MS-Project Report

Title: Cell labeling by Colloidal Janus Nanoparticles

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Declaration

I hereby declare that the works reported in the project is based on scientific observations and findings and is not plagiarised from any sources and neither any data have been fabricated, manipulated or misrepresented.

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Ilora Maiti

Abstract

Colloidal Janus nanoparticles have significant biomedical application potential but their interaction with cells is relatively less studied. Here, we have synthesized 60 nm porous silica particles where one side of each particle is terminated with decylamine and other side is terminated with dextran. We expect our Janus nanoparticles to show faster cellular membrane attachment in comparison with the isotropically functionalized decylamine/dextran. Also, we are trying to develop a urease powered Janus nanomotor which is expected to help in crossing blood brain barrier with proper surface modification.

Introduction

Janus nanoparticles possess two dissimilar compartments/surfaces in a single particle.[1-3] Colloidal form of Janus nanoparticles has unique properties that are suitable for wide variety of applications. In particular, the nanoscale asymmetry in a single particle and different physicochemical properties in its different parts offer surfactant like property, interesting assembly behavior and directional interaction/dynamics.[1-3] As a result, they have potential applications as micro/nanomotor,[4-8] emulsion stabilizer,[2] delivery carrier with specific advantages [8,9] and multifunctional theragnostic agents with enhanced performance.[1-3] For example, colloidal Janus nanoparticles have been used with enhanced performances in amyloid fibrillogenesis,[4] sonodynamic therapy,[5] bacterial biofilm eradication,[7] treatment of multidrug-resistant bacterial infections,[8] drug delivery at the tumor site[9] and enhanced brain delivery.[10]

Despite their unique advantages there are several shortcomings for biomedical applications. First, synthesis of colloidal Janus nanoparticles of < 50 nm is challenging and most of reported methods produce Janus structures of > 100 nm size.[11-14] However, cellular and subcellular targeting requires particle size in the range of 5-50 nm.[15] Second, characterization of Janus structures at < 50 nm regime that occurs due to anisotropic surface chemistry and anisotropic functionalization, is challenging due to requirements of sophisticated chemical characterization at such length scale. Third, cellular interaction with Janus nanostructures is not well understood and recent studies indicate that they can lead to interesting phenomena.[16-19] In particular, it has been shown that amphiphilic Janus nanoparticles can lead to cell membrane disruption,[16,18]anisotropically functionalized Janus nanoparticle offers extended cell surface attachment,[19] and amphiphilic-

polycationic Janus nanoparticle offers enhanced antibacterial activity.[19] These results suggest that Janus nanoparticles with anisotropic functionalization have wide range of application potentials that are yet to be explored.

We design colloidal functional nanoparticle of 50 nm hydrodynamic size for cellular and sub cellular targeting and therapy.[20] We have shown that nanoparticle size, nature of surface functional groups and number of functional group per nanoparticle have significant impact on cell-nanoparticle interaction, cell uptake mechanism and subcellular trafficking.[20] In this work, we have shown that nanoparticle terminated with decylamine groups has unique interaction property with cell membrane that can be utilized for non-endocytic cell delivery via temporary membrane pore formation.[21] Here, we have also synthesized 60 nm porous silica Janus structure via urease termination in one side and dextran termination in the other side which will act as a nanomotor. We have selected relatively a larger size as the synthesis strategy and characterization of < 50 nm Janus structure are not well developed. We show that these Janus nanoparticles have enhanced cellular interaction/uptake over the symmetric particles.

Objectives

As previously reported, arginine conjugated Janus particles [21] showed enhanced cellular uptake in CHO cells in comparison with the isotropic control particles, we are expecting that hydrophobic decylamine conjugated Janus nanoparticles will have some unique bioactivity than the previous Janus nanoparticles. We planned to synthesize 60 nm Janus nanoparticles which is conjugated with dextran in one hemisphere and decylamine on the other.

The second ongoing work entails the synthesis of Urease powered Janus nanomotors to move in a particular direction for better cellular uptake and drug delivery. Nanomotors work in the same principle as any other motor, the urease acts as a fuel to convert urea into ammonia and carbon dioxide gases to help in the directional movement in biological fluids. Taking this application as a motivation we have designed a Janus nanomotors for effective biomedical application.

Experimental Section

Materials: Tetraethylorthosilicate (TEOS), [3-(2-aminoethylamino)propyl]trimethoxysilane (AEAPS), tetramethylammonium hydroxide (25 wt %) (TMAH), octadecylamine, methyl

morpholin N-oxide, octadecene, glutaraldehyde solution, decylamine, dextran (MW-6000), fluorescamine, urease from Canavalia ensiforms, sodium dodecyl sulfate (SDS), sodium cyano-borohydride ($NaCNBH_3$) and Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma-Aldrich. Cetyltrimethylammonium bromide (CTAB) and N,N,N´,N´-tetramethylethylenediamine were purchased from Alfa Aesar. Ammonia (25 wt %) was purchased from Merck.

Instrumentation: For hydrodynamic size and ζ potential measurement, a Malvern Nano ZS instrument was used. UV−visible absorption spectra were measured using a Shimadzu UV- 2550 UV−visible spectrophotometer, and fluorescence spectra was measured using a PerkinElmer LS 45. Fluorescence images were captured using Image-Pro Plus 7.0 software with an Olympus IX 81 microscope, Transmission electron microscopic (TEM) study was carried out using FEI Tecnai G2 F20 microscope by putting a drop of nanoparticle solution on a carbon coated copper grid.

Synthesis of nanoparticle incorporated porous silica particles: Synthesis is carried out using our previously reported method. In brief, 2 mL hydrophilic solution of silica-coated iron oxide/gold nanoparticles was taken in a 250 mL beaker. Then 45 mL distilled water and 5 mL 0.015 M CTAB solution were added under stirring condition. After 15 min of stirring 1.5 mL of 25 % NH_3 solution was added to this solution. Next, 0.5 mL ethanolic solution of tetramethyl orthosilicate (300 µL dissolved in 2.5 mL ethanol), 2 mL ethanolic solution of AEAPS (50 µL of AEAPS dissolved in 10 mL of ethanol) and 2 mL DMF were added stepwise at 5 min interval. The whole solution was kept under stirring condition for 3 h. Then, excess ethanol was added for the precipitation of the particles. Next, the particles were isolated by centrifuge and washed three times with ethanol and three times with water. For removing CTAB, the particles were dispersed in ethanolic solution of NH_4NO_3 (250 mg NH_4NO_3 dissolved in 25 mL ethanol) by sonication and the whole solution was heated to 80 ºC for 1 h under stirring condition. The process was repeated three times and finally the particles were dispersed in 5 mL of water and stocked for further use.

Preparation of silica particles stabilized wax microparticle: Silica particle stabilized wax-water emulsion was prepared by following the reported method.[12,17] In a typical process, 20

mg of nanoparticle incorporated silica particle was dispersed in 15 mL water under magnetic stirring condition. Then the temperature was increased to 75 ºC and at that temperature 1 g of wax was added. After that 1 mg sodium dodecyl sulfate (SDS) was added to the above solution. The resulting mixture was kept for 10 min under stirring condition and then cooled to room temperature, giving rise to a solid wax microparticles with attached silica particles at their surface. These wax microparticles were then filtered, washed with water for several times and dried under vacuum for 24 h. By this process, we get 600-700 mg of particles at each batch.

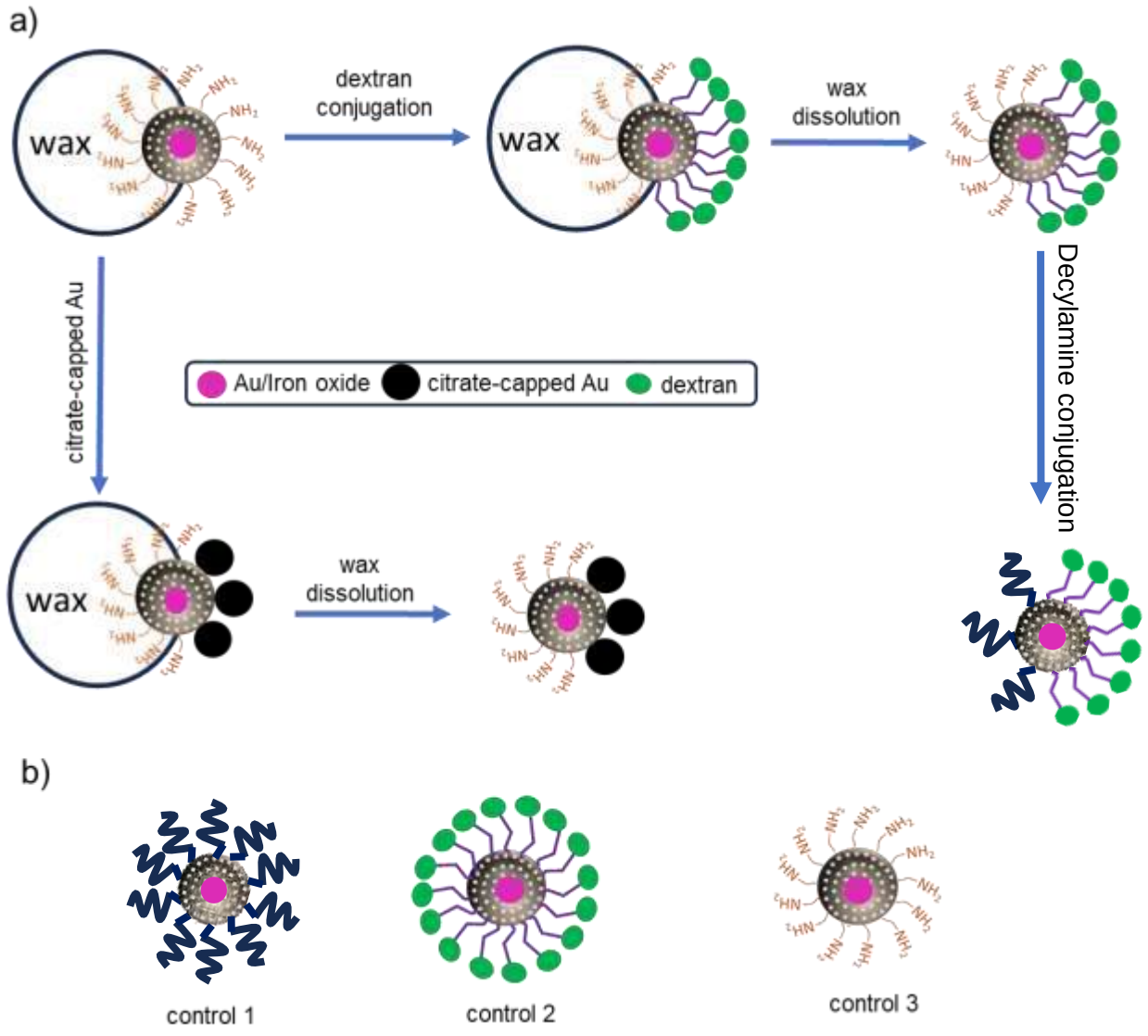

Figure 1. a) Schematic representation of synthesis strategy for making Janus functional nanoparticles. b) Three different control nanoparticles with symmetric surface chemistry that are used for comparative studies.

Preparation of Janus nanoparticles: Solid wax microparticles were used for functionalization with dextran by cyanoborohydride based conjugation chemistry. Briefly, 600 mg emulsion was dispersed in 5 mL water and pH was adjusted to 9 by adding N,N,N´,N´-tetramethylethylenediamine (TMEDA). Next, 6 mg of dextran was dissolved separately in 500 µL water and mixed to the above solution followed by addition of $NaCNBH_3$ solution (18 mg dissolved in 200 µL water) and the solution was kept under magnetic stirring for 6 h. Next, solid particles were isolated from mixture via filtration and washed with water several times to remove the excess dextran. Next, solid particles were treated with cyclohexane-chloroform and sonicated for wax dissolution. The silica particles were then dispersed in 3 mL water and used for decylamine functionalization.

Decylamine functionalization of silica particles was performed using decylamine via glutaraldehyde-based conjugation chemistry. In brief, 18 µL glutaraldehyde was dissolved in 250 µL ethanol and 20 µL was separately dissolved in 250 µL ethanol. Next, two solutions were mixed, stirred for 15 min and then whole mixture was added to dextran functionalized silica particle (3 mL) along with continued stirring. After 30 min, 18 mg $NaCNBH_3$ was added and the stirring was continued for 3 h. Resultant particles are isolated by centrifuge and redispersed twice with ethanol to remove excess glutaraldehyde and decylamine and finally dissolved in 1 mL (about 1 mg/mL) water for further use.

Urease conjugated nanomotors[22] was prepared by using glutaraldehyde as a linker molecule. Typically, the dextran functionalized silica particles were suspended in PBS buffer (pH = 8) solution containing GA (2.5 wt %). The mixture solution was kept stirring for 3 h. Then, the GA activated nanoparticles were washed with PBS buffer (pH = 8) three times before being resuspended into PBS buffer (pH = 8) containing urease (3 mg mL−1). The urease conjugation process was carried out under gentle shaking at room temperature overnight. Then, the urease conjugated Janus nanoparticles were collected and washed with DI H2O three times finally dissolved in DI H2O and stored at 4 °C for further experiment.

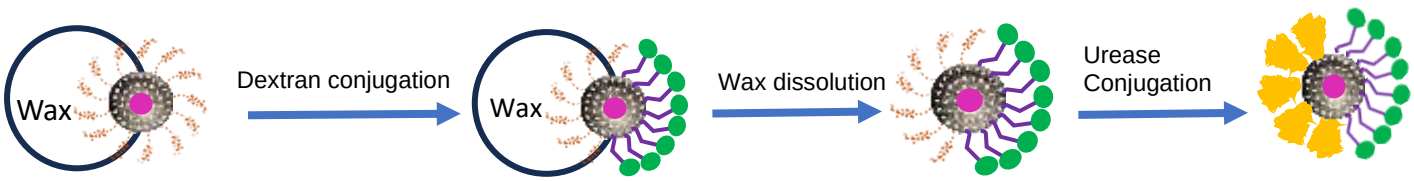

Figure 2. Schematic representation of synthesis strategy for making Janus functional urease powered nanomotors.

Preparation of control particles: We have synthesized two different isotropically functionalized silica nanoparticles terminated with decylamine (control 1) and dextran (control 2), starting from the primary amine terminated silica particles (control 3). For the preparation of control 1, 20 mg control 3 was dispersed in 10 mL water. In a separate vial, glutaraldehyde solution (18 µL glutaraldehyde dissolved in 250 µL ethanol) and decylamine solution (20 µL decylamine in 250 µL ethanol) were prepared and mixed together and after 10 min of mixing, it was added to the 10 mL nanoparticle dispersion under magnetic stirring condition. After 30 min, $NaCNBH_3$ solution (18 mg dissolved in 200 µL of water) was added and the stirring was continued for 3 h. Then, the prepared particles were isolated by centrifuge and washed once with ethanol and once with water. The resultant control 1 particles were dispersed in water.

For the preparation of control 2, 20 mg control 3 particles were dispersed in 10 mL of water and mixed with dextran solution (6 mg dextran dissolved in 500 µL water) and $NaCNBH_3$ solution (18 mg $NaCNBH_3$ dissolved in 200 µL water) and allowed to stir for 6 h. After that the solution was centrifuged and purified two times with water. Finally, the particles were dissolved in water for future application.

Cell labeling study: We have used KB cell in our study. KB is a mouth cancer cell line that is commonly used as model cell for in vitro study. For cell labelling study, we have conjugated nanoparticle with fluorescein using NHS-fluorescein. Cells were cultured in DMEM medium with 10 % heat-activated fetal bovine serum (FBS) and 1% penicillin streptomycin at 37 °C and 5 % CO_2 atmosphere. Next, cells were cultured overnight in a 24-well plate with 500 µL of DMEM medium and then 50 µL of particle dispersion was added followed by 4-12 h incubation. After incubation, cells were washed with PBS buffer solution and washed cells were used for imaging study.

Results and Discussion:

Janus nanoparticle via anisotropic functionalization with decylamine and dextran. We have selected gold or iron oxide nanoparticle incorporated porous silica particles of 50-100 nm size for this study. These silica particles are terminated with primary amines that can be used for functionalization. Figure 1 shows the synthetic strategy for making Janus functional nanoparticle.

At first, wax microparticles are prepared using nanoparticle incorporated porous silica particles as stabilizer. It is known that nanoparticles localize at liquid-liquid interface to minimize the total free energy. In the present case of wax-water system, hydrophilic silica particles adsorb around oil-like wax droplet and make them dispersible in water phase. This arrangement offers aqueous exposure of hydrophilic silica particles and keeping wax away from water exposure. Under this condition, wax can only block one side of the silica particle.

In this microparticle, primary amines of one side of each silica particles covered by wax are not accessible. The exposed/outer surface of silica particles, not covered by wax, are functionalized with dextran using the accessible primary amines. Next, wax is removed to unblock the primary amines at the other side of silica particles and used for conjugation with decylamine. We have also synthesized three different control silica nanoparticles with symmetric surface chemistry and they are used for comparative studies. They are terminated with decylamine (control 1), dextran (control 2) and primary amine (control 3). Figure 3 shows the SEM image of wax microparticles that are stabilized by silica nanoparticle. Results show wax beads of 400 microns in size.

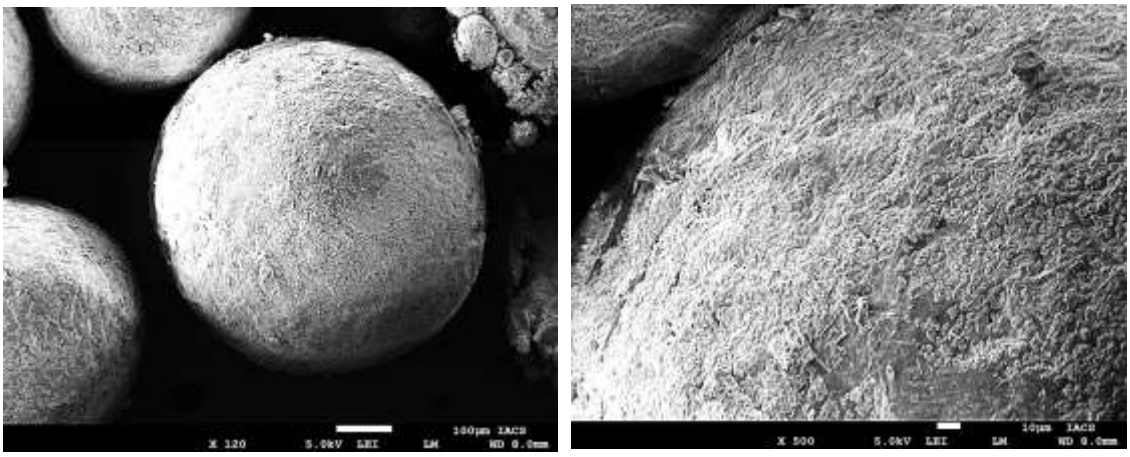

Figure 3. SEM image of silica nanoparticle stabilized single wax microparticles (left) and higher resolution SEM image of wax microparticles surface (right).

In order to establish the accessibility of one side of silica particles and to identify the Janus structure in electron microscope, silica particle stabilized wax microparticles are incubated with colloidal solution of citrate-capped anionic gold nanoparticle of 20 nm size. Next, wax is removed

via gentle washing with organic solvent and used for TEM study. Results show that single or multiple gold nanoparticles are attached to one side of each silica particle (Figure 4), suggesting anionic gold nanoparticle to be attached only at the exposed amine groups of the silica particles. They are unable to attach the other side/surface as amines are not accessible (covered by wax). This result further suggests that silica particles as wax microparticles surface react with chemicals only at the one side, the side that is exposed to the outwards direction.

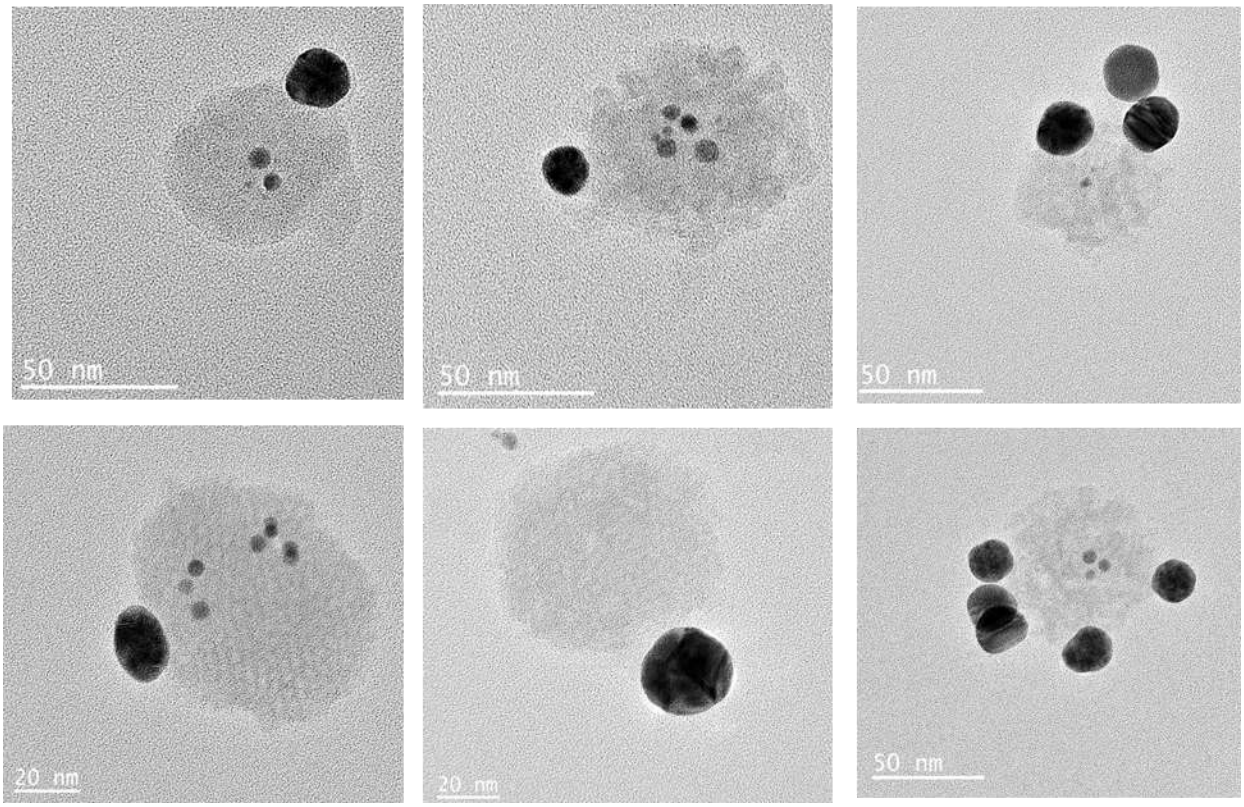

Figure 4. Evidence of accessibility of one side of silica particles and to identify Janus structure via electron microscopy. Dispersion of silica particle stabilized wax microparticles is incubated with gold nanoparticle (20 nm) and then wax is removed. All the TEM images show the gold nanoparticles attached to one side of silica particle. Small gold nanoparticles (3-5 nm) that are incorporated inside silica is observed as well.

Enhanced cell uptake of Janus nanoparticle. We have investigated the cell uptake of Janus nanoparticle and compared with control nanoparticles having isotropic functionalization (control 1). Typically, KB cells are incubated with fluorescein conjugated nanoparticle for different time and then washed cells are used for fluorescence imaging. Results are summarized in Figure 5. The

result is showing that Janus nanoparticle with anisotropic decylamine functionalization has rapid cell uptake as compared to nanoparticle with isotropic decylamine functionalization. For example, significant cell uptake of Janus particles occurs within four hours but nanoparticles with isotropic decylamine functionalization take 7 hours for significant uptake.

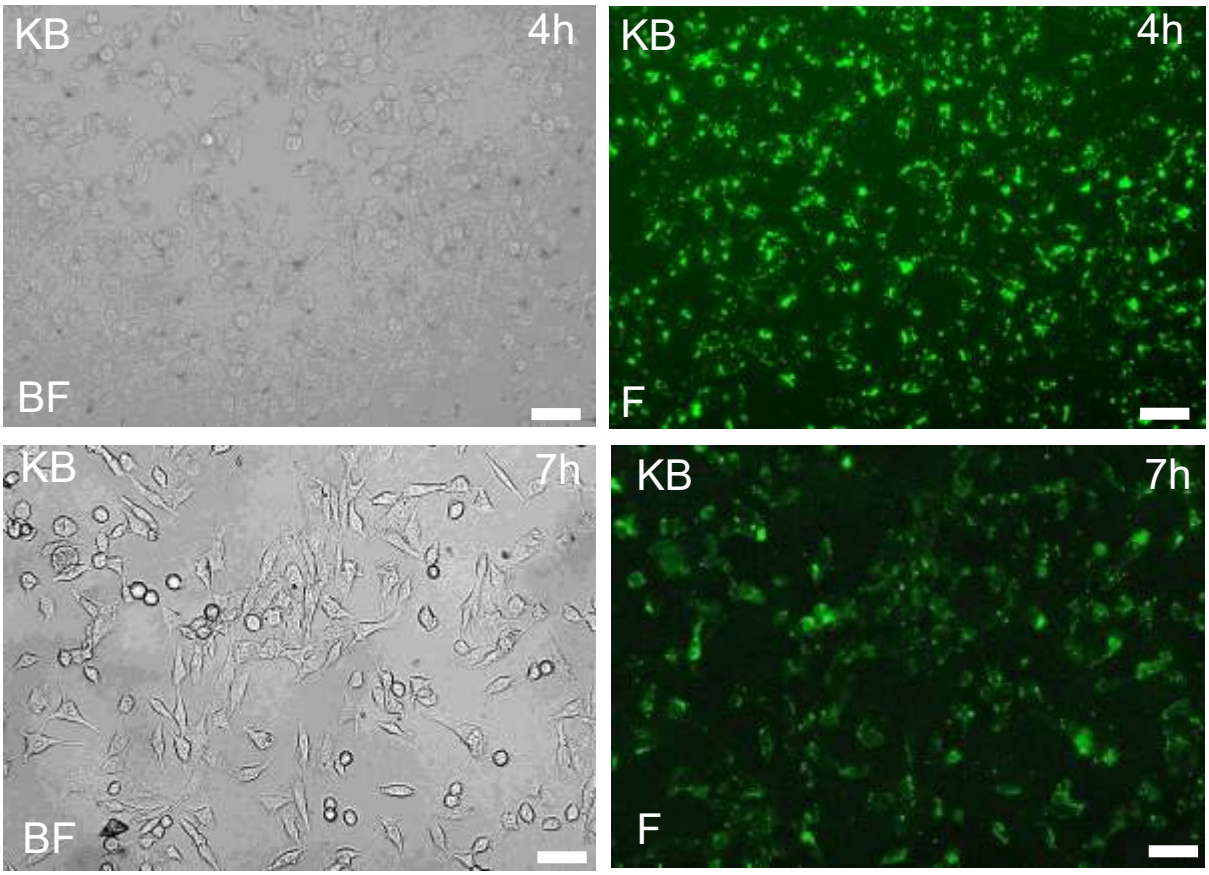

Figure 5. Evidence of enhanced cell uptake of colloidal Janus nanoparticle in KB cells. Typically, KB cells are incubated with fluorescein conjugated nanoparticle for 4h/7h and then washed cells are used for imaging under bright field or fluorescence mode and then merged images are shown here. Scale bar represents 50 microns.

Optical video recording: Zeiss AxioObserver Z1 epifluorescence microscope with a 63× objective was used for the observation of the motors' movement. The microcapsule motors were placed in a Petri dish filled with aqueous solution containing varied concentrations of urea. A

coverslip was placed onto the well of the Petri dish to seal the well and minimize the drifting effect. The videos of the motors' movement were recorded with a CCD camera at a frame rate of about 20 fps under phase contrast.

Conclusion

We have synthesized 60 nm Janus silica particles where one side of each particle is terminated with decylamine and other side is terminated with dextran. It has been demonstrated that these Janus nanoparticles have enhanced cellular interaction/uptake that arise due to anisotropic functionalization. We have shown that urease powered nanomotor of 60 nm is under Brownian motion and can travel larger distances in presence of 100 µM urea solution as compared to nanomotors in absence of fuel.

Future Work Plan

We will study the long-term cellular uptake of Janus nanoparticle with decylamine and dextran as compared to symmetric nanoparticle conjugated with decylamine and compare its colloidal stability. Further we will synthesize control nanoparticles with symmetrically conjugated urease to compare its directional movement and speed with Janus nanoparticles.

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