ present before the drug loading procedure.

The entrapment efficiency was then calculated using the following equation:

$$\text{Entrapment Efficiency}\,(\%) = \frac{\text{amount of drug entrapped}}{\text{amount of drug used}} \times 100 \quad (1)$$

2.4. Synthesis of poly (dopamine-co-acrylamide)-compl-poly(boronic acid-co-acrylamide) hydrogel loaded with 5-fluorouracil

The polymerization followed a simple radical process. First, 30 ml DMSO was purged with N_2 gas for 30 min, and 2.2 Mol at 99.5:0.5 ratio of acrylamide and 4-vinylphenylboronic acid was prepared, and subsequently added to the solvent under constant stirring and N_2 gas blanket. The temperature was raised to 65 °C, and maintained for 24 h. The resulting turbid white solution was then washed using diethyl ether to isolate the solid copolymer. The solid copolymer was next dissolved in pure ethanol, and further washed using diethyl ether for a second time, to removed unreacted monomers and residual solvent. The same process was followed for the polymerization of poly(dopamine-co-acrylamide) copolymer, but with DMF as a solvent, and the molar ratio was changed to 2.3 Mol at 99.5:0.5 ratio. The resulting copolymers were then dried for 24 h using a vacuum pump.

Once the copolymers were synthesized, they were dissolved in ultrapure distilled water at 5 wt/vol%. The dissolved copolymers were then added at 1:1 ratio in a vial, and 10 wt% of 5-FU in respect to the copolymer weight was added to the final solution. The pH of the solution was raised to 9 by adding 10 μl of 1.0 M NaOH solution. After the solution was stirred, the gelation process followed. The viscous gel was then loaded into 12 ml syringe with a 27 gauge needle, and the hydrogel was injected into the CTS.

2.5. Characterization of the chemothermal seed

The morphology of the coating and hydrogel were observed using Field Emission Scanning Electron Microscopy (Carl Zeiss Supra 40VP). The samples were sputter coated under argon, in order to make them electrically conductive. The excitation voltage used to capture the images was set at 2 kV.

Fourier transform Infrared spectroscopy data was obtained using the Spectrum-GX FTIR spectrometer (PerkinElmer Co., USA). The scanning range was set at (500–4000) cm^{-1} with a resolution of 1 cm^{-1}. The elemental composition and surface state of the samples were checked using X-ray photoelectron spectroscopy (XPS, AXIS-NOVA, Kratos, Inc.) with an Al Kα irradiation source.

Thermogravimetric analysis was obtained using TMK 0017 universal analysis (TA Instruments Co., USA) and (Q50, TA Instruments), respectively. The heating rate was set at 10 °C/min, and ranged from (10 to 400) °C. The gas flow rate of nitrogen was set at 40 ml/min for both analyses, which were to determine the thermal stability of the polymers and drugs used in this study.

Rheological studies of the hydrogel were conducted using HR-1 Discovery Rheometer (TA Instruments, USA) to determine the injectability of the hydrogel into the narrow nitinol tubes, as seen in Fig. S2 of the SI. Thermal analysis of the synthesized copolymers was performed using Thermal Analyzer (TAQ20, TA Instruments, USA) (Fig. S3 of the SI). Gel permission chromatography multi-angle light scattering (MALS) was conducted to evaluate the degree of polymerization of the synthesized copolymers; the solid copolymers were dissolved in ultra pure water, and analyzed (Fig. S4 and Table S1 of the SI). H^1 NMR spectroscopy (400 MHz/FT NMR spectrometry, JNM-AL400, JEOL,