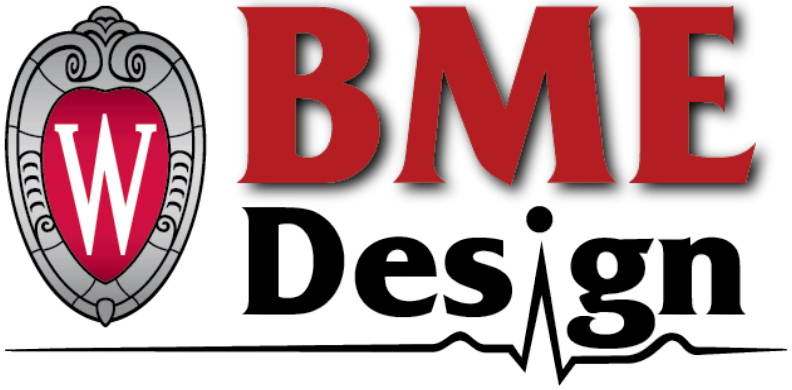
Tissue Model of The Epithelial Mesenchymal Trophic Unit



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BME 402

Product Design Specification

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Function:

Dr. Allan Brasier and his research team have a need for a 3D model of the small airway of the lung that varies in extracellular matrix (ECM) thickness and composition. This tissue model will be designed with fibroblasts that facilitate ECM production and effector cells during injury response. The model will include an air-liquid interface (ALI) that allows for *in vitro* research of the small airway to explore how the ECM, epithelium, and fibroblasts orchestrate reparations after damage. This model will be produced as a bioprinted 3D scaffold that has mechanical and biochemical properties that will be compatible with the lung epithelial cells used for experimentation. The ECM scaffold will allow for cellular communication and function similar to that of an *in vivo* environment.

Client Requirements:

- The product should be amenable to analysis via various techniques such as microscopy and should allow for the encapsulation of lung fibroblasts and cell culture of epithelial cells. Provide a suitable microenvironment for cell-cell interactions and ECM remodeling, as well as enable comprehensive analysis of changes in cell behavior.
- Model should include an air-liquid interface to reflect the polarization of the epithelium in the presence of air.
- Scaffold should have tunable mechanical properties to reflect that of native ECM.
- Product needs to be capable of cell encapsulation and be cell adhesive.
- The product needs to provide an environment that allows for ECM remodeling by encapsulated cells and/or cells coating the scaffold surface.
- The products must be replicable and fabricated using a Cellink bioprinter.

Design Requirements:

1. **Physical and Operational Characteristics**

a. Performance Requirements:

The tissue model will consist of a bioprinted scaffold, encapsulated fibroblasts, and seeded human small airway epithelial cells (hSAECs). The scaffold must be able to function as a cell culture platform; therefore, it must provide the proper biochemical and mechanical signals for cell growth and viability. Additionally, the tissue model system must be sustained for as long as the client needs to run experiments on the cultured cells which will take an estimated two weeks.

b. Safety:

The tissue model of the EMTU will include human small airway epithelial cells as well as fibroblasts. Since human cells will be used, all cell culturing and scaffold seeding must be conducted in a BSL-2 lab. When working with human cells, the concept of “Universal Precautions” is used to reduce the risk of bloodborne pathogens. This concept states that all unfixed tissues and cells are assumed to be infectious, which requires them to be handled using

BSL-2 practices and procedures. Proper PPE must be worn while handling the cells. Additionally, anyone who works with the cells must have completed UW-Madison's Biosafety Required Training, as well as any other additional training required by the BSL-2 lab in use [1].

c. *Accuracy and Reliability:*

i. *Mechanical Properties:*

The scaffold will undergo testing to ensure it meets the mechanical properties necessary to accurately represent the lung ECM. Measuring the Young's Modulus (E) quantifies the stiffness of the hydrogel and can be used to determine whether the hydrogel will accurately mimic its respective tissue properties. The scaffold must have a tunable Elastic Modulus ranging from 3.5-16.5 kPa to reflect the environment that fibroblasts experience through healthy lung tissue to fibrotic lung tissue. The scaffold will be considered mimetic of healthy lung ECM if the mechanical properties are within 5% of the values of native tissue.

ii. *Cell Adhesion and Viability:*

The scaffold will have cell culture of hSAECs cultured upon it for ideally two weeks. To allow for this, the scaffold must mimic the small airway ECM and allow for cell adhesion necessary for proliferation. Beyond this, fibroblasts will then be encapsulated to make the model further resemble the *in vivo* environment of the EMTU. Furthermore, the composition of the scaffold along with the process involved in making the model must be replicable in order to build confidence in the merit of results obtained from scaffold use. To ensure that the scaffold is capable of providing an environment for viable cell adhesion, testing will be performed on the initial sets of scaffolds. This will involve microscopic imaging of the cells to study their attachment to the scaffold, as well as their shape and viability. The shape of the cell within the scaffold can be compared to their shape in the native state to gain insight to their functioning within the hydrogel. Additionally, cell viability will be monitored to ensure the success of the hydrogel to support cell culture. The scaffold will be considered capable of providing an adequate microenvironment if at least 80% of seeded cells are able to proliferate.

d. *Life in Service:*

The bioprinted tissue-model product will be able to be maintained for at least one month. During this period, the product will continue to be compatible with and allow researchers time to implement various microscopy techniques for in-depth analysis while facilitating the encapsulation and culture of lung fibroblasts and epithelial cells within an ALI that encourages cell-cell interactions and ECM remodeling. Importantly, the 3D tissue model will remain replicable, consistently fabricated using a CELLINK bioprinter, following the client's technology requirement.

e. *Operating Environment:*

Once the 3D scaffold is assembled in sterile conditions, the testing will be performed in a cell culture environment. The scaffold will be left to swell for at least 24 hours in DMEM incubating at 37 ℃ and the air inside will have a 5% CO_2 concentration. Cell seeding will be conducted in a sterile environment and will incubate at 37 ℃ and the air inside will have a 5% CO_2 concentration.

f. Ergonomics:

A clear, concise protocol must be written so that bioprinting the scaffolds will be easily replicable as to increase the efficiency of fabrication and make use of the product easy for the client.

g. Size:

The cylindrical scaffold will have a diameter of 9 mm and should be at least 10 microns thick to allow for the embedding of fibroblasts into the scaffold. The cells will then be cultured in a 12-well or 24-well plate with diameter of 22.4 mm [4].

h. Materials:

The tissue scaffold must support three main criteria. First, it must be able to produce a variable range of stiffnesses, as described in the mechanical properties section (1.c.i). The material must also be conducive to cell adhesion, contain RGD peptides to allow for integrin binding. The material must also be conducive to matrix remodeling, containing motifs which are sensitive to matrix metalloproteinases.

The hydrogel will be fabricated from Gelatin Methacrylate (GelMA). Work completed in the previous semester has demonstrated GelMA is capable of spanning a range of stiffnesses, mimicking the mechanical microenvironment of both healthy and fibrotic lung tissue. The team has access to a 3D bioprinter from CELLINK, a company which also sells GelMA bioinks. The team will fabricate the tissue model using CELLINK bioink such that we can be consistent with the recommendation of CELLINK for effective prints.

i. Aesthetics, Appearance, and Finish:

The scaffold should have an overall appearance that resembles the small airway ECM. Additionally, the scaffold must be translucent for optical clarity – the scaffold will be imaged in order to visualize the cell culture. As it is intended to accurately model the stiffness and composition of the ECM, the main focus of the scaffold will be for the tensile strength to be similar to *in vivo* environments as well as allowing for the incorporation of fibronectin and collagen to mimic a natural state. These functional properties take priority over the aesthetic aspects of the design. This will allow for the epithelial cells to attach to the scaffold with a normal morphology in order to create a realistic model of the EMTU.

2. **Production Characteristics**

a. Quantity:

Models of both healthy and fibrotic lung tissue are desired, so scaffolds with mechanical properties matching the aforementioned conditions must be fabricated. Therefore, at least 6 scaffolds are needed, three of each condition, for the purpose of replicability and statistical analysis.

b. Target Product Cost:

The materials for the scaffold should cost no more than $500. In the prior semesters, the team used $1091 of the $5000 budget, so there is $3909 left to spend. The new scaffold design will be made using the client's bioprinter, so the only cost should be the GelMA bioink cartridges.

3. **Miscellaneous**

a. Standards and Specifications:

FDA approval is required for synthetic 3D scaffolds when they are brought to market. The standards and regulations for these products are governed by ASTM F2150-19: Standard Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products [5]. However, the FDA does not have specific standards or specifications for the use of 3D synthetic scaffolds to study epithelial-mesenchymal transition (EMTU). Therefore, proper protocols for biocompatibility, sterilization, and labeling must still be followed. The standards and regulations for the use of bioinks and extrusion-based bioprinting are still under development, but there are some relevant standards that are currently being developed, such as ASTM WK72274: New Test Method for Printability of Bioinks for Extrusion-based Bioprinting and ASTM WK65681: New Guide for Bioinks and biomaterial inks used in bioprinting.[6] These standards do not yet provide specific guidance on logistics or compliance criteria. Additionally, there are many FDA requirements surrounding the use of cell and tissue culture products, which are outlined in Standard 21CFR864 [7]. The purpose of these regulations is to ensure that all research is conducted ethically and with appropriate oversight.

b. Customer:

The client has recently purchased a 3D bioprinter from CELLINK, intended for the team's development of the tissue model. As such, the client would like us to make use of the bioprinter. The client has also expressed a desire to use commercially available GelMA from CELLINK, rather than GelMA synthesized and characterized by the team. This will make replication and fabrication much simpler for the client once the team is no longer available.

c. Competition:

Tissue engineering models to provide in vitro means to study the body has in recent years created many impressive novel designs. For models looking specifically at the lung epithelium, there are currently both 2D and 3D models that mimic the in vivo environment. Unfortunately, these models are oversimplified and do not provide accurate research results from experiments done on these models.

Looking into 2D models, these are typically layers of cells on top of polymer or glass dishes. In the past several years, many experiments have been conducted on these 2D models, but while they have allowed some study into cell function, disease, and the microenvironment, the models greatly lack the typical native environment cell behavior. For reference, the 2D models have a stiffness range of 2-4 GPa while the human lung ranges from .44-7.5 kPa [8]. The differences in stiffness significantly change the behavior of the cells, and thus the experimental data found on them are reflective of in vivo behavior.

While there are many varieties of 3D models on the market, one of the most favorable is a co-culture model using ECM protein gel (matrigel). These models are produced by embedding cells in matrigel and culturing them directly on the surface [9]. These 3D models have variations in methods for each experiment, but generally all involve an ECM gel 3D environment that is more similar (with some limitations) to in vivo than the 2D models. Matrigel is a mouse tumor extracellular matrix mixture, so there are variations for every batch and consists of proteins that don't accurately represent healthy ECM. Matrigel ECM is much similar to the ECM of a tumor with significantly more laminin glycoproteins, which can cause the microenvironment to be unlike native tissue.

The gaps between these models and the in vivo environment result in a lack of data and findings that accurately represent what is happening in the body. As a result, a bio-scaffold of the lung ECM is a model that would bridge the gap between in vitro studies and in vivo actions at the cellular level.

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