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Optimizing Fluorouracil Delivery for Enhanced Efficacy in Colorectal Cancer Treatment

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Abstract

Fluorouracil (5-FU), a mainstay in colorectal cancer therapy, has demonstrated significant efficacy, yet challenges persist in maximizing its clinical impact. This article aims to explore recent advancements in optimizing fluorouracil delivery systems to enhance its therapeutic effectiveness. We delve into the molecular mechanisms underpinning fluorouracil's antitumor activity, examine its current clinical applications, and critically evaluate emerging strategies for targeted drug delivery. The article synthesizes findings from recent studies on nanoparticle formulations, personalized medicine approaches, and combination therapies, providing a comprehensive overview of the evolving landscape in fluorouracil research. The synthesis of these insights contributes to our understanding of how tailored drug delivery systems can potentially revolutionize the treatment paradigm for colorectal cancer.

Keywords : Fluorouracil , colorectal cancer , maliganancies , suboptimal drug delivery.

Introduction

Over time, researchers have consistently shown interest in utilizing agricultural waste for the production of environmentally friendly materials, aiming to address issues such as toxicity and the reduction of landfill space. An example of such waste is Paddy straw, ranking as the second most significant agricultural waste globally, which, when burned, poses potential dangers to health through contamination of the air and depletes organic substances containing significant depletion of nutrients. RS, similar to other plant-derived materials, comprises a substantial proportion of cellulose-fibers (CF) ($C_6H_{10}O_5$), making it an abundant and unique organic polymer with versatile applications.

CF, characterized by a high glucose content linked in a linear chain format by β-1,4-glycosidic bonds, possesses exceptional structural benefits, such as elevated porosity, interconnected pores, extensive surface-to-volume ratios, reduced density, and hydrophilic properties. Among various methods for isolating CF, alkaline treatments are recognized for their simplicity and cost-effectiveness, effectively removing extracting lipids, lignin, hemicellulose, and pectin from plant-derived substances. This process enhances CF crystallinity and purity while maintaining high thermal stability. Alkali-treated CF exhibits potential advantages, such as the ability to reduce fiber size, adjustability, and water dispersibility, making it suitable for various biomedical applications.

This environmentally friendly approach, utilizing bio-waste materials like RS, holds promise for the production of carbon fiber with superior physical and chemical characteristics compared to high scale CF. In the context of cancer, statistics reveal a considerable global burden, with colorectal cancer (CRC) being a major contributor to cancer-related deaths. Conventional cancer treatments present significant drawbacks, prompting the exploration of alternative strategies. Nanosized conjugates, particularly plant-based and low-cost nanomedicines, offer potential benefits in terms of enhanced anticancer properties and reduced side effects. Polysaccharides, including cellulose, have been explored as innovative polymer-anticancer drug conjugates because of their enzymatic degradation by colonic microbial agents. Polymeric nanoparticles (NPs) originating from natural sources, like cellulose and chitosan, possess gained popularity in the context of drug delivery systems , exhibiting optimal physicochemical and biological features. These NPs leverage the enhanced permeability and retention (EPR) effect in tumors, leading to improved drug accumulation and efficacy.

The study also discusses the application of 5-fluorouracil (5-FU), a widely used chemotherapy drug, in different delivery systems, emphasizing the need to minimize its side effects. The utilization of CF extracted from RS for loading 5-FU presents a novel approach for treating nasopharyngeal and colon cancer, addressing the limitations and potential toxicity associated with conventional chemotherapy. The research explores the synthesis and analysis of cellulose-based 5-FU, shedding light on its effectiveness in eliminating cancer cells while also considering potential applications for normal cells. Overall, this study contributes to the ongoing efforts to develop sustainable and effective drug delivery systems using eco-friendly materials.

In this investigation , fibers of cellulose (CF) obtained from paddy straw (RS) ligning removal by alkali & bleaching steps . These CF exhibited sufficiently potential as carriers for 5-fluorouracil (5-FU) in the treatment of colorectal cancer (CRC) and nasopharyngeal carcinoma (NPC). The successful isolation of CF from RS was confirmed through various analytical techniques, including diffraction of gama rays in powder (XRD), Infrared light analysis by (FTIR).

The linking together of 5-FU & CF achieved, and the absorption and release of the drug were assessed using ultraviolet study (UV) spectroscopy. The characteristics of the medication-filled CF were further examined through a device for measuring zeta potential, FTIR .

To evaluate the toxicity to cells and anticancer potential, MTS in vitro cytotoxicity assays were conducted. The study assessed the impact of CF alone, 5-FU alone, and the combination of 5-FU with medicine-loaded CF on regular colo-rectal cells, colorectal cancer cells, regular nasopharyngeal cells, and nasopharyngeal cancer cells at eight's various levels of concentration . This comprehensive analysis aimed to determine the efficacy and safety of the developed 5-FU-loaded CF as a potential treatment strategy for CRC and NPC.

Material and method :

Materials

1. Rice Straw (RS): Sourced from the Agricultural R & D Institute at malasiya (MARDI).
2. Chemicals: Ingredients used is of hplc grade and purchased from Sigmaaldrich ..
3. Reagents for lightening Process and CF extraction:

Potassium hydroxide (KOH, 80%, EM Science)
Sodium chlorite ($NaClO_2$, 75 %, Fluka)
GlacialAcetic acid (CH_3COOH)

4. Water: Deionized Water with a specific conductivity lower than 2 µ ohm/cm is used for all aqueous solutions.
5. Glasswares: All glassware utilized in the experiments underwent cleaning with a mixture of nitric acid (HNO_3) and hydrochloric acid (HCl) in a ratio of 3:1 (v/v). This cleaning process was followed by rinsing with double deionized water and subsequent drying.
6. 5-Fluorouracil (5-FU): 99% pure 5-Fluorouracil with a mole. mass of 120.00 g/mol is procured from CRYSTAL ORGANICS, branch of Thermo Fisher Scientific at USA.

Note: All solutions were freshly prepared, and meticulous attention was given to maintaining the purity of reagents and the cleanliness of glassware throughout the experimental procedures. The materials and chemicals utilized in this study were carefully selected to ensure the accuracy and reliability of the obtained results.

Cellulose thread extraction from Paddy Straw :-

The process of isolating cellulose fibers (CF) from rice straw (RS) involved the removal and lightining of undesired ingredients , like lipids and lignin, following a methodology similar to that applied to various bio-fiber resources.

RS Preparation: RS underwent multiple washes with running water to clean from impurity . Subsequently, it was air-dried for seven days at suitable temp. The dry RS was then reduce particle size by passing through a 60 μ-mesh sieve.

Dewaxing Process: To get un-waxed paddy straw (DRS) with liberated lipids, a 29 g RS powder is added subjected to dewaxing. This involved dissolving oils, wax, lipids, and pigments in a 3:1 v/v toluene/ethanol (400 mL) deionized water solvent using a soxhlet apparatus for 12 hours at 69°C in a silicon oil bath.

The resulting deep brown colour underwent ultrasonic sonication at 36 kHz and then cleaned three times with deionized water. Excess liquid was decanted Employing a laboratory suction filtration unit fitted with Whatman filter paper, followed by drying in a 61°C oven for 24 hours.

Lignin Removal: The powder, now free of lipids, is trichurated with a 1000 mL water solution of NaCl (1.5 %) at 70°C. Acetic acid drops were added to achieve a pH of 3.0–4.0, and the mixture was gently stirred for 5 hours. The resulting light yellowish solid sample underwent cleaning & dry of excess liquid for repeated number of times.

Leaching Process: Undergone drug leached by introducing a 500 mL water solution of KOH (6%) at optimum temp. for 12 hours. Ice cubes were promptly added (10-fold) to the solution, followed by centrifugation at 12,000 rpm. The aqueous sample was then dried using a freeze-dryer apparatus to get the final product as colourless thick thread sample named CF.

Schematic Process: A schematic representation of the isolation process is depicted in Figure 1. This comprehensive procedure ensured the successful isolation of cellulose fibers from rice straw, involving successive steps of dewaxing, lignin removal, and leaching to obtain a purified cellulose fiber sample for further analysis and application.

Depiction of Cellulose Thread Extraction from Paddy Straw

The cellulose fibers obtained from rice straw were subjected to thorough characterization using various analytical techniques:

X-ray Powder Diffraction (XRD): A Philips X'pert XRD instrument with copper Kα employed to assess The crystalline stages of the samples . Measurements were conducted at 2θ over the range of 10–90° by compressing the samples linking two sleek glass films. XRD study is performed under ambient conditions with a step size of 0.02°, a scanning rate of 2 s/step, and a dispersion of 2θ angles from 5° to 40°.

SEM : SEM images captured using an Electron-Scan equipped with an energy scattering gamma spectrometer . A low-voltage of 10 kV was applied to precautefrom detoration of cellulose fibers and dewaxed rice straw (DRS).

Fourier-Transform Infrared Spectroscopy (FTIR): Chemical and supramolecular structural analyses were performed using FTIR spectroscopy .

The drug , trichurate with KBr at a ratio of 1:100 w/w, was converted in pellet for transmittance mode analysis. Spectra is captured in the range of 5000 to 401 cm 1 at pixel of 4 cm 1 and a collection of 129 scans.

Zeta Potential Measurement: It determined using Anton Paar instruments . Drug is substandard with distilled water to achieve a conc of 6% v/v.

The test spanned a temp range between 9°C to 700°C. This comprehensive characterization provided a detailed understanding of the structural, morphological, and heat character of the isolated cellulose thread from paddy straw.

5-FU Loading Study:-

Preparation of Drug-Loaded Sample: A 252 mL solution is substandard in water having 5-FU & cellulose fibers (CF) in a proportion of 1:3 .

The solution was agitated in sealed bottles at 320 revolutions per minute for 12 hours to facilitate the loading of 5-FU onto CF.

Washing and Centrifugation: The medicated-filled sample underwent washing with deionized water to remove unentrapped drug molecules.

Centrifugation was performed using a lab Centrifuge with a 12 mL centrifuge tube at 3000 rpm for 10 minutes.

Analysis of Unentrapped Drug: The supernatant obtained after centrifugation was subjected to analysis using UV–visibl .

Drying and drug Collection: After centrifugation, the drug-loaded sample (CF/5-FU) was collected.

The drug is dry in oven at 46°C to remove any residual water content.

The loading capacity (LC)% and drug encapsulation efficiency (EE)% were calculated using Equations (1 and 2), respectively.

Equations: Loading Capacity (LC)% = [(Weight of loaded drug) / (Weight of CF/5-FU)] × 100

Encapsulation Efficiency (EE)% = [(Weight of loaded drug) / (Total weight of added drug)] × 100

This methodology ensured the effective loading of 5-fluorouracil (5-FU) onto cellulose fibers, and subsequent analysis allowed for the determination of loading capacity and encapsulation efficiency, providing crucial information on the efficiency of the drug-loading process.

Cell Strains and Chemicals :-

Cell Lines: Human colorectal cancer (CRC) cells and regular colorectal cells procured from (American Type Culture Collection) .

Cell Culture: The HCT116 and CCD112 cell lines were cultured following ATCC recommendations Cell Authentication: Cell validation was conducted through STR profiling.

The product indicated Both cell lines were confirmed to be genuine, demonstrating a 92.7o% and 99.99% similar as standard cell lines respectively.

Cell Maintenance: The cell group is properterated in DMEM instrumented with 9% FBS and 2% penicillin/streptomycin.

All cell group utilized for Experiments assessing cell toxicity within a clearance value of 11.

This comprehensive information outlines the cell lines used, their sources, culture conditions, and the authentication process, ensuring the reliability of the experimental results and maintaining the integrity of the cell lines in the study.

Results and Discussion

Reaction:

The transformation of rice straw (RS) into cellulose fibers (CF) was visually observed as the brown color of RS gradually diminished, resulting in the final acquisition of white CF. The process yielded a commendable 35% CF, aligning closely with findings reported in various studies. This visual change in color and the quantitative yield indicate the successful The extraction of cellulose fibers from paddy straw. The validity of The sequential procedure for isolating cellulose fibers (CF) further confirmed through gamma radiation Diffraction using XRD method and FTIR characterization.

XRD & FTIR Characterization: The confirmation of successful cellulose fiber isolation was substantiated through the application of XRD and FTIR techniques. These analytical methods are instrumental in providing structural and compositional insights into the obtained CF.

XRD Analysis: XRD examined & employed to assess the crystalline phases of the drug.

The results from XRD characterization supported the successful isolation of cellulose fibers, confirming the presence of distinct crystalline patterns characteristic of cellulose.

FTIR Analysis: FTIR spectroscopy was utilized to examine the chemical and supermolecular structural aspects of the samples.

The FTIR results further validated the stepwise process of cellulose fiber isolation, demonstrating characteristic peaks and patterns associated with cellulose.

The correlation between the visual changes, the quantitative yield, and the structural characterizations through XRD and FTIR collectively reinforce the efficacy of the cellulose thread extraction process from paddy straw. These results lay a solid foundation for subsequent investigations into the properties and applications of the obtained cellulose fibers.

Gamma rays diffraction study with the XRD apparatus

The XRD examination of rice straw (RS), dewaxed rice straw & cellulose fibers (CF) is presented in structure respectively. The XRD patterns for all samples exhibit striking similarity, indicating the actice ingredient applied did’n adversely affect the cellulose moiety . The discernible threshold point observed nearly $2\theta = 14.5°$, 16.6°, and 22.5° aligning typical primary cellulose shape , specifically corresponding to the 110, 200, and 004 planes.

These characteristic peaks are indicative of the preservation of cellulose crystals throughout the isolation process. Notably, the intensity of these peaks exhibits a gradual increase in successive treatments. This phenomenon can be attributed to the progressive removal of unusual ingredients like lignin and lipids, from paddy straw during the mixing , leading to the isolation of cellulose fibers with heightened purity.

The primary constituents residing in the changeable area of RS, namely lipids, hemi-cellulose, and lignin, undergo peeling off & hydrolysis with the basic treatment. Consequently, each treatment step contributes to the gradual removal of the changable area , with the crystal region always safe . This observed trend is consistent with findings in the literature and suggests that the treatments not only eliminate impurities but also potentially enhance the stability of cellulose fibers.

In summary, the XRD analysis affirms the structural integrity of the cellulose fibers obtained from RS, demonstrating a cellulose-I pattern and underscoring the effectiveness of the chemical treatments in purifying the material.

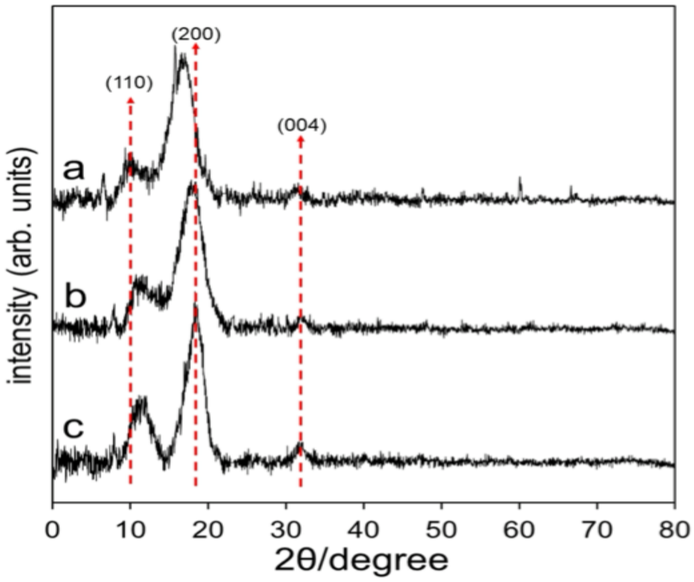

Figure X-ray powder diffraction of (A) RS, (B) DRS and (C) CF.

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier graph of rice straw (RS), dewaxed rice straw , cellulose fibers loaded with 5-fluorouracil (CF/5-FU), and pure 5-fluorouracil (5-FU) are depicted in the graph given below .

Isolation of CF from RS: The FTIR analysis demonstrates the successful isolation of cellulose fibers from RS following sequential treatments involving dewaxing, delignification, and the removal of silica and hemicellulose.

In the course of the bleaching procedure, subjecting to alkali treatment RS likely induced The generation of aromatic hydrogen groups involving carbon-hydrogen bonds (C-H) , as evidenced by the graph at 1523 cm^{-1} , merely associated with pyrane ring structure pf C–O–C bonds of cellulose.

The spectrum reveals the clearance of hemi-cellulose at 1751 cm^{-1} & the loss of silica (Si–O–Si stretching) at 761 & 490 cm^{-1}. Graph at 3351, 2892, and 1101 cm^{-1} Illustrate the stretching vibrations of –OH groups, C–H stretching, and the cellulose structure, resp ectively.

Within the different region (951–701 cm^{-1}), the small graph at 886 cm^{-1} for indicates the glycosidic –C1 –O –C4 degradation behaviour of the β-glycosidic bond in cellulose, affirming the successful isolation process.

FTIR Patterns for CF Before and After Delignification: Same as XRD findings, the FTIR steps remained same for cellulose before

& after lignin removal, suggesting high structural stability and minimal hamper to cellulose rings .

FTIR Spectra of CF & 5-FU and 5-FU: As mentioned in the graph below the FTIR spectra for CF/5-FU and 5-FU .

The CO elaborative group in the CF/5-FU spectrum shifted to 1625 cm^{-1}, indicating efficient conjugation of the anticancer drug into CF. Both CF&5-FU & regular 5-FU exhibited same FTIR reading, confirming successful drug loading into CF.

Showing charaters of medicine-loaded CF, including OH–elongation at 3067 cm^{-1} and methy bands C–H at 2828 and 2351 cm^{-1}, were observed in the CF/5-FUzspectrum.

The Graph of 5-FU displayed a graph range at 2751–3301 cm^{-1} with NH selongation moiety, which moved to 2751–3501 cm^{-1} in the CF/5-FU graph on superimposition of OH and NH in 5-FU.

Peaks corresponding to C–F elongation of 5-FU at 1271 cm^{-1} also appeared in the CF/5-FU spectrum at 1279 cm^{-1}, indicating compatibility between the drug and CF without any sign of incompatibility.

In summary, the FTIR analysis provides valuable insights into the successful isolation of cellulose fibers, preservation of cellulose structure during delignification, and efficient conjugation of 5-fluorouracil into cellulose fibers without any compatibility issues.

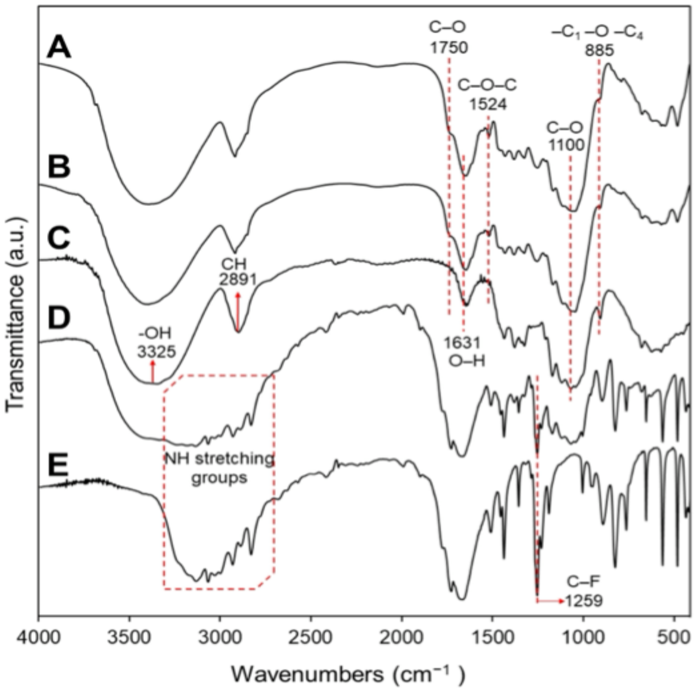

Figure: Fourier-transform infrared spectroscopy of (**A**) RS, (**B**) DRS (**C**), CF (**D**) CF&5-FU and (**E**) 5-FU.

Zeta Potential

The zeta potential of cellulose fibers (CF) & CF loaded with 5-fluorouracil (CF/5FU) is illustrated in the figure. The suspensions containing cellulose fibers (CF) and CF/5-F . Exhibited Significantly elevated negative zeta potentials were observed, measuring -33.61 mV for one and -30 mV for the other. These findings indicate that CF possesses excellent steady in water solutions, irrespective of the presence of 5-FU. The substantial negative zeta potentials imply strong electrostatic repulsion among the fibers, contributing to the colloidal suspension's stability.

The negative charge experienced a slight reduction upon the addition of the drug, indicating that The drug encapsulated the pores of cellulose fibers (CF) . This observation Indicates a successful mixing between the drug and cellulose fibers (CF). The H-bonded structure of CF is conducive to accommodating 5-FU. Pure 5-FU typically crystallizes in a single structure by 4 mole in the irregular assembly. The mole of 5-FU, with their hydrogen-bonded features, can interact with the coo- groups in the CF moiety, forming intermolecular complexes.

In summary, the CF network, characterized by a high negative zeta potential, efficiently conjugates with the anticancer drug 5-FU. This unique interaction holds promise for achieving excellent payload capacity and optimal retention during prolonged dosing showcasing the potential of CF/5-FU as a promising therapeutic formulation.

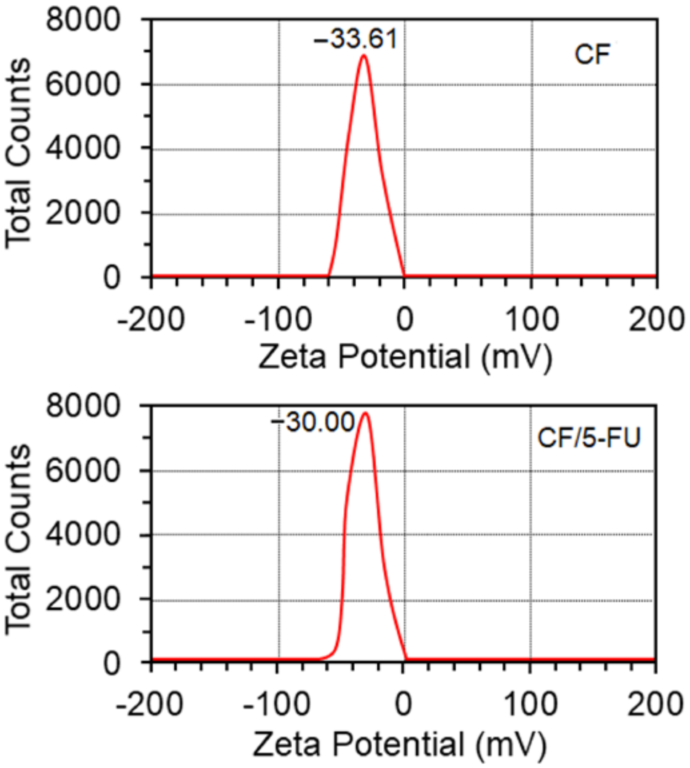

Figure Zeta potential of CF & CF/5-FU.

Inference

In this investigation simply & environmentally friendly basic pH process is employed to extract cellulose fibers (CF) in rice straw (RS) waste. The subsequent loading of the cancer treatment drug 5-fluorouracil (5-FU) onto the CF network was successfully achieved. The study systematically assessed the In vitro cytotoxicity assessment involved pure 5-FU, CF, and CF/5-FU samples at eight differentzconcentration. This evaluation spanned across colorectal and nasopharyngeal cell lines, encompassing both healthy and cancerous cells, and extended over a 72-hour treatment period .

X-ray powder diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) results demonstrated the efficacy of the lightning & lignin removal processes on RS, resulting in Cellulose fibers (CF) characterized by high purity and crystallinity. Comparative analyses revealed that CF exhibited a fourfold increase in coverage area & higher BJH combine pore capacity compared to RS. The FTIR graph for CF/5-FU nearly shows that of 5-FU, indicating potential exchange and conjugation between the drug and its carrier.

The zeta potentials of CF & CF/5-FU samples were found to be -33.62 mV & -30 mV , respectively, reflecting their excellent stability in aqueous solutions. Thermogravimetric analysis (TGA) readings indicated that the cellulose fiber (CF) network is markedly enhanced the heat stability of 5-FU. UV shows estimated the drug loading onto the CF carrier system, revealing an encapsulation efficiency of 82 ± 0.9% and a loading capacity (LC) of 23 ± 3.2%. Moreover, drug release studies conducted in media with changed pH readings within a day shows a highest 5-FU secretion of 78% and

45% for secretion for pH values of 7.4 and 1.2, respectively.

In conclusion, this research underscores the tendency of utilizing the CF cluster as a carrier for the cancer treatment drug 5-FU. The findings suggest that this approach is effective in colorectal cancer (CRC) curement compared to nasopharyngeal cancer (NPC), offering a greener and environmentally safe alternative to conventional chemotherapy with the potential to reduce side effects.

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