Controlled Drug Release Using Core-shell Hydrogel Nanoparticles for Pancreatic Cancer

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Current Challenges in Pancreatic Cancer Therapy

- No available early detection tool
- Poor drug delivery
- Drug resistance
- Poor survival with available therapeutics
  - Gemcitabine (6.6 months)
  - nab-paclitaxel plus Gemcitabine (8.7 months)
  - FOLFIRINOX (11.1 months)
Nanoparticle based therapy

• Avoid the unwanted adverse events of chemotherapy

  • Improves solubility, toxicity, dosage and short circulating half-lives of chemotherapeutics

• Enhance deep-tissue penetration

• Cancer cell uptake (Passive or specific)
Long-term goal

Development of biodegradable hydrogel-silica core-shell nanoparticles (HSCSNPs) for targeted drug delivery and controlled systemic release of chemotherapeutics to PDAC tumor or TME to improve therapeutic efficacy.
Proposed hydrogel drug delivery

Hydrogel:
- polyacrylic acid (PAA)
- chitosan (CS)

Drug:
- gemcitabine (GEM)

Ligands:
- GE11

Aim 1: Engineer hydrogel-silica core-shell nanoparticles (HSCSNPs) and examine their drug loading and drug release profile.

Specific Hypothesis:

*HSCSNPs provides multiple-level of controlled drug release through manipulating gel properties, silica shell structures and GE11 peptide on HSCSNPs enable the targeted drug delivery to PDAC tumor.*

Approach:

1.1. Optimization of GEM incorporation in the nanocomposites;
1.2. Optimization of silica shell thickness to control the drug release kinetics;
1.3. Functionalize HSCSNPs with GE11 peptide (YHWYGYTPQNVI) for targeted delivery;
1.4 Analysis of stability and release kinetics of the engineered core-shell nanoformulations.
Aim 2: Evaluate the therapeutic efficacy of nanoformulations in PDAC cells and in PDAC mouse model.

Specific Hypothesis:

HSCSNP –GEMs with targeting peptide possesses distinguished surface architecture that enhances the therapeutic efficacy.

Approach:

2.1 Evaluate therapeutic efficacies of drug-loaded HSCSNPs in established PDAC cells.

2.2 Analyze the therapeutic efficacy of drug-loaded targeted nanoparticles in animal models of pancreatic cancer.
nanoparticle fabrication (current study)

nanoparticle fabrication (future work)
Preparation of nanoparticles

• Briefly, 25% PAA solution was diluted to 1% by DI water, and Gemcitabine (GEM) and surfactant (1%) was added under stirring.

• Chitosan (0.05%, w/v) was dissolved in 50 mL of an aqueous solution of acetic acid (0.05%, v/v).

• Then 5mL of chitosan solution was added dropwise (2.5 mL/min) to 5mL of PAA/GEM/surfactant solution under mechanical stirring (1200 rpm), and stirring was continued for 30min before EDC was added (molar ratio of PAA:EDC = 10:1) to obtain the aqueous suspension of PAA/chitosan nanoparticles.

• The colloidal suspension was stirred for another 24 hours and then centrifuged at room temperature (30 min at 15,000 rcf, Thermo Fisher Scientific sorvall MTX 150 Micro-Ultracentrifuge)
Determination of the Amount of Gemcitabine Loaded to Chitosan Nanoparticles

• Drug loading measurements were performed by spectrophotometric determinations of the drug remaining in the supernatant after nanoparticle ultracentrifugation at room temperature (30 min at 15,000 rcf).

• Drug incorporation in nanoparticles was expressed in equation (1) and gemcitabine loading was expressed in equation (2).

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\text{Drug entrapment efficiency (\%)} = \frac{\text{Mass of drug incorporated (mg)}}{\text{Initial drug added to the suspension (mg)}} \times 100
\]  

\[
\text{Drug loading (\%)} = \frac{\text{Mass of drug incorporated (mg)}}{\text{Mass of PBCA NPs (mg)}} \times 100
\]
Release study
Release study

• Briefly, the bags were soaked in water for 12 h before use.

• The dialysis bag, with a cutoff of 12000 Da (Spectrum Spectra/Por 6 dialysis membrane tubing, U.S.A.) retained the chitosan nanoparticles and

• allowed the free gemcitabine to diffuse into the dissolution media.

• Practically, GemChit nanoparticle suspensions were poured into the bags with the two ends fixed by clamps. The bags were placed in a conical flask filled with 100 mL of the receiving phase and were stirred at 200 rpm.

• At different time intervals (0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 9, 24, 48, 72, and 96 h), 3 mL samples of the medium were withdrawn for UV-vis spectrophotometric analysis at 268 nm.

• An equal volume of water was added after sampling to ensure sink conditions.
Releasing profile with different GEM concentration

- PAA:CS mass ratio 20:1
- Surfactant: 1% PEG
Releasing profile with different GEM concentration

- PAA:CS mass ratio 20:1
- Surfactant: 1% Pluronic F68
Releasing profile with different GEM concentration

- PAA:CS mass ratio 20:1
- Surfactant: no surfactant
*In vitro* toxicity study

- N171-1: PAA:CS:Fe nanoparticles dispersed in water (~ pH 6) ~100 nm
- N171-2: PAA:CS:Fe nanoparticles Dispersed in PBS (~ pH 7) ~200 nm
In vitro toxicity study

- Hydrogel polymer NP: PAA-CS-EDC

72 hrs

96hrs
Future work

• Optimization of silica shell to establish control drug release

• Conjugation of PDAC targeting peptide

• Evaluation of therapeutic efficacies in PDAC cell line and murine PDAC model

• Evaluation of biodistribution
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