Chemotherapeutic drug-conjugated Microbead technology for selective binding with Methylated and Unmethylated small DNA in the application of Disease Detection and Biomanufacturing

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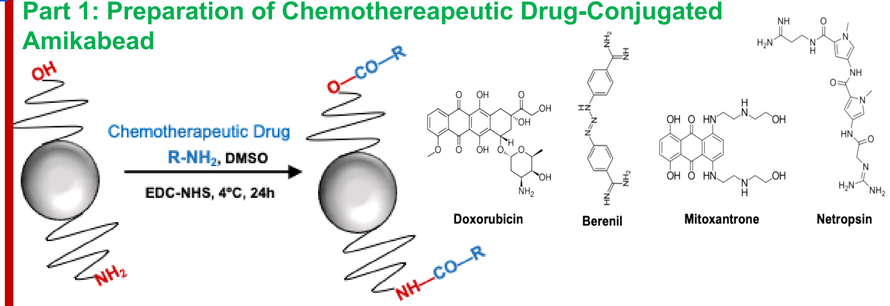
Research Motivation

Circulating tumor DNA (ctDNA) is fragmented and small DNA (typically 150-200 bp long) that is tumor derived and found in the bloodstream as a result of necrosis and apoptosis of tumor cells. Detecting a variety of diseases like various cancers, Alzheimer's, etc.; DNA methylation plays a vital role when the normal genetic sequence of an individual is mutated, leading to the disruption of normal healthy genes to a diseased pattern. Researchers found that the analysis of ctDNA methylation and its interactions have been a very innovative and sensitive approach for cancer diagnosis and prognosis. Usually ctDNA can be effectively isolated from blood or urine samples, but the sequencing techniques used for this methylation analysis is very time consuming and extremely costly. Hence there is an urgent need for a quick and cost effective way for ctDNA analysis, especially in low resource settings. An ongoing study from Dr. Kaushal Rege's Bioengineering lab has discovered that an aminoglycoside-based polymer microbead (named as 'Amikabead') has a greater binding affinity with methylated plasmid DNA, demonstrating the bead's high potential in the field of diagnostic and biomanufacturing applications.

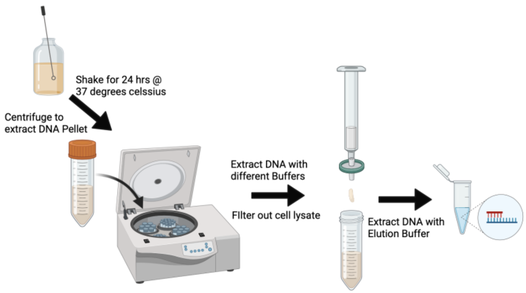
Experimental Methods

Part 1: Preparation of Chemothereapeutic Drug-Conjugated Amikabead

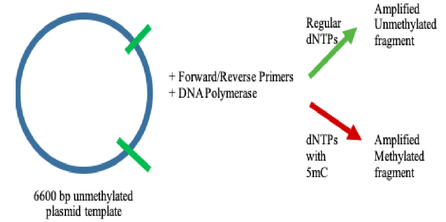


Parental Amikabead Synthesis: Emulsion polymerization between Amikacin hydrate and PEGDE. Characterization with Ninhydrin assay, FTIR, and optical imaging.
Chemotherapeutic drug ligand modification: The four different chemotherapeutic drugs were conjugated on the parental Amikabead using aqueous phase homogeneous catalyzed reaction.

Part 2: Preparation of DNA for Future Binding Studies



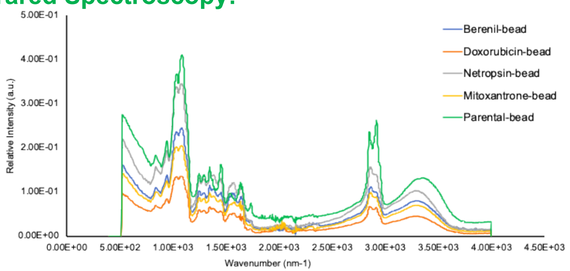
Process of DNA extraction: Bacterial culture is shaken overnight for 24 hours and then centrifuged. The pellet is then broken down and the DNA is extracted with a series of chemicals and buffers



PCR DNA Amplification: The extracted DNA is then amplified in a PCR reaction with both methylated and unmethylated DNA fragments as shown above.

Experimental Findings

Characterization of Chemobeads by Fourier Transform Infrared Spectroscopy:



Characterization of Chemobeads and Parental Beads with Optical Imaging:

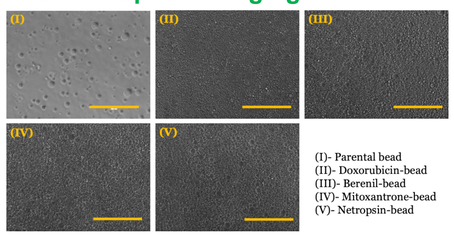
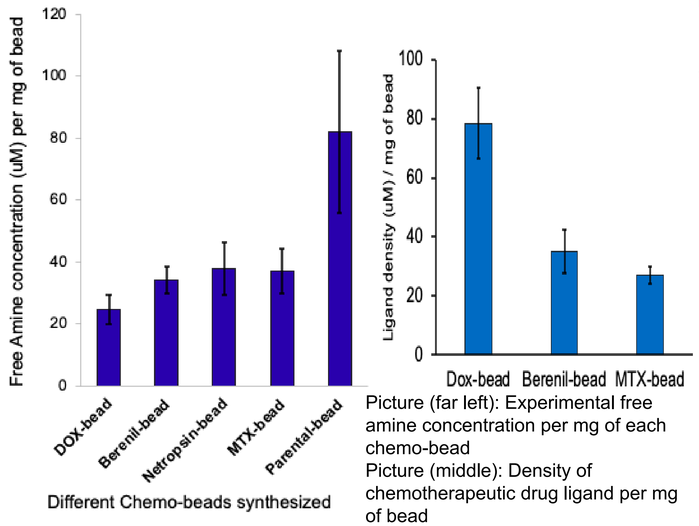


Figure: The scale bar represents 100 microns

(I)- Parental bead
(II)- Doxorubicin-bead
(III)- Berenil-bead
(IV)- Mitoxantrone-bead
(V)- Netropsin-bead

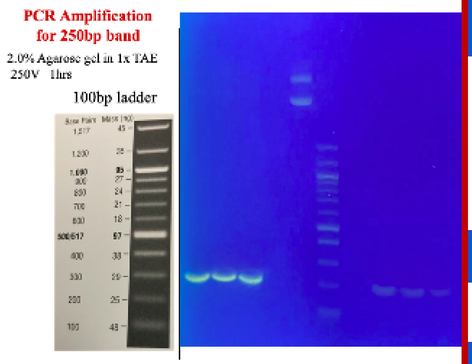
Experimental Findings

Characterization of Chemotherapeutic drug-conjugated beads by Ninhydrin Assay:



Picture (far left): Experimental free amine concentration per mg of each chemo-bead
Picture (middle): Density of chemotherapeutic drug ligand per mg of bead

Characterization of PCR DNA amplification with Gel Electrophoresis and UV-Visualization:



Picture (far right): Experimental UV-visualization of the agarose gel electrophoresis. From left to right, lanes 1-3: methylated DNA, lane 5: DNA 100bp ladder, Lane 6: GM272 template DNA, Lanes 8-11: unmethylated DNA

Obstacles

A significant obstacle that was encountered during the semester was time. There was a significant amount of research to be done and different types of experiments to learn and then execute. Because of this it was difficult to be able to make mass progress in two months.

Conclusion

- Parental Amikabeads were synthesized and characterized successfully with Ninhydrin assay.
- The Parental beads were successfully conjugated with chemotherapeutic drug molecules.
- DNA was successfuly cultured and extracted and was quantified using UV-Visualisation, showing successful methylation.

Acknowledgements

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References

1. Luo, H., Zhao, Q., Wei, W., Zheng, L., Yi, S., Li, G., Xu, R.-hua. Circulating tumor DNA methylation profiles enable early diagnosis, prognosis prediction, and screening for colorectal cancer. *Science Translational Medicine*, **2020**, *12 (524)*, eaax7533.
2. McMahon, K. W., Karunasena, E., & Ahuja, N. The Roles of DNA Methylation in the Stages of Cancer. *Cancer journal*, **2017**, *23(5)*, 257.
3. Huang, J., & Wang, L. Cell-Free DNA Methylation Profiling Analysis-Technologies and Bioinformatics. *Cancers*, **2019**, *11*, 1741.
4. Grandhi, T. S. P.; Mallik, A.; Lin, N.; Miryala, B.; Potta, T.; Tian, Y.; Rege, K. Aminoglycoside Antibiotic-Derived Anion-Exchange Microbeads for Plasmid DNA Binding and in Situ DNA Capture. *ACS Applied Materials & Interfaces* **2014**, *6(21)*, 18577.
5. Kunnath, A. P., & Priyashini, T. Potential Applications of Circulating Tumor DNA Technology as a Cancer Diagnostic Tool. *Cureus*, **2019**, *11(6)*, e4907.

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