Abstract

In order to overcome the limitations of fluorescent dyes in imaging and studying disease progression over time, it is necessary to develop new nanomaterial systems that have therapeutic and diagnostic properties (theranostics). Establishing methods for controlled introduction of new theranostic materials into cell lines will be useful in studying diseases of aging as well as in the development of specific individualized therapies for various diseases as part of the vision for an era of personalized medicine. Since gold nanoparticles are a candidate platform for creating nanoparticle-based theranostics, a study on how to best induce their cellular uptake is necessary. The aims of this study are to determine the optimal cellular microenvironment for osteoblast uptake of gold nanoparticles. The osteoblasts will be cultured on a 2D living bone analog made of nanocellulose and imaged using darkfield microscopy in order to determine how various parameters affect particle uptake.

Project Description

In order to study bone disease and diseases of aging such as cancer, which involve the propagation of genetic errors, it is necessary to study cell lineages over time. Currently, fluorescent molecules dominate as the standard for labeling and contrast generation in microscopic techniques despite their known limitations.1,2 These limitations include a small number of fluorescent agents available in the near infrared region, background autofluorescence produced by the sample which decreases sensitivity, and other phenomena including blinking, saturation, and photobleaching.1,2 Photobleaching is particularly problematic for studying cell lineages over time when attempting to monitor disease progression.2

Along with the inherent limitations of fluorescence imaging, there has been a push to combine diagnostic and therapeutic capabilities into a single agent.3 The marriage of diagnosis and therapeutics would allow for a more individualized approach to medicine and would allow for a better understanding of the effects of different therapeutics in real-time.3 Theranostics, which can be defined as nanoplatforms that allow for the co-delivery of therapeutic and imaging functions, are one way to achieve this.3 The combination of diagnostic imaging and therapy makes a lot of sense because both require a sufficient accumulation of agents in the diseased area.3

Gold nanoparticles have the potential to overcome many of the limitations of fluorescent imaging while simultaneously being good candidates for the delivery of therapeutic agents. Unlike fluorophores, they are not prone to photobleaching and can be protected with various molecular coats allowing them to become virtually insensitive to their surroundings and survive in vivo and in

vitro for long periods of time.1 This makes them ideal for the study of cell lineages over time in order to better understand various disease progressions. Gold nanostars are also able to be effectively used in surface enhanced Raman spectroscopy (SERS) which can be done in the near infrared region.1 This allows for improved optical penetration into biological samples relative to many commonly used fluorophores.1 In terms of therapeutics, gold nanoparticles can be functionalized quite easily with diverse molecules due to the strong interactions between gold and thiol and a number of therapeutics have already been loaded in this manner.3 These and other advantages make gold nanoparticles an attractive choice for studying nanoparticle-based theranostics.

This study would likely be broken down into three main phases. In the first phase, several types of gold nanoparticles would be synthesized, including various sizes of gold nanospheres and nanostars. These would be purified by ultracentrifugation and characterized using Dynamic Light Scattering (DLS), UV-Vis Spectroscopy, Transmission Electron Microscopy (TEM), and possibly Inductively Coupled Plasma (ICP), all tools commonly used in the Mason lab. After this, the particles would be functionalized with various polymers, such as wheat germ agglutinin, a known uptake promoter, for determining which promote particle uptake best by osteoblast cells under varying biochemical conditions.

In the second phase, MC3T3-E1 osteoblast cells purchased from Sigma Aldrich, and currently available in the Mason lab, will be cultured on a 2D living bone analog made of nanocellulose and kept in a continuous cell culture dish that supplies a constant stream of nutrients. The media and/or the nutrient supply will be dosed with a constant concentration of gold nanoparticles which will come in contact with the cells allowing them to be differentially uptaken. The nutrient supply will then be modified to test its effect on cell health and gold nanoparticle uptake. Parameters to be varied will include pH and ion strengths.

The third phase will include imaging the cells and doing analysis on the images. Each group of cells will be imaged using a darkfield microscope and ToupView software. Images will be taken every 2 hours over the first twelve hours of the experiment and then again after 24 and 48 hours. Images will then be analyzed for gold nanoparticle uptake using ImageJ software from the NIH. As many iterations of the three phases as possible will be performed over the course of the summer in order to provide sufficient statistics to adequately describe the process of uptake.

The results of this study will help in understanding the parameters that most influence cellular uptake of gold nanoparticles in osteoblasts. Because the efficacy of many theranostics is dependent on the accumulation of the theranostic agent in cells of interest, this study is a

necessary step in the development of gold nanoparticle-based theranostics to be used in individualized therapies for various diseases.3 Unlike fluorophores, gold nanoparticles can also be used to study cell lineages over time which is also largely dependent on how well the particles are uptaken in the first place. This study will help provide answers to the question of which conditions allow for the greatest uptake of gold nanoparticles among osteoblast cells.

[1] Allgeyer, E. S., Pongan, A., Browne, M., & Mason, M. D. (2009). Optical signal comparison of single fluorescent molecules and raman active gold nanostars. Nano Letters, 9(11), 3816–3819. https://doi.org/10.1021/nl902008g

[2] Keren, S., Zavaleta, C., Cheng, Z., de la Zerda, A., Gheysens, O., & Gambhir, S. S. (2008). Noninvasive molecular imaging of small living subjects using Raman spectroscopy. Proceedings of the National Academy of Sciences of the United States of America, 105(15), 5844–5849. http://doi.org/10.1073/pnas.0710575105

[3] Xie, J., Lee, S., & Chen, X. (2010). Nanoparticle-based theranostic agents. Advanced Drug Delivery Reviews, 62(11), 1064–1079. http://doi.org/10.1016/j.addr.2010.07.009

Budget Justification

Total Budget Requested: $3,000

Budget: Stipend Amount: $2,250

Budget: Materials and Supplies: $625

Budget: Travel: 0

Budget: MISC: $125
Budget Justification:

Supplies:

- TEM Use - $100/hour for approximately 5 hours of use over the summer ($500) -

One gram of Gold(III) Chloride from Sigma Aldrich ($125/gram) Remaining supplies

provided from current lab inventory:

- Wheat Germ Agglutinin

- Two other nanoparticle shuttling polymers (one anionic and cationic) -

Polyethylene Glycol (PEG)

- Polyvinylpyrrolidone (PVP)

- MC3T3-E1 cell line

- Cell Nutrients

- Pipette tips

- Sodium Citrate

-18 MΩ DI water

- Centrifuge Tubes

Miscellaneous costs:

$125

Student Stipend:

$2,250

The requested funds of $3000.00 are for a combination of supplies, instrumentation, and a student stipend. Required supplies include MC3T3-E1 cell line, gold(III) chloride, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), 18 MΩ DI water, wheat germ agglutinin, two other nanoparticle shuttling polymers, cell culture media, centrifuge tubes, and various disposable supplies such as pipettes, cell nutrients, and cell culture flasks. All of these supplies will be provided from the Mason lab aside from the gold(III) chloride which will be purchased from Sigma Aldrich for $125. Instrumentation costs are primarily for use of a transmission electron microscope (TEM) which costs approximately $100/hour to use for a total of $500 for the summer. $125 is requested for any miscellaneous or unforeseen laboratory costs for the project. $2,250 is requested for a student stipend to assist with living costs while the project is being completed.