Synthesis of a pH Responsive Polymeric Drug Delivery – tailoring functionalization for effective delivery

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ABSTRACT

The molecular modelling characterization and synthesis of the folate functionalized biomaterial poly(styrene-alt-maleic anhydride), PSMA predicted the folic acid functionalized SMA as a good candidate for targeted drug delivery. Folate was attached to the pH responsive SMA polymer via a biodegradable linker 2,4-diaminobutyric acid, DABA. The computational results predicted DABA as the optimal linker and showed that the functionazlied PSMA is linear at neutral condition and its linearity remains pH responsive. However, the earlier synthesis route displayed difficulties in characterization and purification due to the complexity of polymeric products. In this paper, a different synthesis route is shown along with NMR and IR characterization. Finally, we show some preliminary results of applying biomaterials in cell lines. Curcumin, a natural ingredient was selected as a fluorecent marker to study polymer-cell binding efficiency as well as cellular uptake.

Keywords: folate receptor, drug delivery, pH-responsive, amphiphilic co-polymers, curcumin

1 INTRODUCTION

Drug delivery systems capable of unloading drugs in response to pH changes have received much attention in recent years. Poly(styrene-alt-maleic anhydride), PSMA, is an amphiphilic alternating copolymer that self-assembles into nanotubes in aqueous solution, physiological-pH environments[1]. Its self-assembled structure with a 2nm hydrophobic inner diameter is stable in neutral pH solution, but collapses when pH is increased or decreased. These unique features of PSMA make it a great candidate for controlled release drug delivery vehicle for cancer cells for which the pH is significantly lowered compared to primary cells. PSMA has been considered as drug delivery vesicle as early as 1980s by Maeda and coworkers[2]. Its been shown that PSMA has low toxicity in cells and binds to albumin and therefore has an increased circulation time in blood stream[3–5].

To increase the specificity of the drug delivery vessel, folic acid is attached. The folic acid has extermely high affinity (K_D~10M) towards its receptors which are over-expressed in certain types of cancers[i]. Indeed, the folate-conjugated nanotubes could potentially transport small, highly toxic, hydrophobic chemotherapy agents safely through the body to be released selectively inside target cells. Once the drug is unloaded, the biocompatible SMA, folate, and linker groups would clear through the excretory system.

The synthesis was conducted in our lab using DCC/NHS coupling reagents linking carbolylic acids with amines. Amide bonds were therefore made among SMA, linker and folic acid. Previously we reported a synthesis route where polymer SMA was the starting material, linker and folic acid were added subsequently. However, due to the complexity of characterizing polymeric compounds, the second method starts with folic acid and linker and SMA polymer were attached in order. All structures were purified in each step and characterized using IR and NMR techniques.

The polymers were labelled using curcumin, which is the active ingredient in tumeric. Curcumin is an effective and safe anticancer agent, however its hydrophobicity inhibits its clinical application[6,7]. Nanotechnology provides an effective method to improve the water solubility of hydrophobic drug. In this research, curcumin is incorporated to the hydrophobic interior of polymeric biomaterial by diffusion as a drug mimic and its fluorecent property serves as a biomarker. Human pancreatic cancer cell lines PANC-1 was culcutred in the lab and the polymer-cell binding efficiency and cellular uptake activities were analysed by fluorecent microscopy

2 METHODS

2.1 Materials

13% poly(styrene-alt-maleic anhydride) (Mw=350,000) was purchased from Sigma Aldrich and freeze dried into white powder. Boc-2,4-diaminobutyric acid (DABA), N-hydroxy-succimide (NHS), dicyclohexylcarbodiimide (DCC), and folic acids were purchased from Sigma Aldrich and were used directly without further processing.

2.2 Synthesis of folic-conjugate

First, folic acid was dissolved in DMSO and allowed to react with DCC/NHS at room temperature overnight. The product was filtered using 0.2 μl filter. The resulting activated folic-NHS was added dropwise to a solution of boc-protected linker boc-2,4-diaminobutyric acid (DABA). DCC/NHS were added as coupling reagents along with boc-2,4-diaminobutyric acid at a 1:10 ratio. The reaction was carried out at room temperature over night. The crude product was dialysed against water to remove DMSO solvent and the product freeze dried.

The boc protecting group was removed by trifluoroacetic acid and dichloromathane TFA/DCM at 30 degrees for 6 hours. TFA was then evaporated, and the remaining product was taken up by dichloromethane (DCM) and recrystalized in ether. The product was then filtered, air dried and characterized by ^{1}H NMR.

PSMA as added to the deprotected FA-daba oligamer and the reaction was carried out over night with DCC/NHS coupling reagent in DMSO. The product was dialysed against water and the final product was obtained after freeze drying. The dry product was characterized using ^{1}H NMR in DMSO and IR by making KBr pellets.

2.3 Cell lines

Human pancreatic cancer PANC-1 cells were maintained in monolayer culture at 37 degrees in DMEM containing 10% heat-inactivated newborn calf serum and 3 μg/ml plasmacin. The cells were seeded to wells one day prior to treatment.

2.3 SMA-curcumin nanoparticle

Curcumin was added to various SMA concentraions in media to saturation. The excedss curcumin was removed by centrifuge at 3000 rpm speed in 7 mins. The supernant was obtained and added to the wells containing cells lines.

2.4 Fluorecent microscopy

The SMA with saturated tumeric was dissolved in DMEM media of concentrations of 0.3 uM, 1uM, 3uM and 10 uM. Spectra were recorded with a fluorescence spectrophotometer

(FP-6600; JASCO) with excitation at 480 nm and emissions between 500 and 700 nm In vitro cytotoxicity assay.

3 RESULTS

Polymers are complicated to analyze, especially when only a few of the bonds are being traced. Therefore starting the synthesis with folic acid allows us to fully characterize FA-daba prior to PSMA attachment. Figure 1 shows the NMR spectrum after folic acid was attached to DAB(boc)-OH (after deprotection). The existence of folate is confirmed by peaks at 6.61 and 7.64 ppm which correspond to its para-aminobezoic acid group and the signal at 8.63 ppm, attributable to the pteridine moity proton from the folate. The peaks at 4.31and 4.47 ppm confirmed the formation of amide bond between the two. The clearance around 1.6 ppm showed that the boc protecting group was sucessfully removed.

PSMA was added to the system by coupling reagents DCC/NHS and the resulting product was characterized by NMR (Figure 2) and IR (Figure 3). The presence of folate is confirmed by peaks at 6.61 and 7.64 ppm, which correspond to its para-aminobezoic acid group, and the signal at 8.63 ppm, corresponding to its pteridine moity proton.

In Figure 3, the loss of peaks 1850 cm^{-1} and 1780 cm^{-1} indicates the conversion of maleic anhydride groups; the strong peak at 1731 cm^{-1} is produced by the remaining carboxylic acid. Furthermore, bands at 1626 and 1530 cm^{-1} are attributed to the formation of amide C═O and N–H functionalities respectively, demonstrating the link between SMA and folic acid [7].

The biomaterial PSMA and functionalized PSMA-daba-FA were briefly tested in PANC-1 cell lines with curcumin serving as a biomarker. As figure 4 shows, 10 μM SMA with curcumin were added to the cell lines, after 12 hours the cells seem to internalize some curcumin and cells appear relatively healthy. However when functionalized FA-daba-SMA were used in the treatment, cells are quickly loosing their morphology and the tumeric uptake is a lot higher. It shows that SMA-daba-FA has great potential as a drug delivery vesicle.

4 CONCLUSION AND FUTURE WORK

In this study, we presented an alternative synthesis route of SMA-daba-FA. The synthesis was characterizaed by NMR and IR and the results confirmed the structure of the biomaterials. The polymers were tested in human pancreatic cancer PANC-1 and monitored by fluorescent microscopy. The preliminary results presented here showed that FA-daba-SMA has great drug delivery potential. The study is still ongoing and cell viability assays and drug release will be tested to provide quantitave data.

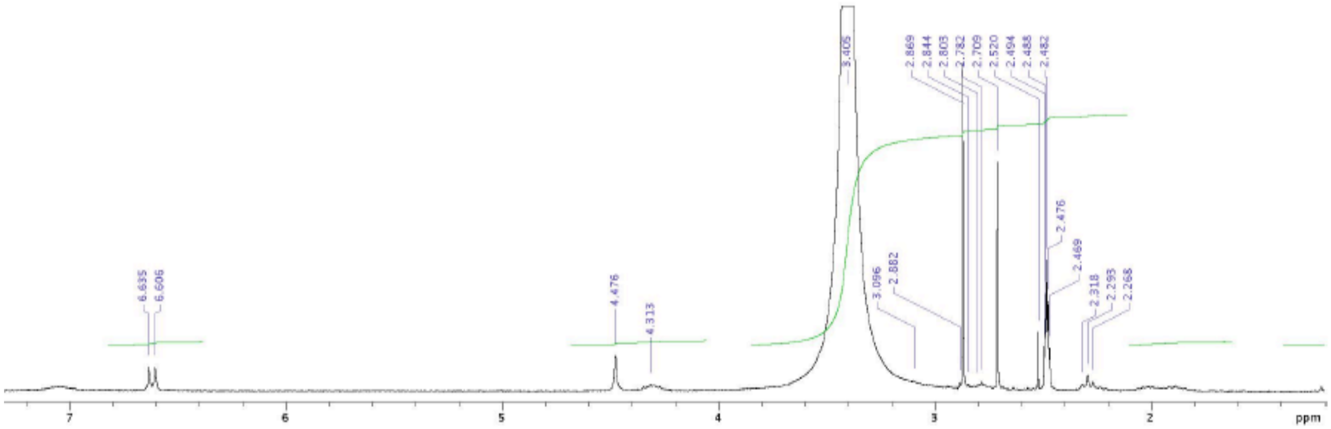

Figure 1: the NMR spectrum after folic acid was attached to DAB(boc)-OH, after deprotection

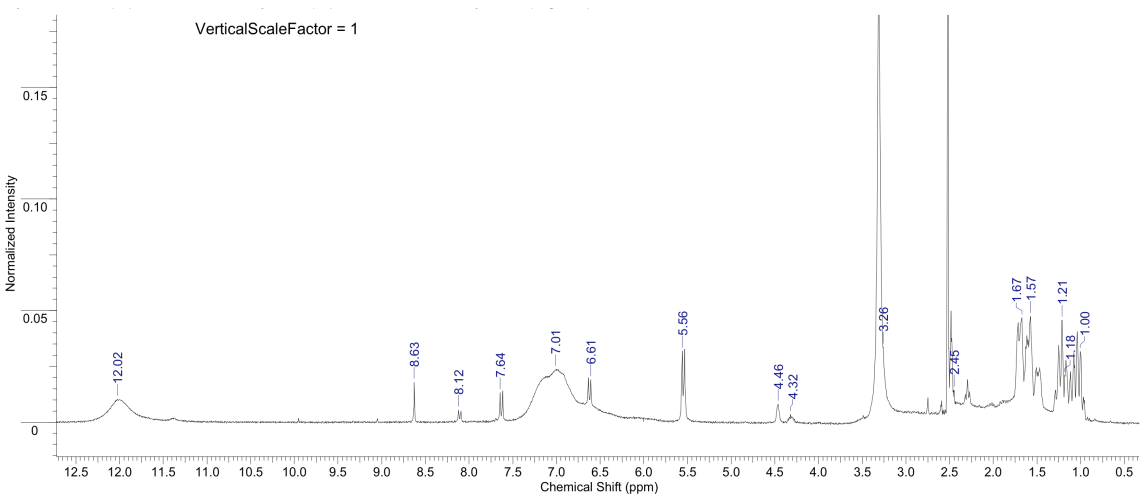

Figure 2: ^{1}H NMR spectra of folic acid-DABA-SMA conjugates in d-DMSO

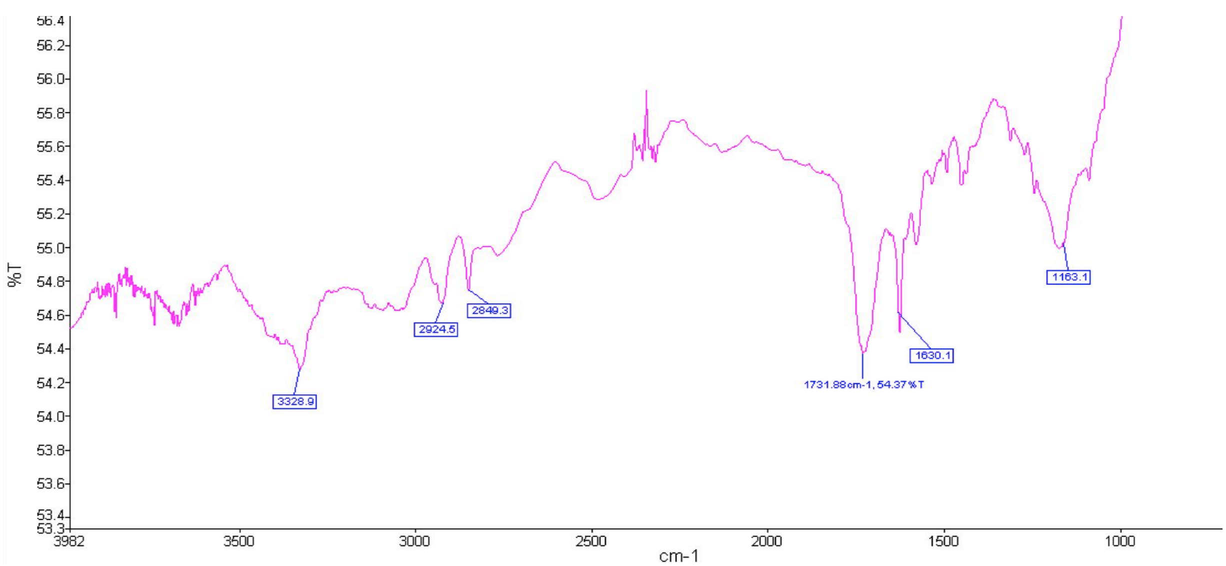

Figure 3: IR spectra of folate-DABA-SMA conjugate

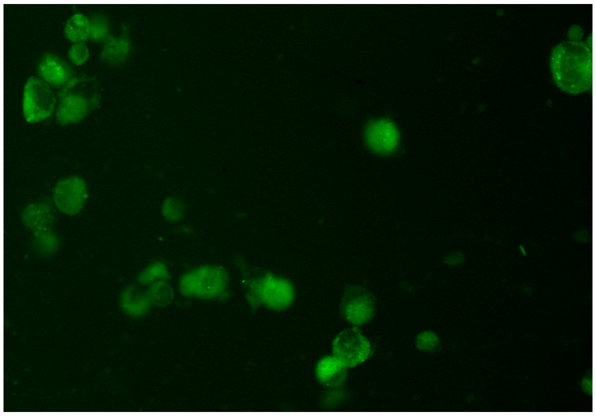

Figure 4: SMA with tumeric (10µM) in PANC1 cells after 12 hours

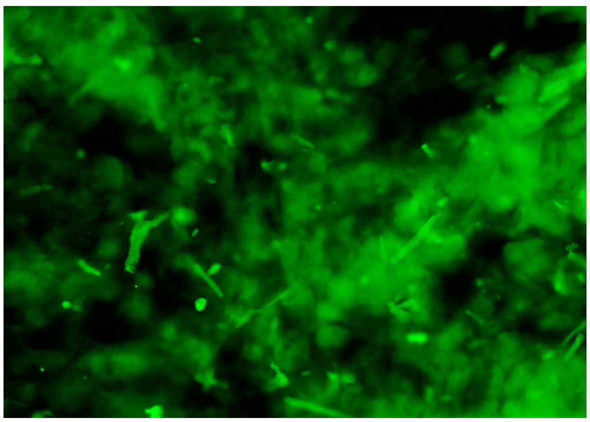

Figure 5: SMA-daba-FA wth tumeric (10µM) in PANC-1 cells after 12 hours

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