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Chapter 1 : HER2 Targeting Peptides Screening and Applications in Tumor Imaging and Drug Delivery

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Curcumin is a promising anti-cancer drug, but its applications in cancer therapy are limited, due to its poor solubility, short half-life and low bioavailability. Alginate and chitosan were deposited on Fe3O4 magnetic nanoparticles based on their electrostatic properties. The nanoparticle size ranged from â€” nm, within the optimum range for drug delivery. Controllable and sustained release of curcumin was obtained by altering the number of chitosan and alginate layers on the nanoparticles. Confocal fluorescence microscopy results showed that targeted delivery of curcumin with the aid of a magnetic field was achieved. The fluorescence-activated cell sorting FACS assay indicated that MDA-MB cells treated with curcumin loaded nanoparticles had a 3â€”6 fold uptake efficiency to those treated with free curcumin. The sustained release profiles, enhanced uptake efficiency and cytotoxicity to cancer cells, as well as directed targeting make MACPs promising candidates for cancer therapy. Introduction Curcumin CUR is a yellow, hydrophobic, polyphenolic compound of turmeric that is extracted from the rhizomes of Curcuma longa, which are widely cultivated in Asian countries, such as India and China, and have been historically used as a spice [1]. CUR is generally recognised as safe GRAS by the Food and Drug Administration FDA [1], and has been widely used in medicine due to its anti-oxidant [2 , 3 , 4], anti-inflammatory [5 , 6 , 7], wound-healing [8 , 9] and anti-bacterial [10 , 11] properties. Recent research has demonstrated that CUR has the ability to inhibit carcinogenesis in various cell lines, including breast, colon and gastric cancer cells, which has resulted in an increased interest as a promising anticancer drug [12 , 13 , 14 , 15]. However, CUR exhibits poor solubility in aqueous solutions, limiting its applications for cancer therapy [16 , 17 , 18]. As a result, the bioavailability and anti-cancer efficiency of CUR is limited by its low solubility [16 , 17 , 18]. In order to improve the bioavailability, various nanocarriers have been used, including lipid-based nanoparticles [20 , 21 , 22 , 23 , 24], polymer nanoparticles [17 , 25 , 26 , 27 , 28 , 29 , 30] and inorganic nanoparticles [31]. The main advantages of the CUR loaded nanocarriers are their small size and large surface area, which enable them to pass through the cell membranes with an enhanced uptake efficiency [1 , 29]. Research has increasingly focused on the fabrication of biopolymer nanoparticles for CUR delivery, due to advantages of low cytotoxicity, excellent biocompatibility and biodegradability [29]. Two of the most commonly used biopolymers in medical applications are alginate and chitosan CHI. CHI based nanoparticles can be prepared by via polyanion of tripolyphosphate TPP without introducing harsh cross-linking agents or organic solvents [35]. Electrostatic interactions between positive CHI chains and negative drugs, such as CUR, enable the retention of the drug in CHI based nanoparticles providing a prolonged drug release profile [36]. These advantages of alginate and CHI make them promising candidates as nanocarriers for drug delivery [37]. Targeted delivery by magnetic nanoparticles MNPs has been reported as a promising strategy for cancer therapy with the advantages ranging from visualisation of the targeting process, rapid targeting and accumulation of drug carriers at the tumour sites via magnetic forces. The MNPs can be heated in a magnetic field to promote the drug release [42]. MNPs have been reported to have very low toxicity within the human body [43]. Their small size and large surface area make them suitable for polyelectrolyte layer-by-layer deposition. The incorporation of MNPs has shown the targeted delivery of drugs to tumour sites with the help of external magnetic fields [42]. For example, Mancarella et al. In another example Pavlov et al. MNPs were incorporated into the particles and the resulting MNPs improved the delivery of enzymes and plasmids into T cells. In addition, MNPs could be efficiently navigated to cells with a magnet below the targeted tissue culture wells [45]. By altering the number of layers deposited, it is possible to encapsulate a high payload of drugs and control the drug release rate [47 , 48 , 49 , 50]. Materials and Methods 2. Preparation of Nanoparticles 2. The solutions were then mixed in a mL round-bottom flask and 15 mL of ammonium hydroxide was added

under vigorous stirring under a nitrogen atmosphere at room temperature. The solution was then vigorously stirred for 2 h and the synthesised MNPs were collected with strong Neodymium magnets and washed several times with DI water until neutral pH. Finally, the magnetic nanoparticles were dried over night at room temperature and stored for future usage. The resulting suspension was vigorously stirred for 30 min at room temperature. Subsequently, mL of Ca OH 2 solution 0. The suspension was then stirred for a further 12 h at room temperature and the resulting MAPs were collected with strong Neodymium magnets, washing thoroughly with ethanol and water to remove any excess salts. Finally, purified MAPs were re-suspended in DI water before being used for the layer-by-layer coating process. The first layer was deposited by adding 1 g of MAPs into mL of the chitosan solution under vigorous stirring for 20 min at room temperature. For each layer the previously described purification process was used. Particles with more layers were fabricated by alternatively coating positively charged CHI and negatively charged SA on MACPs until the desired number of layers was reached.

Chapter 2 : Folic acid targeted Mn:ZnS quantum dots for theranostic applications o | IJN

When fluorescent and thermo-responsive CNCs were used as the carriers for drug delivery, the fluorescence made it possible to observe the distribution of the drug-loaded CNCs and monitor the drug.

How to cite this article: Theranostics ; 6 8: Particularly one of the peptides, P51, has nanomolar affinity and high specificity for HER2 in ex vivo and in vivo tests. Moreover, doxorubicin DOX -loaded liposome nanoparticles were modified with peptide P51 or P25 and demonstrated to improve the targeted delivery against HER2 positive cells. Our study provides an efficient peptide screening method with a combination of techniques and the novel screened peptides with a clear binding site on HER2 can be used as probes for tumor imaging and targeted drug delivery. HER2 targeting peptide, tumor imaging, drug delivery, breast cancer, MD simulation. HER2 is a unique receptor molecule due to its lacking of a ligand but functions as a co-receptor to form homodimers and heterodimers with the other three HER 1, 3 and 4 family proteins [1 , 2]. Therefore, HER2 has become an important validated therapeutic target in breast cancer. Computational aided virtual peptide library screening can provide peptides with a clear structure information of binding site and mechanism [18], but the efficiency of screening is limited when compared to high throughput screening. OBOC library screening [19 - 21] is efficient and has been applied in discovery of novel peptides targeting at cancer and other diseases. In addition, OBOC library combining in situ mass spectrometry MS sequencing and identification by using a microfluidic chip [22 - 24] with magnetic trapping and sheath flow sorting functions have been successfully employed in sorting a large number of peptide beads library in our previous works [25]. However, the screening efficiency of this method can be further improved by applying computational simulation in designing the library to limit mutations of residues in peptide for favorite interactions rather than aimless random mutations. In this study, computational simulation was performed to design the OBOC peptide library for screening, Combined with in situ mass spectrometry MS sequencing using a microfluidic chip, 72 positive peptides were obtained as candidates for targeting HER2 protein. The nude mice imaging further verified that two novel peptides P51 and P25 have strong affinity and high specificity for HER2 in ex vivo and in vivo test. The molecular dynamic MD simulation leaded OBOC peptide library design is successfully applied in screening for novel targeting peptides efficiently. And the results of simulation also indicate that the binding sites of the peptides remain the same as their starting model. These peptides can be used as novel probes in HER2 positive breast cancer imaging and targeted drug delivery, as demonstrated in our study of the improved cytotoxicity of peptide modified liposome nanoparticles loaded with DOX. Materials and Methods Peptide screening strategy The whole design strategy of this study is shown in Figure 1. There is currently no reports of the crystal structures of human epidermal growth factor receptor-2 HER2 homodimer or heterodimers with three other HER 1, 3 and 4 family members, therefore the crystal structure of homodimer of HER1 extracellular domains PDB entry: Molecular dynamics MD simulations followed by binding free energy calculation and decomposition provide the key residues in the four members of HER family proteins forming homodimers or heterodimers with HER2. An initial screening using experimental analysis in vitro showed that WP1 peptide has the highest affinity among eight peptides. It was chosen for the starting sequence for peptide library design. Virtual single mutation for OBOC peptide library was constructed based on preferred amino acids for each residue in WP1 and the properties of their interacting residues of HER2 receptor. The positive peptide beads were selected and sequenced using in situ microarray technique. Finally the candidate peptides were synthesized and validated for their affinity and specificity of targeting HER2 by in vitro and in vivo assays. Figure 1 Overview of the procedures for screening HER2-targeting peptides using a strategy combining computational modeling, OBOC in situ single-bead screening and sequencing. Click on the image to enlarge. The single mutants of WP1 and HER2 complexes were acquired by mutating the amino acids based on ligand sequences. Briefly, positive peptides were trapped by magnetic field on a microchip. Positive peptide beads will be surrounded by the magnetic beads through the interaction

bridge of peptide-HER2-biotin-streptavidin, while native beads will remain naked and cannot be trapped. Supplementary Materials and Methods of Supporting Information. Then cells were incubated with FITC-labeled peptides on ice for 20 minutes. The non-bound peptides were rinsed away with PBS for three times. The whole process was performed on ice and with minimal light exposure. A Beckman Quanta SC flow cytometry was used for the test. Finally, the cells were washed three times with cold PBS. Finally, the cells were washed with cold PBS three times. An Olympus FVIX81 confocal-laser scanning microscope was used for confocal fluorescence imaging. Hoechst was excited by a FV5- LD nm laser and collected within the range of nm. All parameters of the microscope were set to be the same for different samples to allow comparisons of the binding ability of different peptides. For detection, all peptides were linked a cysteine Cys in the amino terminal for interacting with bare gold, since the thiol side chain of Cys can form Au-S bond with Au [33]. After the chip was washed with PBS and water, it was dried with nitrogen for use. The SPRi analysis procedure follows the following cycle of injections: After one cycle was finished for a sample, the next sample will start on the same chip until all were completed. The dissociation constant was calculated by fitting the association-dissociation curves. Tumor size was measured periodically using calipers, and the tumors were allowed to grow to mm in diameter. The conjugation of peptides and Cy5. Chemical reaction happens with Cy5. The crude product was purified by semi-preparative reverse phase HPLC. Near-infrared fluorescence NIRF images of nude mice bearing subcutaneous tumors were acquired after vein injection of Cy5. Half an hour after the injection, the mice were anesthetized and placed into the imaging system. For each peptide and control, at least three mice were used. Near-infrared fluorescence NIRF imaging of nude mice bearing subcutaneous tumors were taken with an exposure time of 50 ms using the Cy5. Then the nude mice were sacrificed and tumors as well as the main organs were harvested and the NIRF images were taken individually. After 72 h incubation, the reaction mixture was put into a dialysis bag with cut off molecular weight MW of Da and dialyzed against deionized water for 48 h to remove free ligands. Briefly, lipids were dissolved in chloroform and dried until thin lipid film formed on a rotary evaporator. The dried lipid film was hydrated with PBS and sonicated with a bath type sonicator [34]. DOX was remote-loaded via the ammonium sulfate gradient method [35 , 36]. After replacing with fresh culture medium, cells continue to grow for 24 hours. In order to find the key residues for the dimer interaction, MD simulation was carried out. The results show that the predicted binding free energy between two monomers in HER1 complex is The following energy decomposition analyses based on MD simulation results illustrate that residues GlnLys in HER1 complex are the main residues for the binding between monomers Figures 2 B and 2C. By examining the structure, the mer fragment from Met to Asn of HER1 forms a hairpin loop, which was selected as the template in the following study. These key residues and their corresponding ones in HER2, HER3 and HER4 should have similar type of interactions in the formation of homo- or heterodimers because of structural and sequence similarity Figure 3 A. As a preliminary screening to select the best peptide from these eight ones for the following virtual mutation and screening, confocal fluorescence imaging was first carried out to estimate which peptide binds best to HER2 protein. The fluorescence intensity shows that PS1 and WP series peptides bind better than others. Figures 3 G and 3H show WP1 has the most fluorescence intensity among all peptides. The binding free energy calculation shown in Table 1 indicates that WP1 has the lowest binding energy with HER2, which is consistent with the above confocal fluorescence imaging and flow cytometry results. The binding free energy decomposition results Figure S6 suggest that the last three residues in the mer peptides PS1-PS4 are unfavorable for HER2 binding and residues 2, 9 and 14 in the mer peptides of WP1-WP4 contribute most, while residues 6 and 10 show less contribution Figure S7.

Chapter 3 : OSA | In vivo mouse fluorescence imaging for folate-targeted delivery and release kinetics

A novel drug delivery system based on arginine-glycine-aspartic acid (RGD)-conjugated graphene quantum dots (GQDs) was synthesized and utilized to load the antitumor drug doxorubicin (DOX) for targeted cancer fluorescence imaging as well as tracking and monitoring drug delivery without the need for external dyes.

Received Aug 25; Accepted Apr 4. This article has been cited by other articles in PMC. Abstract Quantum dots QDs , nano-carriers for drugs, can help realize the targeting of drugs, and improve the bioavailability of drugs in biological fields. And, a QD nano-carrier system for drugs has the potential to realize early detection, monitoring, and localized treatments of specific disease sites. In addition, QD nano-carrier systems for drugs can improve stability of drugs, lengthen circulation time in vivo, enhance targeted absorption, and improve the distribution and metabolism process of drugs in organization. So, the development of QD nano-carriers for drugs has become a hotspot in the fields of nano-drug research in recent years. In this paper, we review the advantages and applications of the QD nano-carriers for drugs in biological fields. Quantum dots, Nano-medicine, Targeted drug delivery, Biomaterial Review Nowadays, many anti-tumor drugs in clinical tests have various side effects, such as non-selective, toxicity, poor targeting, etc. So, the heavy side effects of normal tissues produced by a conventional therapeutic dose of anti-tumor drugs would reduce drug efficacy [2]. Severe side effects arise from the use of classical cancer therapeutics due to non-specificity of the cancer drugs, resulting in high toxicity in noncancerous but rapidly dividing cells [3]. Due to the lack of any bioluminescence, tumors can be difficult to be located and removed, particularly on smaller size scales. Therefore, it is highly desirable to create a means by which cancer cells could be targeted with high specificity and be simultaneously imaged in vivo [4]. Nano-carriers for drugs mainly have nano-vehicles including liposomes, etc. QDs possess unique optical properties that make them potential candidates as luminescent nano-probes and carriers for biological applications. Drugs can be loaded into QD nano-carriers for drugs by the means of dissolving, dispersing, adsorption and coupling, etc. Then, the physical and chemical properties such as saturation solubility, dissolution rate, crystal form, particle surface hydrophobicity, and hydrophilicity , physical response, and biological characteristics of drugs are changed due to the role of the carriers; thus, the absorption, distribution, metabolism, and excretion of drugs are affected [8]. Ultimately, QD nano-carriers for drugs can enhance the efficacy and reduce side effects of drug reactions to improve the therapeutic index of the drugs [9]. Moreover, nano-carriers for drugs can effectively promote the absorption of small molecule drugs. At the same time, the research about macromolecular drug delivery has also demonstrated good prospects [10]. As a new kind of inorganic nano-fluorescent probe, QDs have showed outstanding advantages in the long-time, multi-color fluorescence imaging and detection [11 , 12]. The development of QD labeling promotes the research in the nano-drugs in cellular, even at live animal level. The development of fluorescence imaging technology of QDs and the therapy-based multifunctional nano-drugs is expected to apply to diagnosis and treatment of cancers [13 , 14]. At the same time, surface modifications with targeting ligands are also commonly used to increase drug-delivery efficiency [15]. After almost 10 years of development, the technology of QDs surface modifications has realized relative improvements. The surface ligands can be thioglycolic acid, cysteamine or polyethylene glycol PEG , and water-soluble polymers with carboxyl, which can bind the drug molecules with QDs through electrostatic binding or covalent bond and form nano-drug complexes carriers, and then realize fluorescent trace of drug molecules in cells or animals [16]. Advantages of QDs as Nano-Carriers in Targeted Drug Delivery Compared with conventional drug carriers, nano-carriers for drugs have many advantages, such as smaller size, larger specific surface area, higher and more reactivity activity center, stronger adsorption capacity and other characteristics [17 , 18]. At the beginning, the mode of controlling and releasing drugs of nano-particle carriers is generally an outbreak release, and then it shows a constant release for a long period of time. Therefore, nano-carriers for drugs can significantly extend the effectivity of drugs at limited concentration, deliver at fewer intervals, and lower

doses and reduce side effects and the suffering of patients. At present, the commonly used anticancer nano-carriers for drugs are mainly liposome, chitosan, silica nanoparticles, and polymer nanoparticles. QDs have unique optical properties, due to their quantum effect and size effect. When the particle size is of nanometer scale, it will cause quantum confinement effect, size effect, dielectric confinement effect, macroscopic quantum tunneling effect, and surface effect. Consequently, QDs exhibit many optical properties, and have a very broad application prospect in biological fluorescent probes and functional materials. Therefore, QDs will have a meaningful effect on the continued development of life sciences. To our knowledge, an important aspect of developing modified QDs in biomedical applications is their selective targeting. Generally, in recent years, tumor cell targeting in both therapeutic and diagnostic applications have only focused on a small number of candidate ligands whose receptors are over-expressed in tumor cells, such as folic acid and delivery of siRNA. One such receptor is folic acid, which has widely been used as a targeting ligand to deliver therapeutic agents to cancer cells due to its high binding affinity with folate receptor FR. Folic Acid-Quantum Dot Complexes Folate folic acid, FA , which is a donor number of carbon unit in vivo, is a necessary material to biosynthesize nucleic acids, amino and pantothenic acid, and its structure is shown in Fig. FA is a needed vitamin for everyone, and it not only can participate in a variety of metabolic pathways of one-carbon transfer reactions, but also is the targeting ligand of FR. With the development of molecular biology and molecular medicine and in-depth study of tumor molecular level, researchers have found a series of receptors associated with tumor growth on the surface of tumor cells or tumor-associated blood vessels, and found the receptors high affinity to combine with its ligands. Therefore, ligands as targeting molecules of drug carriers can enhance therapeutic efficacy via a receptor-mediating mechanism, and then to achieve targeted therapy. FR is highly expressed in most tumor cells such as ovarian cancer, cervical cancer, endometrial cancer, breast cancer, colon cancer, lung cancer, nasopharyngeal carcinoma choroid, and ependymal cell tumor cells , so as a low molecular weight targeting molecule, FA quickly becomes a hot topic of research [20]. Studies have shown that FA complexes modified by carboxyl deuterogenic remained a strong binding capacity with FR [21]. Typically, protein toxins, small molecule chemotherapeutic agents, radio-therapeutic agents, polymer-wrapped drugs, gene carriers, pro-drug inhibitors, and immunotherapeutic agents can form multimeric complexes bound with FA by covalent coupling. These FA complexes have better targeting ability and more therapeutic effect than the original drug, so the FA complexes have higher potential for drug delivery system [22 , 23].

Chapter 4 : Wood-Based Nanoparticles for Targeted Drug Delivery - VIRGINIA POLYTECHNIC INSTITUTE

Advantages of QDs as Nano-Carriers in Targeted Drug Delivery. Compared with conventional drug carriers, nano-carriers for drugs have many advantages, such as smaller size, larger specific surface area, higher and more reactivity activity center, stronger adsorption capacity and other characteristics [17, 18].

Objective 2 is to test the hypothesis that cellulose nanocrystals are internalized by mammalian cells. Objective 3 is to synthesize targeted drugs with cellulose nanocrystals as drug carrier that release the drug inside the cell. Project Methods Objective 1: Cellulose nanocrystals will be prepared by sulfuric acid hydrolysis of pharmaceutical-grade softwood sulfite pulp. The nanocrystals will be characterized according to surface charge density, size, and size distribution. Surface charge density will be measured by conductometric titration. Particle size and size distribution will be determined by atomic force microscopy. The nanocrystals will be fluorescently labeled with fluorescein isothiocyanate through e. The capacity of fluorescently labeled cellulose nanocrystals to enter cells will be studied by fluorescence microscopy. Fluorescently labeled nanocrystals will be added to mammalian cell cultures. Incubation of the cells will be continued for different time periods. Internalization of the fluorescently labeled nanocrystals by the cells will be analyzed and photographed with a fluorescence microscope. Cytotoxic agents, such as, for example, butyric acid, paclitaxel taxol , doxorubicin, or mitomycin C, will be covalently linked to the surface of the cellulose nanocrystals through a reaction with the surface hydroxyl groups or with carboxyl groups formed by oxidation of the surface hydroxyl groups. Depending on the chemical link between the drug molecule and the carrier particle, release of the drug by the carrier is triggered by either a lower than normal pH, a higher than normal temperature, or certain mediating molecules inside the cell. Drug release will be studied in vitro under physiological pH, temperature, and chemical environment. To differentiate between attached and released drug molecules, the suspension of the drug conjugate will be placed inside a semi-permeable membrane. In a separate experiment, the rate of diffusion of the drug through the membrane will be determined. Each measurement will be carried out multiple times to determine the standard deviation of the measured drug release rates. The cytotoxicity of the nanocrystal-drug conjugates will be determined by metabolic activity assays in microplate format. Cells will be incubated with and without the drug conjugates present. After a certain incubation time, a colorimetric substance, indicating metabolic activity, will be added. Cell survival rate is measured through a change in absorbance or fluorescence of the colorimetric substance as a consequence of enzymatic reduction by metabolically active cells. Cell culture and assay equipment is available in the College of Agriculture and Life Sciences. Several microplates will be analyzed for each set of parameters to ensure reliability and reproducibility of the data. The project fostered an interdisciplinary collaboration between the PI and Prof. Methods were developed for 1 the synthesis of cellulose nanocrystals with optimal properties for applications in targeted drug delivery; 2 covalent attachment of fluorescein isothiocyanate molecules to the surface of cellulose nanocrystals for fluorescent labeling; 3 covalent attachment of folic acid molecules to the surface of fluorescently labeled cellulose nanocrystals for cancer targeting; and 4 covalent attachment of doxorubicin molecules to the surface of folic acid-conjugated cellulose nanocrystals for cancer therapy. Experiments were conducted to assess 1 the toxicity of cellulose nanocrystals to a variety of human, mouse, and rat cell lines; 2 the cellular uptake of fluorescently labeled cellulose nanocrystals; 3 the cellular uptake of folic acid-conjugated, fluorescently labeled cellulose nanocrystals; and 4 the in-vitro efficacy of the targeted drug nanoconjugates on KB cells as a cancer model. Additional activities include mentoring of three undergraduate research projects and a visit to the Institute of Lung Biology and Disease at the Helmholtz Zentrum Munchen in Munich, Germany for the discussion of potential opportunities for collaboration. The findings of the studies were disseminated through a book chapter, a journal publication, and oral and poster presentations at local and national professional meetings. In addition, the research concept was presented to Virginia Tech Alumni in an oral presentation during an event of the Alumni Association.

Collaborator Yong Woo Lee; Maren Roman Project Director was responsible for the supervision of the laboratory work, carried out by a graduate and three undergraduate students, coordination of the interdisciplinary collaboration, and all reporting functions. The drug delivery technology that is being developed in this project will benefit cancer patients, by providing a more effective treatment with fewer side effects, and the Forest Products Industry, by enhancing its product range and potentially opening up new markets. No significant modifications to report during this reporting period. Impacts The Forest Products Industry faces increased challenges that threaten its viability and global competitiveness. The goal of this project was to assess the potential of cellulose nanocrystals, wood-based cellulose nanoparticles, in targeted drug delivery applications to enhance the product range of the Forest Products Industry and possibly open up new markets. The cytotoxicity studies have shown that cellulose nanocrystals have no cytotoxic effects. The demonstrated lack of cytotoxicity is a necessary prerequisite for the use of cellulose nanocrystals in targeted drug delivery applications. The cellular uptake studies have demonstrated that targeting of cellulose nanocrystals through folic acid conjugation leads to uptake of cellulose nanocrystals by cancer cells and that non-specific cellular uptake of cellulose nanocrystals is minimal. These results confirm that cellulose nanocrystals can be targeted for selective uptake by specific cells. The efficacy studies with doxorubicin-conjugated, folate receptor-targeted cellulose nanocrystals have shown that the targeted drug nanoconjugates are more effective in eradicating cancer cells than free doxorubicin. The studies have also shown that doxorubicin-conjugated cellulose nanocrystals that were not targeted to the folate receptor are less toxic to cancer cells than free doxorubicin. The lower toxicity of doxorubicin-conjugated cellulose nanocrystals, compared to that of free doxorubicin, indicates that targeting of the drug nanoconjugates to the folate receptor is crucial for its high efficacy. The findings of this project confirm that cellulose nanocrystals are promising nanoparticles for targeted drug delivery applications. Synthesis of FITC-labeled, folate-targeted cellulose nanocrystals. Electronic conference proceedings abstract. The activity enabled continuation of the previously established interdisciplinary collaboration with Dr. Yong Woo Lee from the Department of Biomedical Sciences and Pathobiology, mentoring of an undergraduate research project, and a visit to the Institute of Lung Biology and Disease at the Helmholtz Zentrum Munchen in Munich, Germany, for the discussion of potential opportunities for collaboration. Moreover, a chemical method was developed for the covalent linking of doxorubicin molecules, a cancer drug, to the surface of folic acid-conjugated cellulose nanocrystals and experiments were conducted that assessed the in-vitro efficacy of the targeted drug nanoconjugates on KB cells as a cancer model. The results were disseminated through an oral presentation at a graduate student research symposium at Wake Forest University, two poster presentations at local meetings with industry representatives at Virginia Tech, and a poster presentation at a national meeting of the American Chemical Society for a list of publicly available abstracts see Publications. Maren Roman Project Director was responsible for the supervision of the laboratory work, carried out by a graduate and one undergraduate student, coordination of the interdisciplinary collaboration, and all reporting functions. If successful, the drug delivery technology that is being developed in this project will benefit society in general. Impacts The efficacy studies have shown that doxorubicin-conjugated, folate receptor-targeted cellulose nanocrystals are more effective in eradicating KB cells than free doxorubicin. The studies have also shown that doxorubicin-conjugated cellulose nanocrystals that were not targeted to the folate receptor are less toxic to KB cells than free doxorubicin. These new findings further support our hypothesis that cellulose nanocrystals are promising nanoparticles for targeted drug delivery applications. The activity fostered an interdisciplinary collaboration with Dr. Yong Woo Lee from the Department of Biomedical Sciences and Pathobiology and enabled mentoring of an undergraduate research project. Experiments were conducted that assessed the uptake of folic acid-conjugated, fluorescently-labeled cellulose nanocrystals, developed during the preceding reporting period, by different types of mammalian cells from human, mouse, and rat tissues. Furthermore, different pathways for the synthesis of folic acid-conjugated, fluorescently-labeled cellulose nanocrystals were evaluated. The results were disseminated through a book chapter as well as a poster presentation at a local

professional meeting see Publications. Impacts The uptake studies have shown that folic acid-conjugated, fluorescently-labeled cellulose nanocrystals are taken up by mammalian cells. In combination with the findings of the previous reporting period, that non-targeted, fluorescently-labeled cellulose nanocrystals show little or no uptake by cells, the findings of the current reporting period support the hypothesis that cellulose nanocrystals are promising nanoparticles for targeted drug delivery applications. The findings confirm that, by folic acid-conjugation, cellulose nanocrystals can be selectively targeted to cancer cells. Cellulose Nanocrystals for Drug Delivery. Chapter 12 In K. ACS Symposium Series Washington, DC in press. During this reporting period, the toxicity of cellulose nanocrystals to a variety of cells from different human, mouse, and rat tissues was studied in collaboration with Dr. The internalization of the fluorescently-labeled cellulose nanocrystals, developed during the preceding reporting period, by the same cell lines was also investigated. Furthermore, a chemical method was developed for attaching folic acid molecules to the surface of the fluorescently-labeled cellulose nanocrystals. The folic acid molecules are intended to interact with certain receptors on the surface of cells and facilitate internalization of the nanocrystals by the cells. The results were disseminated through a journal publication as well as an oral presentation at a national meeting of the American Chemical Society ACS and several local poster presentations see Publications. Nothing significant to report during this reporting period. Impacts The toxicity studies have shown that cellulose nanocrystals are not toxic to animal cells, which is an important requirement for drug delivery applications. The cellular uptake studies of fluorescently-labeled cellulose nanocrystals revealed little to no uptake by the cells. This result is encouraging because it indicates that the nanoscale drug carriers will not be internalized by healthy cells but that internalization has to be triggered through one of the available mechanisms and can thus be targeted to diseased cells. Journal of the American Chemical Society, Novel Applications of Cellulose Nanocrystals: From Drug Delivery to Micro-Optics. Cellulose nanocrystals for targeted drug delivery applications. During this reporting period, a systematic study was conducted of the synthesis parameters that affect the properties of cellulose nanocrystals. The synthesis of cellulose nanocrystals was optimized for application in targeted drug delivery. In addition, a chemical method was developed for covalently attaching molecules of fluorescein isothiocyanate, one of the most widely used fluorescent labels, to the surface of cellulose nanocrystals. For future studies involving cell cultures, a collaboration was established with Dr. The results were disseminated through poster presentations to members of the Chemical Industry as well as the Forest Products Industry at national meetings of professional societies see Publications. Furthermore, the research concept was presented to Virginia Tech Alumni in an oral presentation during the Summer Around the Drillfield event organized by the Alumni Association. The topic of the event was "Global Health Issues Today: Laying the Foundation for a Better Tomorrow. Maren Roman Project Director was responsible for supervision of the laboratory work, carried out by a graduate student, and all reporting functions. The audience of the Alumni Association event, during which the research was presented, was a group of alumni and their families, ranging in age from teenagers to retirees. Impacts The systematic study of experimental parameters governing cellulose nanocrystal properties revealed that the reaction conditions producing maximum yield are different from those producing maximum particle surface charge. Thus, different cellulose nanocrystal applications will require different reaction conditions during synthesis. The method for fluorescent labeling of cellulose nanocrystals, developed as part of Objective 1, enables the use of fluorescence techniques, such as spectrofluorometry, fluorescence microscopy, and flow cytometry, to study, the interaction of cellulose nanocrystals with cells and the biodistribution of cellulose nanocrystals in vivo. Cellulose nanocrystals as targeted drug delivery systems.