Matrix F.T. Microcarriers and Scaffolds:

Product Feature: Evaluation of Cells Grown on Microcarriers and Scaffolds

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Matrix F.T's microcarriers and scaffolds are uniquely edible and animal-component free; once cells are grown on them, cell yield, growth rate, differentiation, morphology and metabolism can be determined in a number of ways as described in this document.

Introduction

Matrix F.T. microcarriers are designed to support cell attachment and growth for adherent cells grown in suspension, while scaffolds support adherent cell growth and expansion 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]. Edible scaffolds and microcarriers allow for scale-up of adherent cells capable of differentiating. The vast majority of commercially available microcarriers and scaffolds for cell culture are not edible and require additional steps and reagents to harvest the cells from the microcarriers or scaffolds. Matrix F.T. microcarriers and scaffolds are designed to be edible in order to be incorporated into the final cultivated meat product.

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. These variables need to be optimized based on cell type to increase performance and yield.

Evaluation of the cells on the microcarriers and scaffolds after seeding and growth can provide quantitative and qualitative information on cell number, identity, differentiation status, morphology, viability and metabolic characteristics.

Technical Methods Overview

Cell characteristics and growth can be evaluated on microcarriers and scaffolds both qualitatively and quantitatively. The methods of which are common to cell and molecular biology and can be tailored to the requirements of the cultivated meat industry. Due to the edible nature and properties of the Matrix F.T. products, certain modifications of traditional methods may be needed and are noted where relevant. Please note: Matrix F.T. does not have formal affiliation or agreements with the companies or materials described, understandably customers will have

different needs and availability to equipment and reagents, the protocols included here are for general reference and guidance as relevant to the use of microcarriers and scaffolds in the field.

Qualitative Methods

Qualitative methods of evaluation, such as imaging, can provide valuable information about cells grown in a 3D environment on scaffolds and microcarriers. Staining for viability, intracellular proteins and nuclei can show how well cells have established and spread across and within the surface of a scaffold or microcarrier. Observing the morphology of the cells can show compatibility, confluence, alignment, invasion and differentiation to help inform studies of cells and products for cultivated meat.

- **Fluorescent Staining**
  - Viability can be evaluated using Live/Dead cell staining, using live cell green calcein AM or fluorescein diacetate stain with red staining of dead cells using ethidium homodimer or propidium iodide [3,4,5,11]
  - Structure, differentiation and protein expression can be observed using cells that are fixed and stained using immunohistochemistry to show protein expression of muscle fibers such as myosin heavy chain (MHC)[3,4], actin and phalloidin.
  - Lipid accumulation and fat deposits can be stained using oil red O, nile red or other similar fat specific stains
  - Note: traditional wash and buffer reagents such as PBS can compromise the structural integrity of some microcarrier and scaffold products, it is advised to substitute culture media (phenol-red free if available, for steps traditionally requiring PBS)
- **Histology**
  - Can be used on preserved and/or frozen sections to stain for structural features using H&E or other desired histological stains. [4]
- **Image Analysis**
  - Can provide quantitative data based on the staining and imaging results. Software programs (ImageJ, CellProfiler, Biodock) can quantify the number of nuclei per microcarrier and/or the number of live and dead cells based on the color, cell size and other characteristics.
- **Experimental Design**
  - Experiments run in parallel with replicates can be used with imaging or other metrics to compare the outcomes of visually improved cell attachment over time, such as observations of established cells, confluence, microcarrier to microcarrier transfer and similar outcomes by changing variables such as cell seeding concentration and time,

with or without intermittent mixing, microcarrier loading concentration, cell growth and expansion time, different media or vessel, etc.

Quantitative Methods

The quantitative methods available to evaluate cell yield, gene expression, metabolism and other molecular characteristics are extensive. Below are several that have been used in commercial and published methods with microcarriers.

- **Quantifying cells and assessing cell proliferation and cytotoxicity** using a fluorescent dye and plate reader to measure DNA in a sample can be performed using seeded microcarriers, the DNA is collected from frozen and lysed cells. For example CyQUANT® Cell Proliferation Assay from Thermofisher, Cat. C7026
- **Quantitation of viable cell number using a colorimetric proliferation and cytotoxicity assay** Cell Counting Kit - 8 (Sigma, Cat. 96992), can be used by reading the supernatant of seeded microcarriers as described[6] and/or according to the manufacturers recommendations.
- **MTT assay to measure cellular metabolic activity related to viability, proliferation and cytotoxicity** (i.e. Sigma (Roche) Cat. 11465007001) reading the supernatant of seeded microcarriers using a plate reader according to the manufacturers recommendations.
- **RT-qPCR to quantify expression of genes of interest** can be prepared as described [7,8,9,11] or according to standard protocols. For cultivated meat, genes of interest may include Myh4, Mef2C, Pax7, MHC or other genes specific to cell/animal type (fish, fat etc.).
- **Quantitative metabolic Resazurin assay** of cells on scaffolds as shown[10] or microcarriers to quantify the number of live cells in a sample, cell viability and/or cytotoxicity.
- **Cell viability using PrestoBlue[3,4] or alamarBlue[11] analysis** using a plate reader as referenced or according to the manufacturers recommendations.

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. is continually adding to the portfolio of products available to customers. As novel materials and microcarrier formats become available and in-house testing capabilities increase, additional information will become available to inform expected yield, product metrics and expanded product usage. If specific method development with cells and media are desired and/or customized microcarriers and scaffolds, 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).

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