Engineering 3D spatiotemporally dynamic hyaluronic acid hydrogels with heterogeneous mechanics to model idiopathic pulmonary fibrosis

(Technical Topic)

Identifying social factors that influence sex-specific policy in the regulation of clinical trials
(STS Topic)

A Thesis Project Prospectus Submitted to the

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On my honor as a student I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

With clinical trials being such a critical step in the release of any therapeutic to market, both the social and technical aspects of trials must be analyzed. The technical project focuses on developing a pre-clinical hydrogel model of idiopathic pulmonary fibrosis (IPF) to develop a more accurate predictor of the success of therapeutics once they are in the clinic. Social factors influencing clinical trials are analyzed in the STS report with a focus on policy regarding sex-dependent responses to medicine in the clinic.

The technical project looks to model IPF using three-dimensional (3D) hyaluronic acid (HA) hydrogels. With this model, characteristics that have been shown to be critical in a cell's response to its surrounding environment will be incorporated in a way that mimics the diseased tissue environment of IPF. Such factors include viscoelasticity and heterogeneous mechanics.

The STS prospectus looks to explore the importance of having clinical trial participants that adequately represent the patient population affected by the disease a drug is meant to treat, specifically with regard to gender. Through the examination of case studies that show the sex-dependent responses to medications, the consequences of the medical field's previous failures in this parameter will be evaluated to highlight the importance of including a diverse population in future clinical trials.

Technical Topic: Engineering 3D spatiotemporally dynamic hyaluronic acid hydrogels with heterogeneous mechanics to model idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by tissue scarring that can become fatal due to respiratory failure. Although IPF is the most common and aggressive form of interstitial lung disease, current therapies have proven ineffective due to a lack of knowledge surrounding how cells sense and respond to dynamic environmental cues

(Wernig, 2017). Accurate *in vitro* disease modeling is important in informing therapeutic design through its ability to explore a broad range of variables that contribute to disease progression as well as the capability to perform high-throughput drug screening that would be unsuitable with *in vivo* animal models. Hydrogels are water swollen polymer networks that are often used to model normal and diseased tissues due to their ability to mimic soft tissue properties (Caliari, 2016). However, many current models are two-dimensional (2D), non-physiologically stiff, and have homogenous mechanical properties – all traits which fail to portray the native 3D tissue microenvironment. Additionally, the majority of models fail to incorporate time-dependent properties such as viscoelasticity, despite its key role in mediating in cell behavior. Previous work has shown that incorporating viscoelasticity into hydrogels impacts cell responses such as spreading, focal adhesion organization, proliferation, and differentiation when compared to solely elastic models (Hui, 2019; Chaudhuri, 2015; Charrier, 2018; Cameron, 2011).

To develop a more accurate disease model of IPF we will engineer a 3D phototunable viscoelastic hydrogel system that allows for independent tuning of mechanical properties implicated in healthy and diseased lung tissue such as stiffness and viscoelasticity. This project includes two major specific aims: (1) optimize photopatterning of 3D HA elastic and viscoelastic hydrogels to mimic native lung tissue mechanics and (2) encapsulate human lung fibroblasts (HLFs) in 3D HA hydrogels with heterogeneous mechanics to observe cell behavior.

3D hyaluronic acid (HA) hydrogels will be fabricated using photopolymerization and include both elastic and viscoelastic mechanical properties. Hydrogel precursor solutions that incorporate either only covalent (elastic) or both covalent and reversible supramolecular (viscoelastic) crosslinks will be irradiated with ultraviolet (UV) light to initiate photopolymerization. The crosslinker used will be matrix metalloproteinase (MMP)-degradable

to ensure that cells are able to remodel their surrounding 3D environment. To incorporate heterogeneity, initially soft hydrogels will receive a secondary irradiation with a patterned photomask transparency placed over the hydrogel surface. The regions not covered by the opaque pattern receive additional irradiation, causing further crosslinking and a higher stiffness than the regions covered by the pattern on the photomask.

Hydrogel mechanics will be tested using an Optics11 Chiaro nanoindenter to ensure pattern fidelity through the full thickness of the hydrogel. We expect regions that did not receive a secondary irradiation will have softer, more viscous mechanics similar to those of native lung tissue (elastic modulus near 1-2 kPa) while patterned regions will show a higher stiffness and loss of viscoelasticity similar to fibrotic nodules seen in IPF (elastic modulus near 15-20 kPa) (Wells, R. G., 2013).

HLFs, known to be the main mediators of lung fibrosis, will be encapsulated within 3D hydrogels and swollen overnight in culture media. Cell-encapsulated hydrogels will then be photopatterned in the method described in Specific Aim 1, and the cells will be cultured within the hydrogels for 7 days. The effects of non-uniform stiffness on migration as well as several cell shape metrics such cell spread area and cell shape index (CSI), which is a measure of circularity, will be quantified. Measures of cell shape are indicators of fibrosis as fibroblasts have been shown to increase spreading behavior on stiffer, less viscoelastic substrates. Additional metrics that are indicative of fibrosis such as focal adhesion formation (paxillin) and α-smooth muscle actin (α-SMA) stress fiber formation will be measured. α-SMA is a key indicator of fibrosis as fibroblasts that become activated under fibrotic conditions will become contractile, α-SMA expressing myofibroblasts. Focal adhesions are macromolecular assemblies that enable cells to participate in mechanotransduction. In fibrosis, cells show increased focal adhesion formation

resulting in greater ability to adhere to and contract the ECM.[8,9] Image analysis to quantitatively measure the stated metrics will be performed. We expect that low levels of viscoelasticity will allow for cell spreading and stress fiber formation even in the stiffer regions, but the soft unpatterned viscoelastic regions will lead to increased cell circularity.

STS Topic: Identifying social factors that influence sex-specific policy in the regulation of clinical trials

The Food and Drug Administration (FDA) defines clinical trials as "…voluntary human research studies designed to answer specific questions about the safety and effectiveness of drugs, vaccines, devices and other therapies…". While organizations attempting to release their treatment to the market are responsible for conducting clinical trials, the FDA is the governing body that sets clinical trial regulations and evaluates the data from trials to approve or reject the treatment ("FDA Encourages More Participation…", 2018). Though the clinical trial phase of a drug's lifetime is a critically important step in determining a drug's safety and efficacy, historical data shows that not all trials have adequate inclusion of women in clinical trials or analysis of sex-specific responses to drugs. While certain lifestyle and environmental differences can contribute to the ways in which a drug affects each specific patient, contrasts in the biological makeup of men and women make inclusion of the sexes in clinical trials in a way that represents the patient population especially crucial (Liu, 2016). In this analysis, a study of the social factors influencing women's inclusion or exclusion from drug clinical trials and the level of evaluation of sex-dependent data will be conducted along with their implications.

The social factors that played a role in sex-specific data analysis and inclusion in clinical trials will be looked at through the social construction of technology (SCOT) framework. This STS framework highlights the criticality of human action in shaping technology rather than the

importance of technology in shaping human action. The reasons for acceptance or rejection of a technology can be understood by evaluating the social world surrounding a technology. In the context of this analysis, human action has largely shaped the direction of clinical trials and as such the ways in which medications are developed. Implementation of key policy changes in clinical trials alongside specific cases they influenced will be analyzed using SCOT to gain a historical perspective.

Regarding the inclusion of women in clinical trials, the case of thalidomide in the early 1960s will be investigated with the SCOT framework. Thalidomide is an immunosuppressant drug used that, in combination with aspirin, was indicated to treat maladies ranging from the common cough and cold to asthma and anxiety. By 1962, around 20,000 patients had taken the drug as part of a largely unmonitored clinical trial. It was later discovered that fetal abnormalities resulted when pregnant women took the drug. Over 10,000 debilitating malformations were reported in children whose mothers were taking the drug, resulting in the strengthening of the regulatory role of the FDA and the passing of the Kefauver-Harris Amendment which categorized pregnant women as an especially vulnerable population during clinical trial research (Vargesson, 2015). While this may be deemed an appropriate response to the thalidomide disaster, further legislation known as the General Considerations for the Clinical of Drugs in 1977 barred women of child-bearing age from participating in Phase I and II clinical trials except in the case of life-threatening illness. Groups opposing the legislation argued women should be given the freedom to elect whether or not they wish to participate in clinical trials and that exclusion of women from these early stages would leave large gaps in data regarding a drug's efficacy. While these advocates worked to have women included in clinical trials, governing

bodies held fast to the view that women of child-bearing potential would negatively impact trial data and legislation to reverse the act would not be enacted until 1988 (Liu, 2016).

A case that will be investigated when analyzing the potential impacts of including sex-specific data in clinical trial results is of zolpidem. Zolpidem is a drug manufactured under varying brand names meant to treat insomnia. In 1993, the drug was approved for the market following clinical trials; however, after its approval it was noted that women were having more severe side effects than men. Subsequent studies through the FDA found that zolpidem levels were 25-35% higher in women than in men after taking the medication leading to daytime drowsiness and driving impairment the following day. As the differences between the genders could not be explained by weight differences, further studies found that there were different rates of drug metabolism in men and women. For this reason, the FDA required sex-dependent dosing of the drug in 2013 marking the first instance of different prescribing information based on sex (Liu, 2015; Jasjuja, 2019). While the FDA released Section 907 of the FDA Safety and Innovation Act (FDASIA) in 2012 which required the organization to present data to congress demonstrating how minority participation and analysis of demographic specific data on the performance of drugs a clear route for collecting and presenting this data was not put in place. With data from the zolpidem studies coming to light the FDA released an action plan in August 2014 to accomplish the requirements outlined in FDASIA Section 907 ("FDA Action Plan to Enhance the Collection…", 2014). The action plan identifies several SCOT social groups key to implementing policy surrounding clinical trials including trial participants, the FDA, congress, and companies running the trials.

Conclusion

In conclusion, the technical topic will look to provide a more physiologically-relevant preclinical model for IPF so that therapeutic efficacy prior to entering the clinic can be predicted with more accuracy. The STS research will outline and analyze historical cases that influenced policy surrounding inclusion of women in clinical trials and analysis of sex-specific data. The topics are related in their potential to provide insight on and improve clinical trials, a key phase of a drug's lifetime.

References

Caliari, S. R., & Burdick, J. A. (2016). A practical guide to hydrogels for cell culture. *Nature Methods, 13*(5), 405-414. doi:10.1038/nmeth.3839

Cameron, A. R., Frith, J. E., & Cooper-White, J. J. (2011). The Influence of Substrate Creep on Mesenchymal Stem Cell Behaviour and Phenotype. *Biomaterials, 32*(26), 5979-5993.

Charrier, E. E., Pogoda, K., Wells, R. G., & Janmey, P. A. (2018). Control of cell morphology and differentiation by substrates with independently tunable elasticity and viscous dissipation. *Nature Communications, 9*(1). doi:10.1038/s41467-018-02906-9

Chaudhuri, O., Gu, L., Klumpers, D., Darnell, M., Bencherif, S. A., Weaver, J. C., . . . Mooney, D. J. (2015). Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nature Materials, 15*(3), 326-334. doi:10.1038/nmat4489

FDA Encourages More Participation, Diversity in Clinical Trials. (2018). Retrieved October 16, 2020, from https://www.fda.gov/consumers/consumer-updates/fda-encourages-more-participation-diversity-clinical-trials

Hui, E., Gimeno, K. I., Guan, G., & Caliari, S. R. (2019). Spatiotemporal Control of Viscoelasticity in Phototunable Hyaluronic Acid Hydrogels. *Biomacromolecules, 20*(11), 4126-4134. doi:10.1021/acs.biomac.9b00965

Jasuja, G., Reisman, J., Wiener, R., Christopher, M., & Rose, A. (2020). Gender Differences in Prescribing of Zolpidem in the Veterans Health Administration (VHA). *Health Services Research, 55*(S1), 79-79. doi:10.1111/1475-6773.13439

Liu, K. A., & Dipietro Mager, N. A. (2016). Women's involvement in clinical trials: Historical perspective and future implications. *Pharmacy Practice, 14*(1), 708.

Oh, S. S., Galanter, J., Thakur, N., Pino-Yanes, M., Barcelo, N. E., White, M. J., . . . Burchard, E. G. (2015). Diversity in Clinical and Biomedical Research: A Promise Yet to Be Fulfilled. doi:10.1101/034538

United States of America, Food and Drug Administration, Office of Women's Health. (2014). *FDA ACTION PLAN TO ENHANCE THE COLLECTION AND AVAILABILITY OF DEMOGRAPHIC SUBGROUP DATA* (pp. 1-39). Washington, DC: FDA.

Vargesson, N. (2015). Thalidomide-induced teratogenesis: History and mechanisms. *Birth Defects Research Part C: Embryo Today: Reviews, 105*(2), 140-156. doi:10.1002/bdrc.21096

Wells, R. G. (2013). Tissue mechanics and fibrosis. *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease, 1832*(7), 884-890. doi:10.1016/j.bbadis.2013.02.007

Wernig, G., Chen, S., Cui, L., Neste, C. V., Tsai, J. M., Kambham, N., . . . Weissman, I. L. (2017). Unifying mechanism for different fibrotic diseases. *Proceedings of the National Academy of Sciences, 114*(18), 4757-4762. doi:10.1073/pnas.1621375114