Fellowships and Symposia in Biomaterials for Nanomedicine and Tissue Engineering

Robert S. Langer

2022 Balzan Prize for Biomaterials for Nanomedicine and Tissue Engineering

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Period: 2023-

Robert Langer since 2009 has been David H. Koch Institute Professor at the Massachusetts Institute of Technology, where he was previously Institute Professor; between 1988 and 2005, he was Kenneth J. Germeshausen Professor of Chemical and Biomedical Engineering at MIT Department of Chemical Engineering; Whitaker College of Health Sciences, Technology, and Management, and the Harvard-MIT Division of Health Sciences and Technology. Since 1999 he has been Senior Lecturer on Surgery, Harvard University, Harvard Medical School.

The project's aims are to provide fellowships in the area of biomaterials for nanomedicine and tissue engineering for four graduate students from multiple locales (Hong Kong, the USA, and the Middle East) and to organize travel symposia to present research results. Four different projects are foreseen.

The first will be in tissue engineering. The long-term success of islet replacement therapy for curing type I diabetes is built upon effective protection against the host immune system and the maintenance of cell viability and function. Immuno-protection has been achieved by various cell encapsulation techniques that sequester islet grafts from host immune cells. However, maintaining long-term graft function is at an impasse. It has been shown that, prior to encapsulation, the depletion of islet extracellular matrix (ECM) during islet isolation is detrimental to islet function and undermines glucose responsiveness. Thus, this student proposes to restore essential islet-matrix interactions by formulating a synthetic matrix containing an optimized mix of islet binding sites to prolong islet function post- encapsulation.

She plans to construct a library of islet-binding short peptides and screen through them individually and then combinatorically using a high-throughput imaging-based assay to identify a formulation optimal for islet growth and function. Islet ECM composition and the expression of islet ECM-binding receptors will be studied using quantitative mass spectrometry and immunofluorescence staining. The peptide library will be constructed by mapping out ligand- receptor pairs that are present between the most

abundant ECM components and islet receptors. Peptide candidates will be displayed on a hydrogel scaffold for islet encapsulation. Samples will be subjected to high-throughput screen based on calcium flux imaging to study the effect of individual peptides on glucose responsiveness. After identifying the effective peptides, a series of experiments will be conducted to identify the optimal formulation, which will be validated in vivo. The most immediate aims are to finalize the peptide library, finish optimizing the high throughput imaging protocol, and complete the individual peptide screens.

The second project involves nanomedicine. Although lipid nanoparticles are a potent and safe delivery method for mRNA, they currently can only be delivered via injection or infusion, both of which cannot target lung tissues, require hazardous sharps and trained administrators, and necessitate liquid cold-storage. Nebulization is being pursued as a solution to the first two of these issues but does not address the costs and logistical difficulties of liquid storage. Dry powders, however, in addition to being easily administered to the lung, also boast long shelf lives at dry ambient conditions. This fellow intends to develop dry powder forms of RNA delivery systems that enable potent delivery to the lung but have substantially improved shelf life.

To date, the student has made progress on the preparation of a dry powder formulation for mRNA lipid nanoparticle (LNP) delivery to the lung. Using spray freeze drying (SFD), he prepared powders from a variety of LNPs consisting of different ionizable and helper lipids, cholesterol, and lipid-anchored poly(ethylene glycol) (PEG lipid). He found that the introduction of certain phospholipids and other cryoprotectants notably improved the stability of LNPs before and after SFD. These powders show the ability to transfect human cells in vitro in both adherent and primary air-liquid interface lung cultures comparable to conventional liquid LNPs. Preliminary in vivo experiments in small rodents confirm that these powders show significant biological activity.

The goals of this proposal are to examine the safety and efficacy of those nanoparticles and to explore applications of gene editing, using powders containing mRNA encoded nucleases. Additionally, this project will combine new ionizable lipids to create maximally potent powders which can more effectively target the lung. The goal of this proposal is to develop dry powders that effectively deliver CRISPR/Cas9 mRNA for gene correction, beginning first with Cre reporter models, and then transitioning to rodent disease models such as for cystic fibrosis.

The third project is concerned with targeted and adjuvanted viral mimicking intranasal lipid nanoparticle mRNA vaccines. Although lipid nanoparticles (LNPs) have shown significant success in mRNA antigen delivery for intramuscular (IM) administration against SARS-CoV-2, a major limitation of mRNA LNPs and other parenterally administered vaccines is their failure to elicit a mucosal response, which could prevent the spread of infectious diseases beyond mucosal surfaces. Therefore, generating strong mucosal responses with vaccines are needed to prevent the spread of respiratory, gastrointestinal, and sexually transmitted diseases. Intranasal (IN) administration can achieve this for respiratory infections, but endogenous obstacles such as mucus, mucociliary clearance, and epithelial cells make current LNPs inefficient for IN vaccines.

This student plans to examine and design LNPs for IN vaccines, resembling natural entry through mucosal barriers of viruses for enhanced mucosal response. In the process, he will develop targeted LNPs and adjuvants for IN use. He hypothesizes that

a) targeting of MCs and dendritic cells (DCs), as done by human immunodeficiency virus (HIV), can improve bypassing of mucosal barriers, enhancing potency of vaccine response, and b) proinflammatory cytokines can allow for a targeting and adjuvanting LNP to activate signaling pathways seen in a natural infection. The first objective is to develop dual liganded/targeted LNPs for IN administration, mimicking the mechanism of infection of HIV entry. HIV targets multiple cells to bypass mucosal barriers and reach target cells. Similarly, he will design targeted LNPs that can transfect MCs and DCs. To conjugate ligands, he will add maleimide PEG into LNPs to form covalent bonds with thiols derived from the reaction of ligand with Traut's reagent. He will examine overall transfection efficiency in the nasal airways of each ligand individually in vivo at a dose of five micrograms of firefly luciferase mRNA. The individual DC and MC ligands that have the highest transfection will be examined together on the same LNP. The second objective is to develop endogenous proinflammatory cytokines as adjuvants to mimic inflammatory responses observed in natural infections, allowing for both targeting and activation of immune cells.

Cytokines are advantageous adjuvants since they can trigger Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways, enhancing inflammatory cytokine secretion. First, he will conjugate cytokines on LNPs to allow them to target cytokine receptors, trigger subsequent signaling pathways, and delivery antigen to these cells. Second, he will create fusion proteins that are mRNA encoded for LNP encapsulation. To achieve these mRNA transcripts, he will design plasmids with an IL2 secretion tag, a peptide linker, the desired antigen, and the cytokine, followed by in vitro transcription to obtain the fusion protein mRNA. For both studies, the mucosal immune response will be measured by secretory IgA titers in nasal washes and bronchoalveolar lavages. Based on these projects, the student will design vaccines that can elicit strong systemic and mucosal immunity with LNPs. HIV is a potent pathogen at bypassing mucosal barriers while current IN vaccine technology is not as adept, evident by only a single clinically used IN vaccine. Designing an IN vaccine based on HIV's mechanism of infection can potentially provide improved translational potential than non-targeted or adjuvanted LNP.

The fourth student envisions a project that will develop new antibody-RNA nanoparticle conjugates. Though promising, antibodies face a number of challenges as a platform for siRNA delivery, including internalization and delivery to the correct subcellular compartment, efficiency of siRNA escape from the endosome, and target selection for optimal efficacy and specificity. This project attempts to address these issues through careful design and efficient screening of antibody-RNA conjugates for high potency.

The first way to increase the efficacy of siRNA conjugates is by developing more advanced linker chemistry. Homogeneity and high drug-to-antibody ratio (DAR) both improve ADC potency with small molecule drugs and can be controlled through linker design. The student will use branched linkers to obtain the benefits of higher DAR and homogeneity for ARCs. A number of designs for branched linkers have been published recently, but none have been shown to conjugate siRNA to antibodies. Branched linkers on ARCs allow for more siRNA molecules to be endocytosed by receptors with low copy numbers and decrease competition for receptors. Increasing DAR can also help to distinguish kinetically between different mechanisms of siRNA release from the endosome, which a yet unsolved research problem. For example, a pronounced effect of DAR increase could indicate a burst-release, rather than a gradual release profile, of

siRNA from endosomes. These effects could result both in more effective ARC therapeutics and in a better understanding of the mechanism of ARC delivery.

He will also attempt to develop ARCs by screening for new antibodies capable of delivering siRNA to cells, particularly to T lymphocytes. Target selection has been shown to be a crucial factor determining the success of antibody-siRNA conjugates—for example, Genentech showed that five out of seven tested antibodies could not deliver siRNA in vitro. T-cells have been shown to be the primary orchestrators of the adaptive immune response to cancer and a number of autoimmune diseases. Many of the differences between T-cell subsets are visible at the level of RNA interference, the mechanism co-opted by siRNA that is used by the body to downregulate expression of certain mRNAs. The importance of RNAi in determining T-cell fate and function gives hope that these attributes can be controlled through siRNA delivery to T-cells. Indeed, some experiments have already succeeded in changing T-cell function through siRNA-induced knockdown of FoxP3, a transcription factor that imparts regulatory function. This student's project attempts to deliver siRNA to T-cells (and possibly other targets later) for therapeutic applications in cancer immunotherapy and autoimmune disease. By expanding the known dataset of cell receptor targets that can or cannot uptake siRNA, he expects that this study will also help to elucidate properties of receptor targets that enable or impair ARC function.

To diffuse the results of these four projects, there are plans to submit manuscripts to high impact journals like *Science*, *Nature*, and *PNAS*. Support from Robert S. Langer's Balzan Research Project will be duly acknowledged.