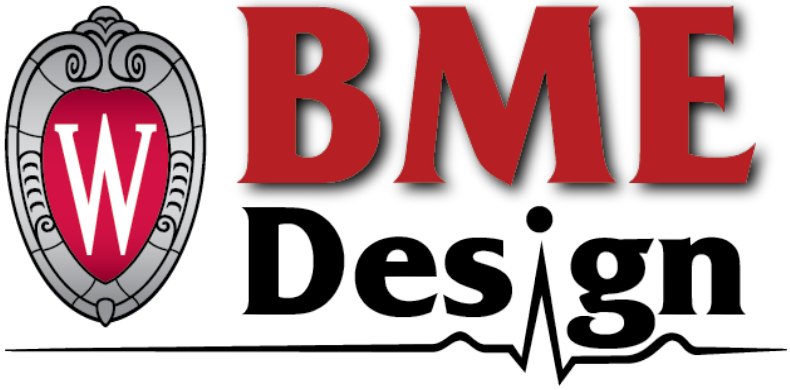
Tissue Model of The Epithelial Mesenchymal Trophic Unit



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BME 301

Product Design Specification

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Function:

Dr. Allan Brasier and his research team have a need for a 3-D 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 3-D 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:

- Product should allow for the exploration of cell-cell interactions and the effects of changes in the extracellular matrix due to respiratory virus on the activity and state of fibroblasts within the lung.
- Model should include an air-liquid interface to reflect the polarization of the epithelium in the presence of air.
- Matrix should have tunable mechanical properties to reflect that of native ECM.
- Product needs to be cable of cell encapsulation or means for cell adhesion
- The product needs to provide an environment that allows for ECM reconstruction via encapsulated or coated cells

Design Requirements:

1. **Physical and Operational Characteristics**

a. Performance Requirements:

The scaffold has both biochemical and structural factors that affect its success at providing an environment that is similar to the native lung ECM. Looking into structure, the scaffold must be in similar tension to that of native tissue because even slight differences can affect how the cells function. For example, tensioned ECM will induce a stretching of the cells' cytoskeleton, and compression of the ECM will result in an altered local charge of cells [1].

Using synthetic ECM materials allows the fine tuning of mechanical and other biophysical properties but has limitations with cell-cell communication which is vital for ECM functionality. As a result, the model will include the ability to encapsulate collagen and fibronectin to facilitate the biochemical communication aspect of the ECM or provide these interactions via incorporated peptides. This model must meet these requirements of biochemical and mechanical properties to best mimic the native tissue environment.

b. Safety:

Working with human epithelial cells and cell culturing requires chemical training as cell or tissue cultures can be associated with human pathogens. The following cell cultures and tissues require biosafety level 2 (BSL-2) practices and procedures:

1. All cultured cells derived from human sources, including immortalized and "well established" cell lines.
2. All cultured cells derived from nonhuman primate tissue.
3. All cultured cells exposed to, or transformed by, a primate oncogenic virus.
4. All human clinical materials, such as samples of human tissue, obtained from surgery, biopsy, or autopsy.
5. All primate tissue.
6. All virus-containing primate cultured cells.
7. All mycoplasma contains cultured cells.

When working with human and tissue 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. Lab personnel must also receive annual OSHA bloodborne pathogens training. The adherence of these standards is key to ensuring safety of all laboratory personnel [2].

c. Accuracy and Reliability:

The scaffold will undergo tension and compression testing to ensure it meets the mechanical properties necessary to accurately represent the lung ECM. In addition to measuring

the Young's Modulus (E), the lungs exhibit viscoelastic properties which require the storage modulus (G') and loss modulus (G'') to also be obtained. The scaffold must have a Young's Modulus ranging from 2-16.5 kPa to accurately mimic the environment that fibroblasts experience in healthy lung tissue and in fibrotic lung tissue. The viscoelastic properties for healthy lung tissue must include the storage modulus (G'= 500 Pa) and loss modulus (G'' = 50 Pa). Fibrotic lung tissue viscoelastic properties must also be met by the storage modulus (G' = 5 kPa) and loss modulus (G'' = 500 Pa)[3]. Additionally, it will be tested with active cell cultures grown for one month to ensure it can mimic the ECM and that the cells attach normally. Beyond this testing, other cell layers and components will then be added to make the model further resemble the in vivo environment of the EMTU. Further, 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 cell adhesion and proliferation 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. 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 measurements with these first hydrogels will provide knowledge on how well the mechanical properties, porosity, and the overall biochemistry of the hydrogel supports proliferation.

d. Life in Service:

The tissue model should be maintained for a minimum of 1 month to perform the human ALI cell culture method. This month of time will allow for optimal cell culturing on the scaffold so that any testing done will reflect not only cell viability but also the degradation and reconstruction of the ECM timeline.

e. Operating Environment:

Once the 3-D scaffold is assembled in sterile conditions, the testing will be performed in a cell culture environment. This environment will include HEPA filtered air. The filter will remove 99.97% of dust and air borne particles with a size of 0.3–10 microns [3]. The cell environment will be kept at 37 degrees celcius and the air inside will have a CO_2 concentration

of 5%. When not being used for research, the scaffold will be stored on a 1’ x 10” x 1’ cell culture rack [2].

f. Ergonomics:

The model should mimic the tension and morphology of the extracellular matrix (ECM) as closely as possible. This involves having the viscoelastic properties as discussed prior, porosity that allows for exchange of material, cell adhesion and proliferation, and degradation of the matrix to allow for ECM reconstruction.

g. Size:

The scaffold will have an area of 1 sq. cm and should be at least 10 microns deep 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 client did not give specific requirements for the material to be used for the project in an effort to not bias the design process. Materials to be used for creating a hydrogel can range from synthetic materials such as polyethylene glycol to a naturally based hydrogel like collagen. Based on the clients requirements for mechanical tunability creating a synthetic to semi-synthetic hydrogel would allow for this precision best. The chemistry of the scaffold needs to allow for proteins, specifically fibronectin and collagen, to be added under both stressed and normal environments. This means that the material must be biocompatible and allow for cell adhesion to the scaffold.

i. Aesthetics, Appearance, and Finish:

The scaffold should have an overall appearance that will mimic the small airway ECM as closely as possible. As it is intended to accurately model the stiffness and composition of the ECM, the main focus of the scaffold will be for the tension to be similar to in vivo environments as well as allowing for the incorporation of fibronectin and collagen to mimic a natural state. 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:

It is intended to produce scaffolds with variable stiffness, beyond that of healthy lung epithelia to model fibrotic or other diseased states. Therefore, as proof of concept to produce variability, a gradient of scaffold stiffnesses will be produced across a 24 well plate. Each scaffold, sized to a well plate as discussed in section 1.g should house 10^4 to 10^5 cells for DNA isolation and flow cytometry [5].

b. Target Product Cost:

The materials for the scaffold should cost no more than $500. In a prior semester, the team used around $1000 of the $5000 budget on the scaffold design, so there is around $4000 left to spend. The new scaffold design will be made from less expensive materials such as gelatin based hydrogels or use of polyethylene glycol materials from the prior semester.

3. Miscellaneous

a. Standards and Specifications:

FDA approval is required for these types of synthetic 3-D scaffolds. The standard and regulations of these products fall under ASTM F2150-19: Standard Guide for Characterization and Testing of Biomaterial Scaffolds Used in Regenerative Medicine and Tissue-Engineered Medical Products [5]. Before reaching the market, the design must abide by these FDA standards and address any risk that the device may have. There are also many FDA requirements surrounding the use of cell and tissue culture products which fall under Standard 21CFR864 [6].

b. Customer:

As of the initial meetings, the client does not have specific preferences for how to proceed through the design process, provided that the requirements outlined above are met. Preliminary meetings suggest the use of a synthetic to semi-synthetic material based on their preference of precise mechanical properties.

c. Competition:

Tissue engineering models to provide in vitro means to study the body has in recent years created many amazing and novel designs. For models looking specifically at the lung epithelium, there are currently both 2-D and 3-D models on the market 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 2-D 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 2-D 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 2-D models have a stiffness range of 2-4 GPa while the human lung ranges from .44-7.5 kPa [7]. The differences in stiffness greatly change the behavior of the cells and thus the experimental data found on them are not as accurate as in vivo.

While there are many varieties of 3-D 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 [8]. These 3-D models have variations in methods for each experiment, but generally all involve an ECM gel 3-D environment that is more similar (with some limitations) to in vivo than the 2-D 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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