Using Nanodiamonds as a Potential Therapeutic for Pyelonephritis Caused by *Escherichia coli*

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Abstract

Escherichia coli (*E. coli*) is an opportunistic pathogen that is one of the main causative agents of infections in the urinary tract. In enters the urinary tract by entering the urethra and causes urinary tract infections by colonizing the urinary bladder. Such uropathogenic *E. coli* account for over 80% of urinary tract infections (UTIs). There are some strains of uropathogenic *E. coli* that have gained the ability to invade into cells and tissues of the urinary tract. UTIs ensuing from invasive *E. coli* strains are often difficult to treat due to the pathogen's ability to evade immune surveillance and the actions of antibiotics. This invasive *E. coli* can also travel to the kidneys from the bladder, resulting in kidney infections known as pyelonephritis. Pyelonephritis induced by *E. coli* is harder to treat, which not only drives the cost of treatment but also increases the time of treatment. Therefore, it is imperative to find therapies that can eradicate invasive uropathogens. Detonation nanodiamonds (NDs) are inert, carbon nanoparticles created by detonating trinitrotoluene (TNT). NDs are promising drug delivery agents due to their small size, biocompatibility, and large surface area that can be functionalized to load various molecules. In the current study, we hypothesized that NDs can interact efficiently with human kidney cells. To test the hypothesis, we utilized the human kidney cell line, HK-2, as our model. Our findings revealed that ND did not induce significant cytotoxicity in HK-2 cells. Furthermore, we were able to observe a concentration-dependent binding of NDs to HK-2 cells by flow cytometry and microscopy. These findings suggest that NDs could potentially be used to deliver drugs intracellularly into kidney cells.

Introduction

Escherichia coli (*E. coli*) is Gram-negative commensal bacteria that is found in the gastrointestinal tract but can cause infections when it colonizes the urinary tract[1]. Treatment of urinary tract infections (UTIs) cost more than $2 billion in the USA. More than 80% of UTIs are caused by *E. coli*, and it is becoming increasingly difficult to treat these infections due to their recurrence and ability to overcome antibiotic treatment[1]. UTIs normally begin at the bladder, but some strains of *E. coli* can traverse to the kidneys and infect the kidneys resulting in pyelonephritis. Pyelonephritis is a more serious and complicated disease to treat, further increasing the cost and time spent on treating the infection. Therefore, it is imperative to invent new and innovative therapies that can help combat these invasive uropathogens.

Nanodiamonds (NDs) are nanocarbon molecules containing many qualities suitable for biomedical and biotechnological applications[2]. They are biocompatible, stable, have a surface that can bind or adsorb various molecules making them good candidates for drug delivery.

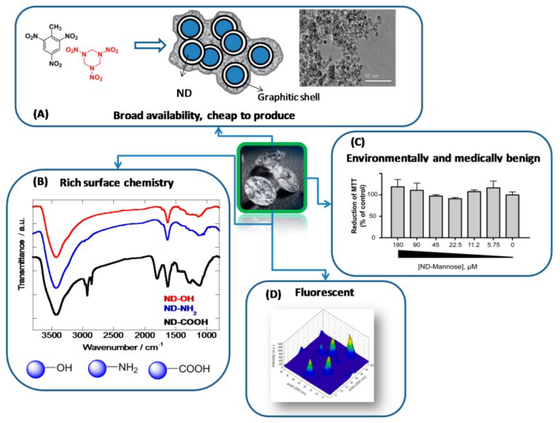


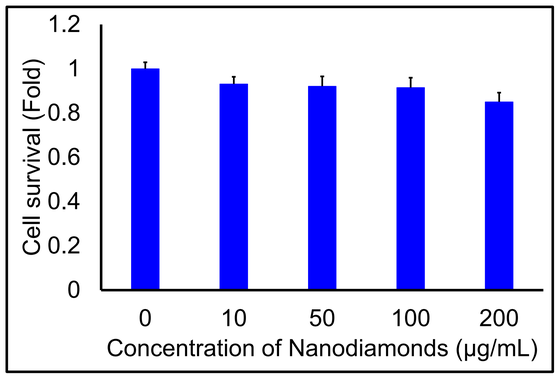
Figure 1: Nanodiamond synthesis and properties.

In the current study, we wanted to determine if NDs can be used in therapies to treat pyelonephritis caused by invasive *E. coli*. We hypothesize that NDs will efficiently interact with human kidney cells. We used an *in vitro* model that comprised of the human kidney 2 (HK-2) cell line and performed experiments to evaluate if NDs were cytotoxic to HK-2 cells at different concentrations. We further analyzed the ability of NDs to interact with HK-2 cells by flow cytometry and microscopy. These studies will help in determining if NDs are suitable for treating pyelonephritis

Results

Nanodiamonds do not exhibit significant cytotoxicity towards human kidney HK-2 cells

Figure 2: HK-2 cells were treated for 6 hours with various concentrations of NDs. Cytotoxicity was evaluated by the MTT assay



An increase in concentration of nanodiamonds reveals an increase in binding to HK-2 cells

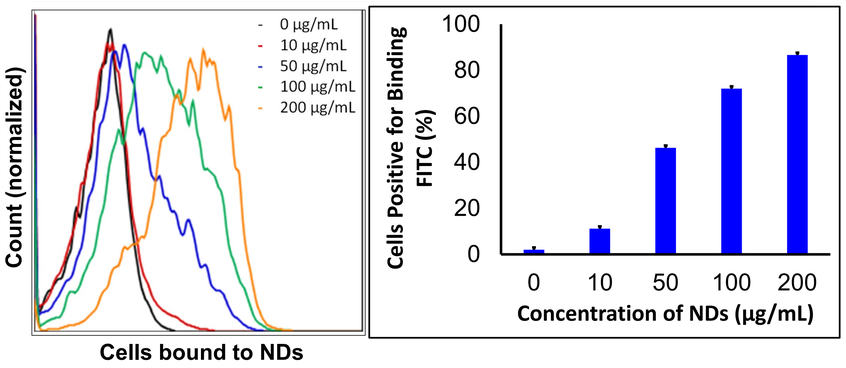


Figure 3: HK-2 cells were treated with different concentrations of NDs for 2 hours and interactions were analyzed by flow cytometry. Cell fluorescence (left) and percent of HK-2 cells binding to FITC-NDs (right) are shown.

Nanodiamonds can be visualized in HK-2 cells by fluorescent microscopy

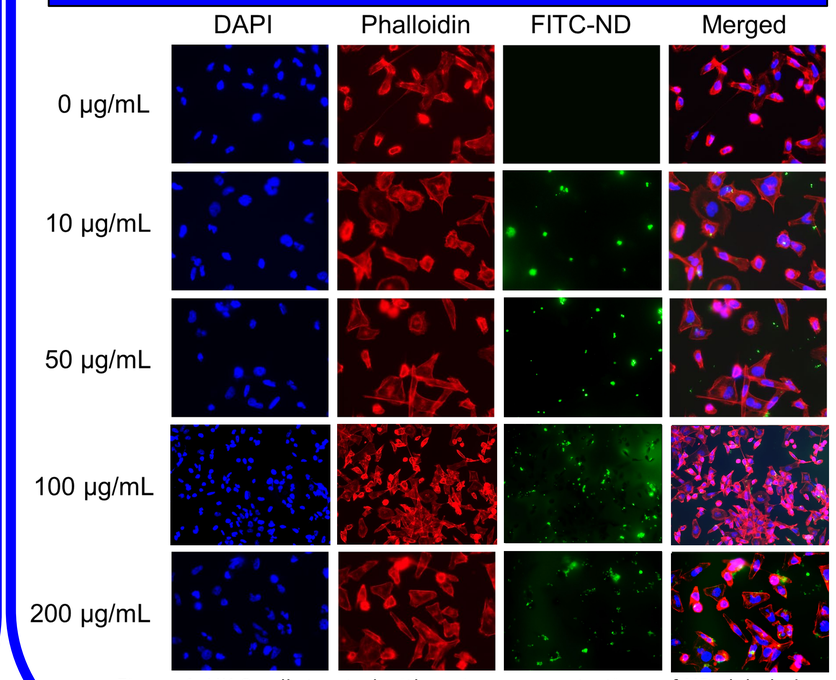
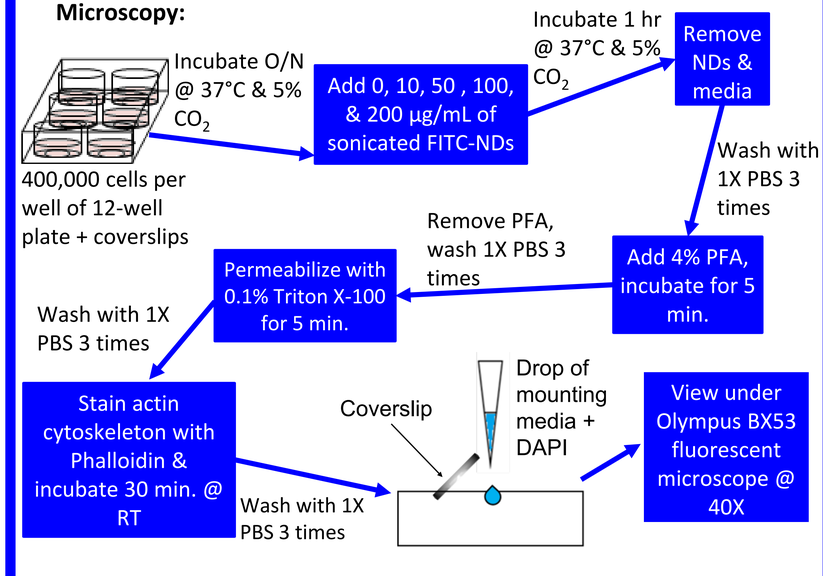


Figure 4: HK-2 cells treated with various concentrations of NDs labeled with FITC to reveal their location within the cell.

Materials & Methods

MTT Assay: HK-2 cells were incubated with different concentrations of NDs for 6 hours at 37°C & 5% CO_2. 5 mg/mL of MTT in 1X PBS was added 1 hour before the end of the incubation period. Cells were washed with 1X PBS, followed by solubilization of the formazan with DMSO. The cells were then centrifuged at 400 rcf for 5 minutes, and 100 µL of supernatant was transferred into new wells for their absorbance to be measured at 595 nm.

Microscopy:



Flow Cytometry: HK-2 cells were incubated with 10, 50, 100, & 200 µg/mL of nanodiamonds conjugated to FITC for 1 hour at 37°C & 5% CO_2. Nanodiamonds and the media were removed, and cells were washed with sterile 1X PBS. Next, cells were detached from the wells of the 12-well plate with cell dissociation buffer and transferred to their respective microcentrifuge tubes. Cells were centrifuged at 400 rcf for 5 minutes, then the supernatant was discarded. The pellet was resuspended in fresh 1X PBS and placed on ice for flow analysis.

Conclusions

NDs did not exhibit significant toxicity towards HK-2 cells as observed by the MTT assay. Flow cytometry analysis demonstrated that NDs were able to interact effectively with HK-2 cells while the we could visualize the interactions of HK-2 cells with fluorescent NDs through microscopy. Our findings suggest that NDs may prove to be promising candidates for intracellular drug delivery applications in kidney cells. Thus, further studies are required to understand the interactions between NDs and host cells.

Acknowledgements & References

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2. Szunerits, S., Barras, A., & Boukherroub, R. (2016). Antibacterial Applications of Nanodiamonds. International journal of environmental research and public health, 13(4), 413. https://doi.org/10.3390/ijerph13040413

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