



**NEXTURE**  
BIO

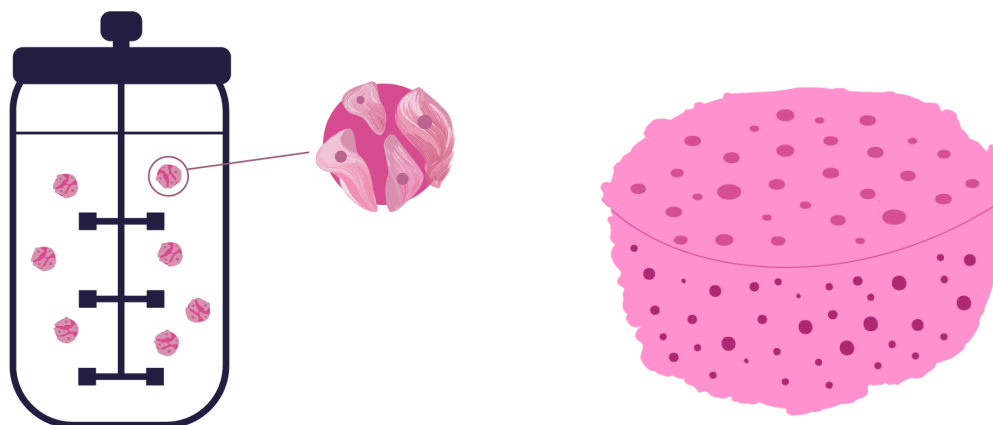
# Evaluation of Cells Grown on Nexture Bio's Microcarriers & Scaffolds

Nexture Bio microcarriers and scaffolds are animal-component-free and made with ingredients that are safe and suitable for use in food. 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

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Nexture Bio's microcarriers are designed to support cell attachment and growth for adherent cells grown in suspension, whereas scaffolds support adherent cell growth and expansion in a fixed or static environment.



**Figure 1.** Adherent cells can be grown in 3D, such as on microcarriers in suspension (left) and on fibrous or porous scaffolds (right)

Microcarriers have been used for decades in cell culture to increase the yield of cells per mL<sup>1,2</sup>. Edible scaffolds and microcarriers enable the scale-up of adherent cells in long-term culture, allowing them to proliferate and differentiate. 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. Nexture Bio's microcarriers and scaffolds are designed to be incorporated into a final tissue engineering or cultivated meat product.

Methods for growing cells on microcarriers in suspension include the use of suspension flasks or bioreactors, which come in a variety of designs and volumes<sup>3</sup>. Factors influencing cell culture performance on microcarriers in these vessels include cell seeding time, cell density, microcarrier loading concentration, mixing speed, media, access to nutrients, gases, 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, functionality, differentiation status, morphology, viability, and metabolic characteristics. Additionally, in some systems, samples can be collected throughout the experiment for evaluation and analysis.

## Technical Methods Overview

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Cell characteristics and growth can be determined on microcarriers and scaffolds both qualitatively and quantitatively. The recommended methods captured below are common to cell and molecular biology and can be tailored to the requirements of tissue engineering, cell therapy, and the cultivated meat industry.

*Please note: Nexture Bio does not have formal affiliations or agreements with the companies or materials described. 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

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Qualitative methods of evaluation, such as imaging, can provide valuable information about cells grown in a 3D environment on scaffolds and microcarriers. Observing the morphology of the cells can show compatibility, confluence, alignment, invasion, and differentiation, which helps inform studies of cells and products for 3D cell culture. 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.

## Fluorescent Staining

- Viability can be evaluated using live/dead cell staining, where live cells are stained green with calcein AM or fluorescein diacetate and dead cells are stained red with ethidium homodimer or propidium iodide.<sup>3,4,5,11</sup>
- Structure, differentiation, and protein expression can be observed in cells that are fixed and stained using immunohistochemistry (IHC) to show protein expression of muscle fibers, such as myosin heavy chain (MHC)<sup>3,4</sup>, 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 that traditionally require PBS.*

## Histology

- Can be used on preserved and/or frozen sections to stain for structural features using H&E (Hematoxylin and Eosin staining) or other desired histological stains.<sup>4</sup>

## Image Analysis

- Can provide quantitative data based on the staining and imaging results. Software programs (e.g., 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 conducted with appropriate replicates can be used in conjunction with qualitative methods and other metrics to compare cell culture outcomes. Visually improved cell attachment over time can be observed, for example, through observations of established cells, confluence, morphology, and microcarrier-to-microcarrier transfer. Optimization of cell attachment can involve adjusting variables such as cell seeding concentration, time, intermittent mixing, microcarrier loading concentration, cell growth and expansion time, different media or vessel, etc.

## Quantitative Methods

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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.** The DNA is collected from frozen and lysed cells. The cell number can be determined based on the DNA content measured using a fluorescent dye and a plate reader. Supernatant can be measured for microcarrier and scaffold cultures. 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 performed by reading the supernatant of seeded microcarriers as described<sup>6</sup> and/or according to the manufacturer's 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 manufacturer's recommendations.
- **RT-qPCR to quantify expression of genes of interest** can be prepared as described or according to standard protocols. For cultivated meat, genes of interest may include Myh4, Mef2C, Pax7, MHC, or other genes specific to the cell/animal type (e.g., fish, fat, etc.).
- **Quantitative metabolic and viability measurements using Resazurin assays** of cells on scaffolds<sup>10</sup>, or microcarriers to quantify the number of live cells in a sample, cell viability, and/or cytotoxicity. Specifically, **PrestoBlue<sup>3,4</sup> or alamarBlue<sup>11</sup> analysis** can be used with a plate reader as referenced, or according to the manufacturer's recommendations.
- **Lysing cells off of the microcarrier or scaffold and counting the nuclei** can be performed using lysis buffer and a live/dead stain such as DAPI and Acridine Orange. A system such as ChemoMetec NucleoCounter® NC-202™ can be used to count the nuclei and determine viability, and is designed for GMP settings

## Nexture Bio

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### Products

Nexture Bio's plant-based microcarriers are engineered for performance, biocompatibility, and regulatory ease. Free from animal-derived components and supportive of multiple species and cell types, they offer a clean, ethical, and sustainable solution for modern anchorage-dependent cell culture systems. Our microcarriers support robust cell attachment, proliferation, and differentiation across a variety of applications. To learn more, please visit [nexturebio.com/products](https://nexturebio.com/products).

### Services

At Nexture, we help you move faster from idea to impact. Whether you would like access to infrastructure, scientific talent, and technical processes that you don't have in-house, or you would like to relieve bottlenecks because your teams are at capacity, our services could be a great option for you. Offerings include electrospraying & electrospinning, cell culture services, bioprocess development, and custom projects. To learn more, please visit [nexturebio.com/services](https://nexturebio.com/services).

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