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Review Article

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Drug Delivery System for Aflibercept Delivery in Metastatic Colorectal Cancer (MCRC)

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ABSTRACT

Background: Metastatic colorectal cancer (mCRC) is a major health burden worldwide and a growing challenge in Asian countries. Treatment options include chemotherapy and vascular endothelial growth factor (VEGF) inhibitor (such as bevacizumab, aflibercept or ramucirumab) or anti-epidermal growth factor receptor (EGFR) therapy. Aflibercept, a recombinant fusion protein, is approved for the treatment of mCRC in combination with FOLFIRI in patients with disease progression during or after an oxaliplatin-containing regimen based on its efficacy and tolerability profile in clinical trials. Hence, the amount, dosage and side effects of chemotherapy even though they are highly reduced are still considerable challenges. The purpose of this report is to provide insight into the future production of a drug delivery system that can be sustainable and targeted and has fewer side effects. To achieve these goals, a DDS system was designed using an electrospinning method and nanoparticles filled with aflibercept.

Keywords: MCRC, Aflibercept, IBB

Introduction

In the United States, colorectal cancer is the most common cancer in men and women and the second leading cause of cancer deaths in both sexes combined. In 2011, approximately 140,000 new cancer diagnoses and 50,000 cancer-related deaths occurred. In the United States alone.1 Worldwide, colorectal cancer caused more than 1.2 million new cases and more than 600,000 deaths during the same period. Therefore, colorectal cancer is a major global health problem. Approximately 60% of colorectal cancer patients are diagnosed at the locally advanced or metastatic stage. Over the past three decades, several methods for treating colorectal cancer have been developed, one of which involves developing agents that target angiogenesis.

Anti-vascular endothelial growth factor agents (antiangiogenic agents), such as bevacizumab and aflibercept, are known to be effective agents for the treatment of colorectal cancer and are known to be among the leading causes of death worldwide [1]. Bevacizumab inhibits and binds to VEGF-A, which leads to the suppression of cell migration and proliferation [2]. In randomized clinical trials, Bmab improved clinical outcomes in patients with mCRC receiving chemotherapy (fluoropyrimidines, irinotecan and oxaliplatin) via both first-line (3-5) and second-line (6-8) regimens. In addition, in ML18147, Bennouna et al. reported the efficacy of second-line Bmab in patients with disease progression after first-line Bmab in combination with standard chemotherapy [median survival (OS): 11.2 months in the Bmab + chemotherapy group versus 9.8 months in the chemotherapy group, hazard ratio]. (HR) = 0.81, 95% CI = 0.69–0.94, p = 0.0062] [3]. Bevacizumab beyond first progression (BBP) is widely used among patients, especially those who receive bevacizumab combined with chemotherapy as a first-line treatment.

Aflibercept is a soluble decoy receptor that inhibits the activity of VEGF-A, VEGF-B, and placental growth factor (PIGF) 1 and 2 with greater affinity than endogenous receptors [4]. In a randomized phase III trial of patients with mCRC previously treated with an oxaliplatin-based regimen, the addition of AFL to FOLFIRI significantly improved the median survival and progression-free survival (PFS) of patients compared with those of patients treated with placebo or FOLFIRI (median OS: 13.50 vs. 12.06 months, HR=0.817, 95% CI=0.713-0.937, p=0.0032; median PFS: 6.90 vs. 4.67 months, HR=0.758, 95% CI=0.661-

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0.869, p<0.0001) [5]. Another study showed that there was no significant interaction between treatment and prior exposure to bevacizumab [6].

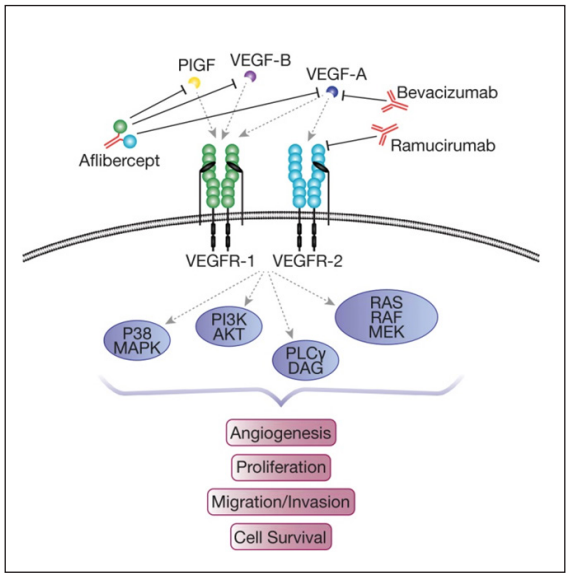

Figure 1: Effect of Anti-Vegf Agents on Mcrc

Figure 1 shows how different anti-VEGF agents respond to growth factors in metastatic colorectal cancer. As shown in the figure, aflibercept binds with most of the growth factors that help the cell survive and proliferate. By inhibiting angiogenesis in tumor cells, nutrients and oxygen are removed from the cells so that the tumor mass can be isolated and cannot grow or spread. Over time, the body has enough time to recover and produce antibodies.

Materials & Methods

The use of nanoparticles as carriers for peptide and protein drugs could effectively protect drugs from inactivation; achieve sustained release, controlled release and targeted drug delivery; significantly enhance bioavailability; and reduce side effects. Such a drug delivery system enables highly effective and predictable release of drugs and reduces drug delivery time, thereby increasing the safety, effectiveness and reliability of drug therapy. It has been reported that chitosan and chitosan derivatives exhibit many biological activities that are beneficial for the human body, such as antitumor effects, immune adjuvant effects, tissue repair promotion and hemostatic effects, which make chitosan and chitosan derivatives ideal sustained-release materials for drugs. Additionally, a controlled-release drug delivery system based on chitosan nanoparticles has become a widely used sustained-release form currently known as one of biomedical engineers' best choices.

Another type of nanoparticle that is widely used in drug delivery system design is PLGA-based nanoparticles. PLGA is known for its ability to preserve and release drugs. Additionally, its surface charge can be controlled (but is mostly positive), which makes it a perfect match for drug delivery to tumors.

In this study, we recommend a new method involving the combination of both electrospinning and nanocarriers to form an injectable biodegradable blazered capsule to achieve long-duration sustained release. To achieve this goal, 0.5 g of poly-Capro-lactane (PCL) was dissolved in chloroform–ethanol (9:1) and stirred overnight at room temperature. A 33-gauge syringe was used to electro spin the solution at 15-20 kV, a humidity of 30% controlled by nitrogen, and 500-800 rpm, and the mixture was left for a full hour to complete the electrospinning process. After that, the electro spun matt was salt leached, placed in distilled water and then dried in an oven.

To produce the aflibercept-loaded nanocarrier, two different particles were synthesized.

The first group included PLGA-loaded nanoparticles, whose preparation method is briefly as follows

Dexamethasone was encapsulated into PLGA (75:25, MW 4–15 kDa) nanoparticles by a modified single-emulsion, solvent evaporation technique 33–34. Dexamethasone and PLGA were dissolved in dichloromethane (DCM) to create the oil phase, and polyvinyl alcohol (PVA) was used as the water phase. The oil-in-water emulsion (o/w) was created by sonication at 100 W for 3.5 minutes. After the solvent was allowed to evaporate, the DEX-np were collected by centrifugation, washed with deionized (DI) water at least three times, lyophilized, and stored at 4°C.

Similarly, aflibercept was encapsulated into PLGA (75:25) microspheres by a modified double-emulsion, solvent evaporation technique 29–30,34. The first emulsion (w1/o) was created by vertexing and then immediately added to the PVA phase (w2) to create a double emulsion (w1/0/w2) by vertexing. Excipients were added to the inner aqueous phase (w1) for protein stabilization and to the oil phase (o) to act as a buffer 29. After solvent evaporation, aflibercept-loaded microparticles (AFL-mp) were collected by centrifugation, washed with DI water at least three times, lyophilized, and stored at 4°C.

Thermo-responsive, biodegradable PEG-PLLA-DA/NIPA Am hydrogels were synthesized by free radical polymerization, as described elsewhere 27,35. NIPA Am (350 mM), N-tert-butyl acrylamide (50 mM), ammonium persulfate (13 mM), and PEG-PLLA-DA (2 mM) were dissolved in 1x PBS (pH 7.4) to prepare the hydrogel precursor solution 30. Varying loading doses of microparticles (0 mg/ml and 20 mg/ml) and nanoparticles (0 mg/ml, 20 mg/ml, 40 mg/ml, 60 mg/ml, and 80 mg/ml) were suspended in the solution to create the composite DDS. N, N, N', N'-Tetramethyl ethylenediamine (168 mM, pH 7.4) was added to the hydrogel precursor solutions to initiate polymerization. The hydrogels were placed on ice for 30 minutes, after which the reaction proceeded to create nanoparticle- and microparticle-hydrogel DDSs. The DDS was then collected, washed with DI water five times, and stored at 4°C.

Aflibercept was radiolabeled with iodine-125 using iodination beads for all characterization and release kinetic studies. To remove any unbound, free iodine, radiolabeled aflibercept was dialyzed against DI water using a dialysis cassette for 48 hours. Then, the radiolabeled aflibercept was collected, lyophilized, and dissolved in PBS to create a 40 mg/ml stock solution. Radioactivity was measured using a gamma counter.

The second group included chitosan-coated PLGA nanoparticles. Aflibercept-loaded chitosan-coated PLGA nanoparticles (CS-PLGA NPs) were prepared by the double emulsion solvent

evaporation method described by Virchowian et al. (2013) with some modifications. Briefly, 100 µL of 1% w/v bevacizumab solution (equivalent to 1 mg) in phosphate-buffered saline (PBS, pH 7.4) was emulsified in 2 mL of dichloromethane (DCM) containing an appropriate amount of PLGA (3.5 mg/mL) by sonication using a probe solicitor) at 60 W in an ice bath. The formed primary emulsion was dropped into an aqueous solution containing chitosan (0.25% w/v) and PVA (1.0% w/v) and sonicated for 10 min to form a w/o/w emulsion. The temperature was maintained throughout the procedure by using an ice bath. The developed w/o/w emulsion was stirred for 6 h at 1000 rpm at room temperature to allow evaporation of the organic solvent (DCM) and hardening of the nanoparticles. NPs were collected by centrifugation at 18000 rpm, washed 3 times with Milli-Q water and finally dried in a lyophilized using mannitol (1% w/v) as a cryoprotectant.

Device Characterization

The morphological characteristics of the capsules were inspected by electron microscopy (SEM). Recently, chitosan/PLGA nanoparticle layers, PCL stringy layers, and cross-sections of blazered movies and mono-layered movies after salt filtering were joined on carbon tape set on aluminum stub mounts and sputter-coated with a layer of gold-palladium. Capsules were drenched and broken in fluid nitrogen to obtain cross-sections for imaging. The normal fiber sizes and pore sizes of the PCL layer and chitosan/PLGA layer were characterized and evaluated from SEM images of three tests utilizing ImageJ (NIH).

Surface chemical investigations of the electro spun materials were performed by employing a Fourier transform infrared (FTIR) spectrometer in weakened addition to reflectance (ATR) mode. The germanium precious stone was put in contact with the tests, and 100 filters were collected at 8 cm-1. Standard crest positions at 1727 cm-1 and 1590 cm-1 were utilized to distinguish PCL (carbonyl crest) and chitosan/PLGA (amine band), respectively.

Drug Release Profile

Empty blazered capsules with two open closes were obtained by evacuating the drum collectors. For the 1.645 mm inward breadth capsule, 2.0 mg of BSA powder (show protein) or 2.0 mg of lyophilized aflibercept powder (aflibercept, anti-VEGF) broken down in phosphate-buffered saline (PBS) at a concentration of 0.1 mg/µL was stacked on the capsule, which was fixed at the closes employing a tube sealer (Doug Care Hardware, TTS-8C) [7,8]. For the 260 µm internal distance across capsules, concentrated 1.0 mg of BSA or 1.0 mg of aflibercept slurry at a concentration of 1.0 mg/µL was loaded into the capsules employing a 31-gauge needle considering the constrained volume. In vitro BSA discharge profiles from PCL mono-layered capsules and PCL-chitosan/PLGA blazered capsules were obtained as described below. Capsules were submerged in 1 mL of PBS in a 1.5 mL Moo authoritative centrifuge tube to decrease the size of the centrifuge tube to meet the needs of the eluted physiological conditions. At 1 h, 3 h, 6 h, 12 h, 24 h, 3 days, 1 week, 2 weeks, 1 month, and month to month from that point, the eluant was collected [9-11]. At that point, new 1.0 mL of PBS was added and kept beneath the brooding. The BSA discharge profile was obtained by determining the retention of eluted BSA by the BCA test and evaluating the concentration by employing a BSA protein-based standard bend. For in vitro aflibercept discharge from PCL monolayered capsules and PCL-chitosan/PLGA blazered capsules, the same convention was used to obtain the aflibercept eluant. The characteristic absorbance of aflibercept was determined at 277 nm by UV–Vis spectroscopy, and the discharge rate of aflibercept from capsules was determined by a microplate reader at 277 nm and measured based on the standard bend of the stock aflibercept arrangement at distinctive concentrations [12]. The tests were performed in triplicate.

To determine the release rate of receptive aflibercept from the 260 µm wide-band capsules, a protein-coupled immunosorbent assay (ELISA) was conducted as previously described [11,13]. Briefly, 100 µL of 1 µg/mL recombinant human VEGF protein at pH 9.6 in sodium carbonate buffer was immobilized on a 96-well Nunc Maxisorp plate at 4°C overnight [14-24]. The plate was blocked with 200 µL of 2% BSA in PBS/T (0.05% v/v tween 20 in pH 7.4 PBS) for 2 h at room temperature and washed with 300 µL of PBS/T three times. At that point, the amount of Aflibercept eluted from the capsules was decreased to between ng/mL and 10 ng/mL (decided by the standard curve) in a 0.1% BSA-PBS/T arrangement, and 100 µL of the test solution was added to each well and incubated at room temperature for another 2 hours. Afterward, the plate was washed with PBS/T three times, and 100 µL of the HRP-conjugated goat anti-human IgG Fc auxiliary counteracting agent PBS/T (1:1000) was added to each well [25-32]. The entire plate was incubated in the dark at room temperature for 1 hour and washed with PBS/T five times. The color was determined by the addition of 100 µL of TMB and the addition of 100 µL of 1 N sulfuric acid. The concentration of dynamic aflibercept in each test was determined by comparing the absorbance at 450 nm with that of the standard bend.

The sedate payload was determined by breaking three BSA and aflibercept stacked monolayered and blazered capsules of diverse sizes in PBS [32-38]. Briefly, three stacked mono-layered and blazered BSA and aflibercept capsules were broken and submerged in 1 mL of PBS. The gadget was energetically washed with 1 mL of PBS five times utilizing a vortex blender. Each washing step took at least ten minutes. The concentrations of the collected eluents of BSA and responsive aflibercept were determined by BCA measurement, UV–Vi's spectroscopy, and ELISA. The medicate embodiment proficiency was calculated as free sedate within the eluent/adding up to the sum of the medicate *100% [39-46]. The sedate stacking proficiency was calculated as sedate payload/capsule weight *100%. The total discharge % was calculated as the aggregate sum of the amount of the medicate eluted from the capsule/[sedate payload * embodiment proficiency] *100%.

Cytotoxicity

The in vitro cytotoxicity's of the PCL mono-layered capsule and the PCL-chitosan/PLGA blazered capsule were evaluated by an MTS test conducted with a human cancerous cell line. Cancer cells were seeded in 48-well plates at a density of 4×104 cells/well for all tests. Cytotoxicity was measured by both the coordinate contact strategy and the extricate presentation strategy [47-54]. For the coordinate contact strategy, a 1cm PCL monolayered capsule or PCL-chitosan/PLGA bilayer capsule was placed within a cell-seeded well plate for 24 hours. For the

extricate introduction strategy, the PCL monolayered capsule or PCL-chitosan/PLGA bilayer capsule was submerged in 1 mL of new media for 1 day, 3 days, 1 week, 2 weeks, or 1 month. At each time point, the capsule-conditioned media was exchanged for the cancerous cell culture, and estimations were performed with brooding times of each test with the cells for 24 hours [55-62]. To perform the cytotoxicity test, the cell culture media were blended with 20 µL of MTS reagent after 3 h of incubation at 37 °C. The absorbance of the supernatants was estimated with a microplate reader at 490 nm [63-68]. The viability of the cells in the exploratory group was normalized to that of the control group (no treatment). All tests were repeated in triplicate, and the data were analyzed by one-way ANOVA with a post hoc Tukey test at an importance level of 0.05. The data are displayed as the mean ± standard deviation.

Results

Our procedure for manufacturing the IBB capsules is based on two-step coating of movies of chitosan and PCL in a rod-shaped format taken after evacuation from the layout. To form a permeable central emptied bilayer structure, electrospinning was utilized, which can offer a tall surface region-to-volume ratio for protein chemo adsorption and tunable porosity for drug dissemination. Electrospinning, as a strategy for nanofiber creation, is based on the use of electric constraints to generate charged polymer arrangements in nanosized filaments. To synthesize the chitosan nanoparticles, we optimized the preparation parameters, namely, the mugginess and voltage. To this end, a layer of PCL was included not because it was physically entangled with drugs but also because of its progressive adaptability. In particular, PCL nanofibers with a distance across 932.57 ± 399.42 nm were coated with the chitosan nanoparticles. In conclusion, nanofiber-based barrels that have a high surface region area, high mechanical adaptability, and solid attachment between distinctive layers were developed as building pieces for IBB capsules.

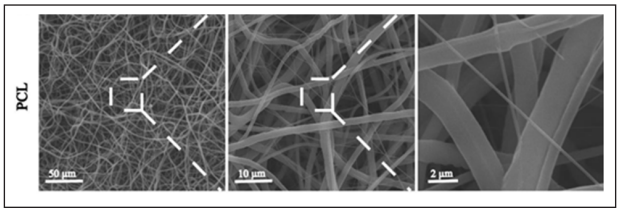

Figure 2: PCL nanofibers

Table 1: Porosity and pore size of PCL membranes prepared with different ratios of PCL to HEPES sodium salt

Sample name	Pore diameter (nm)	Porous channel
0.0% HEPES salt	None	No
1.0% HEPES salt	237.26 ± 96.93	No
5.0% HEPES salt	371.65 ± 156.77	Yes
7.5% HEPES salt	582.21 ± 302.17	Yes
10% HEPES salt	608.55 ± 273.90	Yes

In contrast, the PCL-chitosan/PLGA bilayer capsules did not exhibit self-evident evidence of burst release. Overall, the bilayer capsules moderated the BSA discharge. The discharge profiles of the bilayer capsules exhibited high linearity [69]. After one month, the 1.645 mm internal breadth bilayer capsule appeared to have a better capacity to hold the BSA inside the device, with approximately 15% of the stacked BSA released, which was 60% less release within the same period than that of the monolayered PCL capsule. Additionally, 260 µm internal breadth bilayer capsules essentially decreased the burst discharge [70]. A total of 25% of the BSA was eluted from the 260 µm internal breadth bilayer capsules, which was higher than that of the 1.645 mm capsules due to the moderately more noteworthy range of volume for dissemination. The chitosan/PLGA nanoparticles were able to constrain sedate dissemination, and the porosity of the PCL shell did not play a crucial role in controlling BSA release [71-72]. There was no critical difference between the widths of the monolayered and bilayer capsules ($p>0.05$), so the impact of thickness on the discharge of the medicate was insignificant. Hypothetically, the bilayer structure has the potential to control the discharge of medicate at least one year for capsules of both sizes based on aggregate discharge information.

Conclusion

In conclusion, we have created a polymer-based conveyance stage for the controlled discharge of anti-VEGF agents, which is based on a bilayer microstructure that synergistically combines the electrostatic interactions between chitosan/PLGA and anti-VEGF agents with a defensive hydrophobic layer of PCL to provide a successful course to balance polymers and proteins for controlled discharge. We characterized the bilayer structure in detail and advanced capsule execution for protein conveyance. Most critically, our outlined conveyance stage essentially made strides in the long-term discharge of anti-VEGF in vitro compared to most current gadgets, supporting its potential for treating MCRC. In the future, assessing and re optimizing the helpful impact of anti-VEGF-loaded gadgets on in vivo MCRC will be necessary. We are trusting to bridge the gap between our novel microstructure conveyance stage and clinical anti-VEGF conveyance, which can advance the clinical treatment of MCRC and a few cancers by providing promising options for accessible approaches.

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