Matrix F.T. Microcarriers and Scaffolds:

Product Feature: Optimization for Cell Attachment

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Matrix F.T's microcarriers and scaffolds are uniquely edible and animal-component free; expected yield is dependent on a number of factors that can be optimized by the user for their specific cell line and growth conditions using guidance provided by Matrix F.T.

Introduction

Anchorage-dependent cells require a substrate for growth and proliferation. Matrix F.T. microcarriers are designed to support cell attachment and growth of these cells in suspension on microcarriers and on scaffolds in a fixed or static environment.

Microcarriers have been used for decades in cell culture to increase the yield of cells per mL.[1,2] Methods to grow cells on microcarriers in suspension include suspension flasks or bioreactors that come in a number of designs and volumes.[3] Factors influencing cell culture performance on microcarriers in these vessels include cell seeding time, cell density, microbead loading concentration, mixing speed, media, access to nutrients, gasses and duration of growth (Table 1).

These variables must be optimized to increase performance and yield.

Matrix F.T. microcarriers and scaffolds are designed for use in the innovative field of cultivated meat, therefore, it is expected that users of these products will be developing customized bioreactors, proprietary media and/or novel characterized cell lines that will require additional measures for optimization compared to standard established equipment and cell growth methodologies. Matrix F.T. works with companies in the industry to find the best fit for unique research purposes, products and processes. The summary section below explains Contract Research Service and Sample Kit testing opportunities.

Optimizing Cell Attachment

Table 1. Parameters for cell culture, microcarrier and scaffold optimization

Parameter	Description	Matrix F.T. Recommendations
Cell Seeding Density	Initial cell density should be optimized for best downstream outcome (yield, biomass etc.)	1-5e^5 cells/mL in growth media for microcarriers, 1e^5-1e^6 cells per cm^2 of scaffold depending on cell type
Cell Seeding Method	Variables include time, intermittent or constant agitation, days in culture, desired maximum cell density[5]	Cell line specific; 30 min to overnight for most cell lines
Microcarrier Loading Density	Loading concentration must be optimized and is dependent on the type of microcarrier	0.5-1.0 mg/mL for Matrix F.T. dry microcarriers, 2.0-5.0 mg/mL for Matrix F.T. wet microcarriers
Microcarrier Format	Cells may be optimized on fibrous[4], textured, aligned, specific protein formulations, surface treatments and more[6]	The Matrix F.T. sample kit contains a variety of plant proteins in wet, dry, textured, microsheet, and/or microbead formulations for screening
Mixing Speed	Consider shaker (RPM), impeller speed, shear stress, fluid dynamics, gas and nutrient exchange	Important to optimize, follow current literature and manufacturing guidelines appropriate for the scale of the vessel in use[2,5,9]
Mixing Vessels	Dish, flask, spinner flask, bioreactor	Dishes are ideal for static scaffold testing, microcarriers designed for suspension testing should be tested in suspension vessels
Media	Serum-containing, cell line specific, serum free, differentiation media, proprietary formulations	Change media frequently and gently to prevent accumulation of by-products[7], expect that novel or serum free media may lead to slower growth rates and reduced yield[5,8]
Culture Conditions	Temperature, CO_2/O_2 levels, humidity	Follow current literature and protocols associated with the specific cell line in use

Cell and Microcarrier Loading Optimization

The initial amount of cells added to a microcarrier suspension can influence cell attachment and yield. Starting with a low cell number to generate cell mass over time may be desired when there is a limited number of cells to start with. Additionally, if a more rapid time to confluence is desired, starting with a higher cell seeding density may be preferred. Experiments should be designed to include a range of cell seeding densities that are appropriate for the goals of the user. If cell seeding density is too high, cell sheets or aggregates may be present, if too low, microcarriers may not become adequately covered (Figure 1). Cell to cell contact is important for cell proliferation signaling and contact inhibition may limit overall microcarrier confluence.[9]

Microcarrier loading density is an important factor for optimization as well as cell seeding concentration, if microcarrier loading is too high, nutrient and gas exchange will be limited and cells can become constricted, and undergo stress and damage in a stirred system (Figure 2). Additionally, cell sheets and aggregates may form if microcarrier loading density is low (Figure 3). Intermittent mixing during cell seeding may also improve cell attachment in order to optimize conditions for microcarrier loading and cell seeding density (Figure 4).

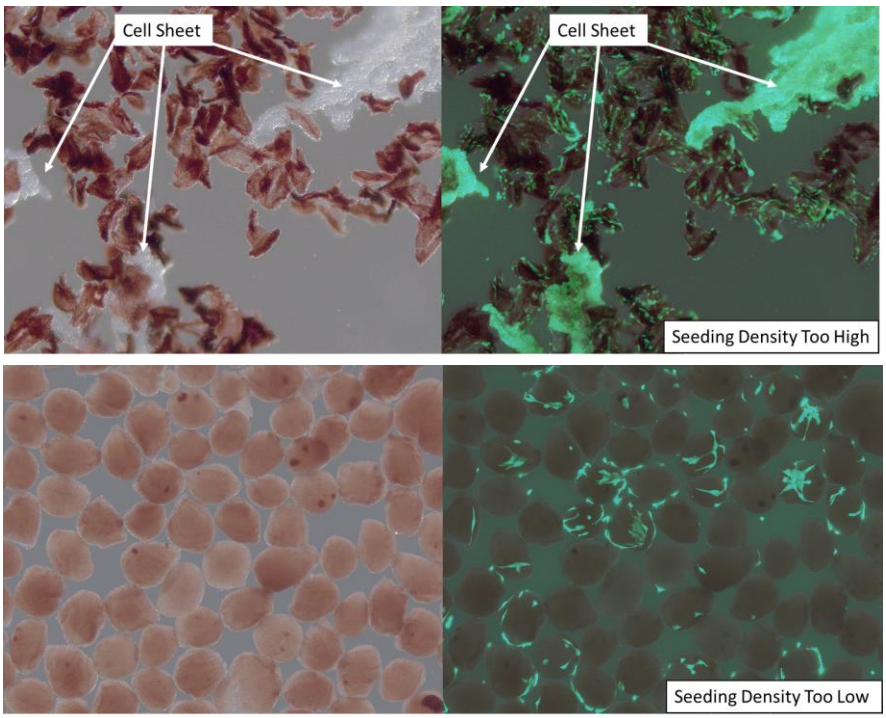


Figure 1. Live cell (C2C12) attachment on Matrix F.T. soy microcarriers (upper panel) and Matrix F.T. pea microbead (lower panel), 4X with brightfield (left) and merged with Calcein live cell stain (right).

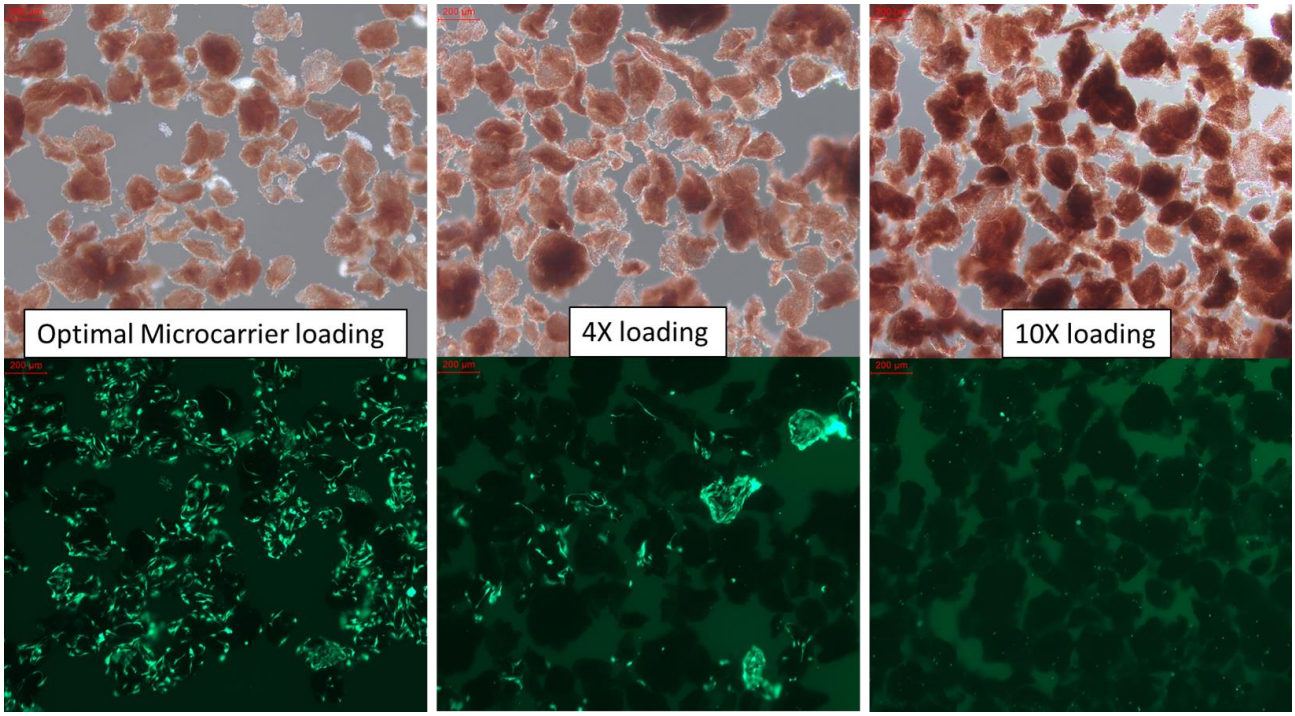


Figure 2. C2C12 cells with increasing microcarrier loading densities at three concentrations on Matrix F.T. pea microcarriers, 4X objective with brightfield imaging (upper) and parallel Calcein live cell stain (lower).

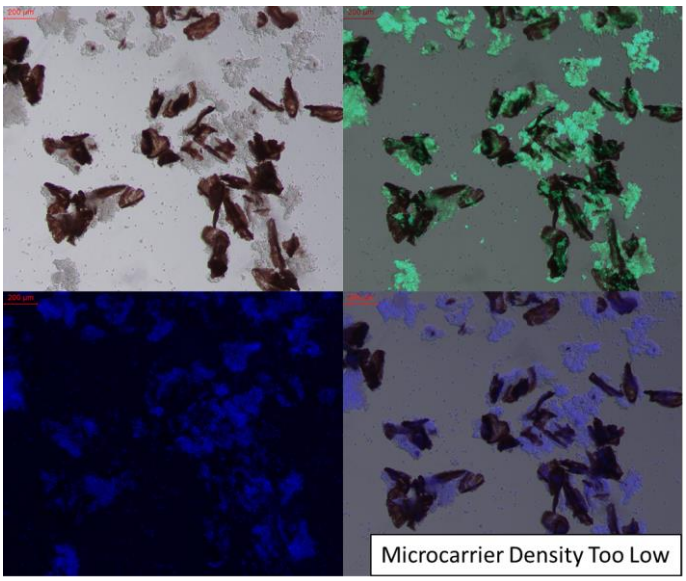


Figure 3. C2C12 cells seeded with Matrix F.T. soy microcarriers, 4X objective with brightfield imaging (top left) merged with parallel Calcein live cell stain (green) and nuclear Hoescht stain (blue).

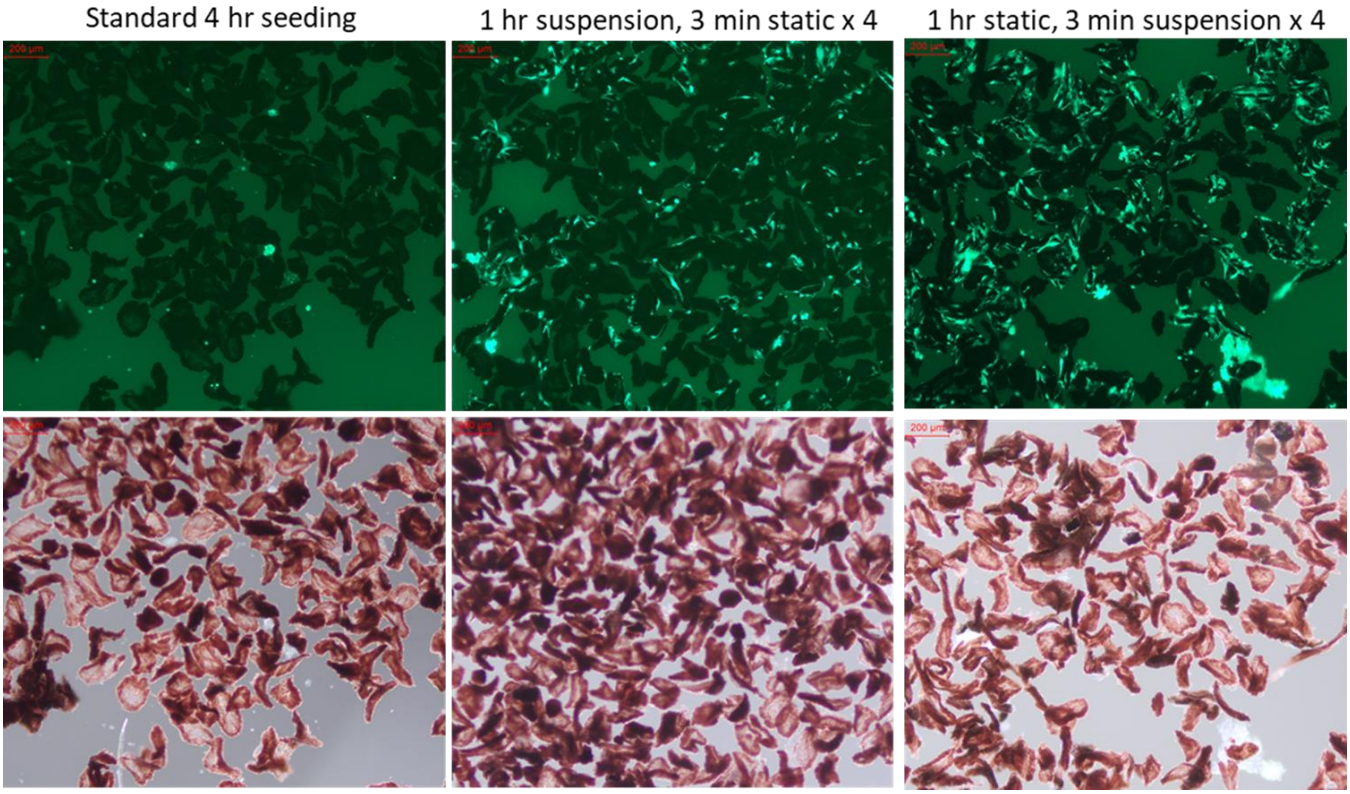


Figure 4. Seeding methodology variations with C2C12 cells on Matrix F.T. soy microcarriers. 4X objective with brightfield imaging (lower) and parallel Calcein live cell stain (upper).

Microcarrier Customization and Optimization

Optimization can be performed with the established Matrix F.T. ingredients and coatings or alternatively, the microcarriers and scaffolds themselves can be customized in formulation or production steps to include surface treatment and ingredients of interest to the user. Customizations can include the scaffold or microbead initial formulation of plant proteins (corn, soy, pea, etc.), surface treatment, size, texture and more. Figure 5 illustrates cell culture outcomes that were optimized by modification of microcarrier processing steps.

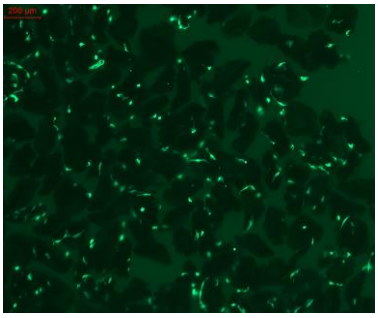
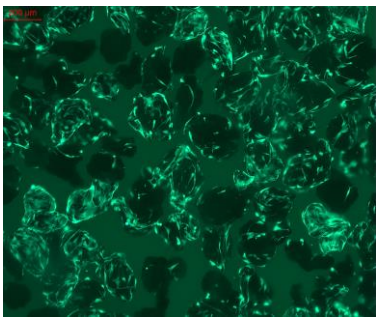
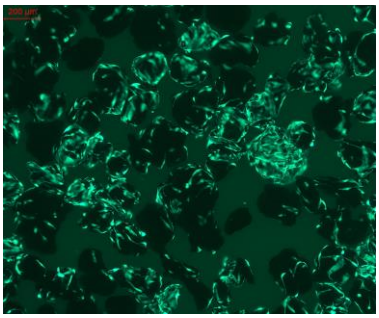
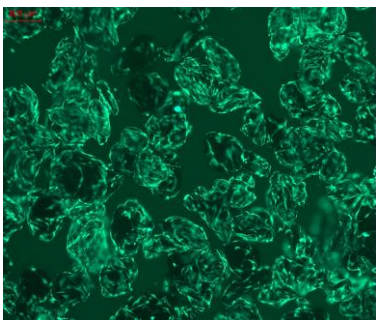
Coating concentration A Coating time A Batch size A Production method A	
Coating concentration A Coating Time B Batch Size A Production method A	
Coating concentration A Coating time B Batch size B Production method A	
Coating concentration A Coating time A Batch size A Production method B	

Figure 5. Microcarrier variations for optimization in the microcarrier production process with associated cell culture outcome (right); microcarriers were seeded with C2C12 cells on Matrix F.T. soy microcarriers. 4X objective using Calcein live cell stain (green).

Summary and Matrix F.T. Services

Matrix F.T. evaluates microcarrier and scaffold designs in-house in order to understand cell culture performance and make recommendations based on the product type to streamline protocol development for customers according to the microcarrier format and composition. Matrix F.T. has a wide portfolio of products available to customers and provides associated usage guides and application notes for guidance on their use. If specific method development with select cells and media and/or customized microcarriers and scaffolds are desired, please reach out to our sales team at sales@matrixfood.tech for Contract Research Service opportunities. Matrix F.T. also provides a free 5-sample kit to customers to evaluate using their cells and technology (https://matrixfood.tech/free-sample-kit-1).

References

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