# Drug-loaded PLGA nanomotors as a new approach for bladder cancer therapy

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Bladder cancer is the 7th most common cancer type worldwide, with over 500,000 new cases and 200,000 deaths annually (1). Current bladder cancer treatments are hindered by drug sedimentation and poor retention in the bladder, leading to high recurrence rates and low long-term survival (2). In recent years, nanomotors (NMs) have been developed as drug delivery systems for therapeutic agents. Nanomotors are self-propelled nanoparticles capable of converting chemical energy from their surroundings into mechanical propulsion (3). This motion enhances their diffusion and mixing capabilities, as well as their internalization into tumors, compared to passive particles (4,5). Given these benefits, they are an excellent tool for improving bladder cancer treatment as has been demonstrated in in vivo experiments, where radiolabeled nanomotors reduced bladder tumor size in mice by 90%(6). However, the designs used so far have limitations for clinical applications due to their inorganic chassis, such as silica. Therefore, there is a need to develop new nanobots based on organic materials, which are more biocompatible, biodegradable, and FDA-approved.

In this study, we developed a new design of nanomotors based on poly (lactic-co-glycolic acid) (PLGA) to enhance the standard treatment for bladder cancer, Mitomycin C (MMC). MMC-loaded PLGA nanoparticles were synthesized using the double emulsion method. To achieve motion, the surface of the nanoparticles was modified for urease attachment by first adding polyethyleneimine (PEI) and then using glutaraldehyde as a linker for the enzyme. The polydispersity, size, and surface charge of the nanoparticles were analyzed by Dynamic Light Scattering (DLS) after synthesis and at each stage of functionalization. Additionally, the enzyme activity of the nanomotors was measured by the pH change promoted by urea catalysis using Phenol Red reagent. Moreover, motion studies were conducted by comparing nanomotors in the presence and absence of urea. After characterizing the drug-loaded nanomotors, their therapeutic efficacy was assessed in bladder cancer cells derived from mice (MB49 line) and compared with the standard treatment (free MMC), demonstrating that the motion and drug encapsulation enhanced MMC-induced cell death. Finally, the mechanisms of action of our formulation were studied by analyzing the nanomotors' cell internalization and their effect on bladder epithelial cells, showing that they can internalize into cancer cells within just one hour affecting only bladder cancer cells.

## References

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