# Product Highlight: Zein Microcarriers

*Animal component free microcarriers to increase cell density for cultivated meat*

*Application Note MC-01-20220401*

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## Introduction

One of the largest challenges facing scale-up of cultivated meat involves increasing cell proliferation and yield in suspension culture; obtaining maximum cell density is crucial for scaling up production in the cellular agriculture field. Microcarriers (MCs) enable “quasi-suspension” culture conditions which are required to allow for high-density culture of adhesive cells.[1,2] Initial efforts to use MCs for cellular agriculture relied upon materials generated by the pharmaceutical industry, leading to high price tags and reliance on inedible or animal-derived materials. At Matrix F.T., making ACF microcarriers and scaffolding is central to our value proposition.

*We do not use animal-derived components in any phase of procurement or manufacturing of our MCs or scaffolds, and we are working on the GRAS conclusion for our product.*

We run a variety of rigorous tests in our lab to understand our zein microcarriers' performance, including incorporating our MCs into Matrix's proprietary plant-based meat recipe to understand how the zein microcarriers may affect the appearance and texture of a consumer-facing cultivated meat product, as described in the ‘Microcarrier Appearance’ section below.

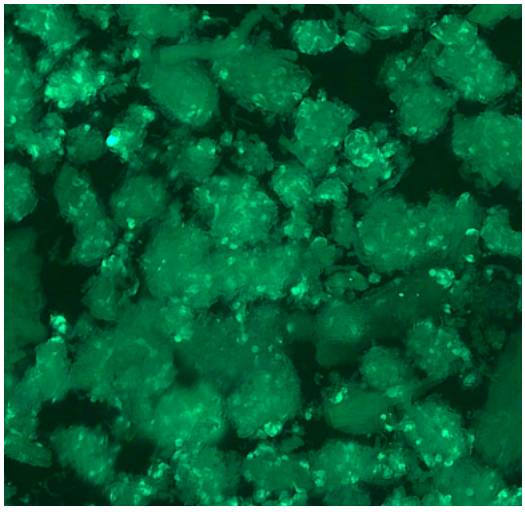


Figure 1: C2C12 cells on zein MCs two days following seeded MC to unseeded MC transfer in suspension.

## Performance

### *Cell Adhesion onto Zein Microcarriers*

Initial tests showed that seeding densities of around 5 x $10^5$ cells/mL with MC loading densities of between 1 mg/mL and 5 mg/mL are potential functional initial inputs to understand cell behavior on zein MCs. Figure 1 shows C2C12 cells that were seeded onto zein MCs at a density of 5 x $10^5$ cells/mL and a MC density of 1 mg/mL. Cells adhered after a 4 hour incubation in static conditions (A) and showed homogenous cell distribution after overnight incubation on an orbital shaker (B-C).

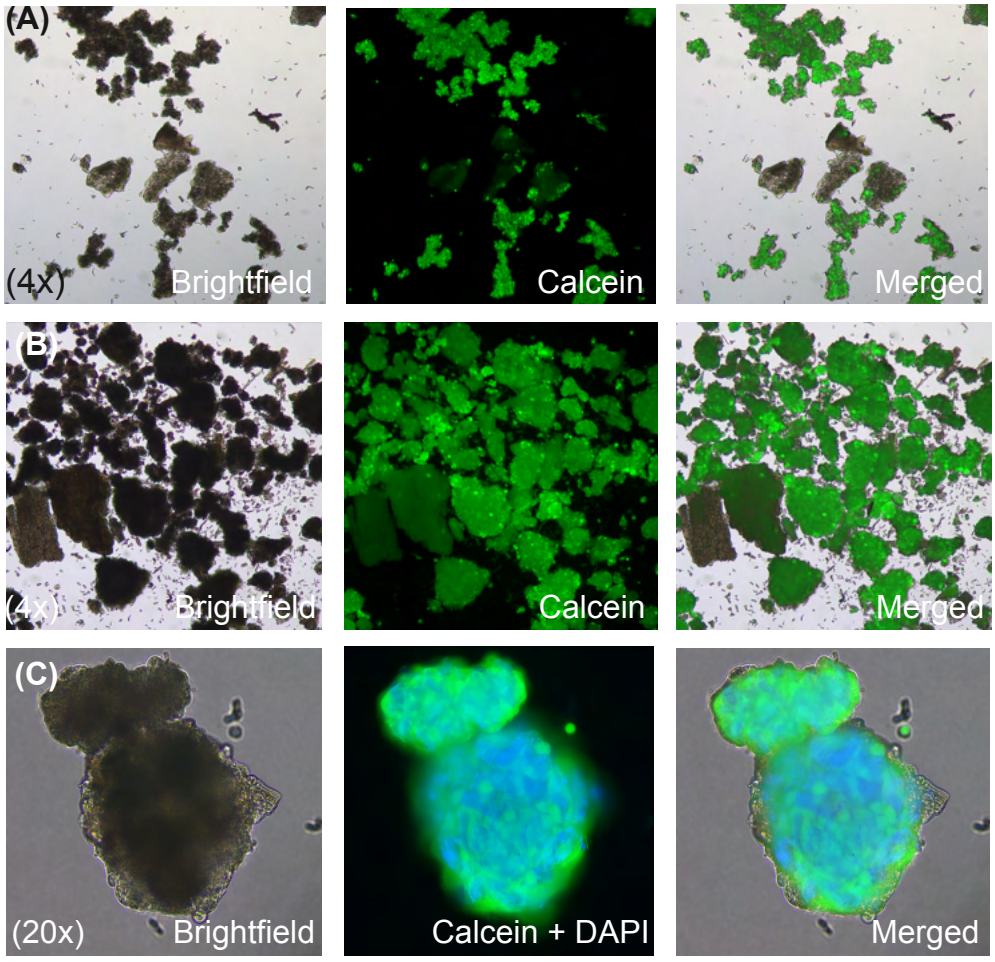
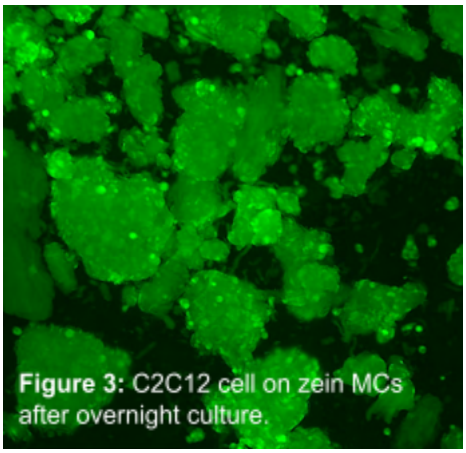



**Figure 2.** C2C12 seeded onto zein microcarriers. Cells were seeded at a density of 5 x $10^5$ cells/mL, and zein scaffolds were loaded with a density of 1 mg/mL. (A) Cells were observed with Calcein fluorescent dye after four hours of incubation in static conditions; (B-C) MCs were stained with Calcein and DAPI after overnight incubation on an orbital shaker.

Cells attached in as little as four hours in static conditions (Figure 1) seeded in a vented conical tube, and were then moved onto an orbital shaker in Erlenmeyer flasks for long-term culture (Figure 2). Cells show the continued ability to attach to the MCs, and data supports that cells are able to infiltrate inside the MC, which significantly increases the growing area of the MC (Figure 2 B). Additionally, MCs exhibit self-micronization throughout the culturing process, emitting smaller fibrous carriers that cells can adhere to and use to create agglomerations.




Since cells adhere and cover the microcarriers so quickly, it is important to monitor the cultures and to image cells in as little as two days after seeding due to the rapid attachment and growth of cells on the microcarrier; imaging routinely captures peak attachment and growth timepoints.

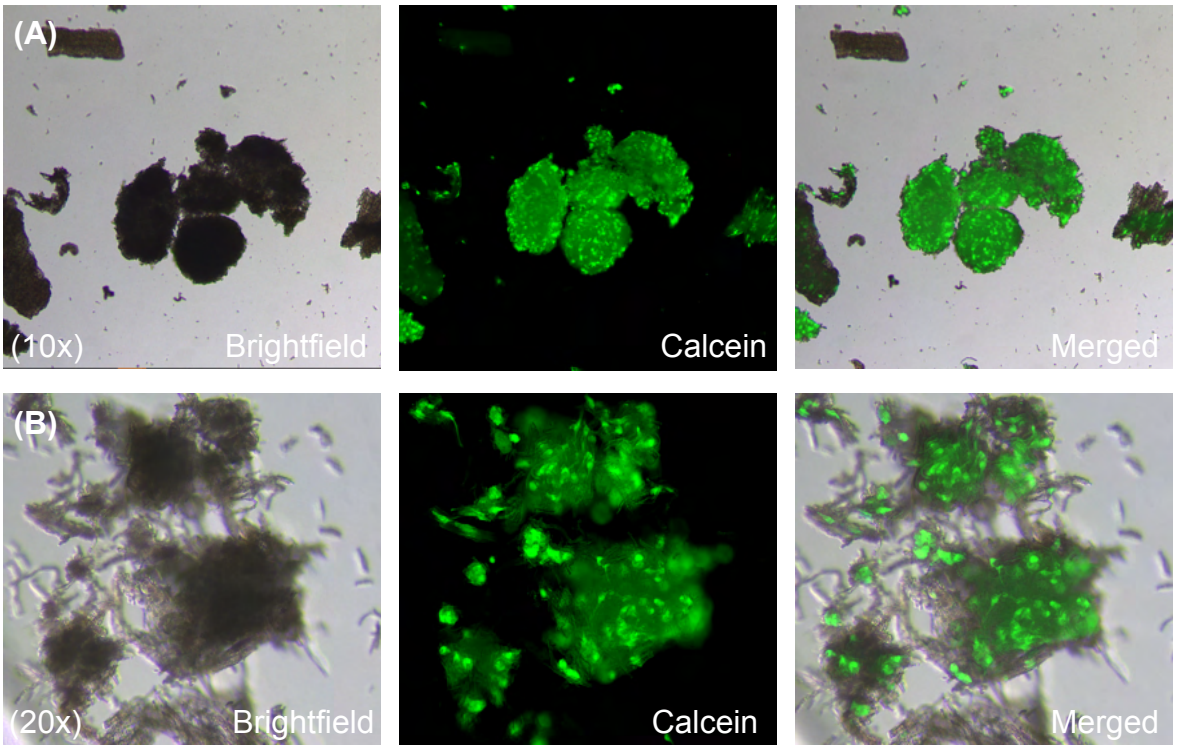



**Figure 4.** C2C12 post-seeding onto zein microcarriers. Cells were seeded with a density of 5 x $10^5$ cells/mL, and zein scaffolds were loaded at a density of 1 mg/mL. Cells were observed with Calcein fluorescent dye after two days of incubation and imaged at (A) 10x and (B) 20x magnification.

## *Carrier-to-Carrier Transfer*

Carrier-to-carrier transfer is a crucial part of MC functionality. Cells must have adequate growing area to increase yield and prevent overgrowth during cultivated meat scale up. Additional growth area can be easily provided by adding fresh, unladen MCs to seeded MCs[3]- however, this process relies upon the ability of cells to leave one microcarrier and transfer to another in a process called carrier-to-carrier transfer.

Carrier-to-carrier transfer was studied by adding fresh MCs to a suspension of cell-laden MCs that had been seeded the day prior. Adding new MCs and media in a 1:1 ratio allowed for the same ratio of MCs to media while giving cells a fresh supply of growth area for proliferation. After the new MCs were added, the suspension was incubated in static conditions for one hour, and then was transferred onto an orbital shaker overnight before observation. If cells were unable to transfer to fresh MCs, half of them would be empty. In this experiment, over half of the MCs showed cell adhesion, showing that cells are able to transfer from seeded MCs to empty MCs rapidly.

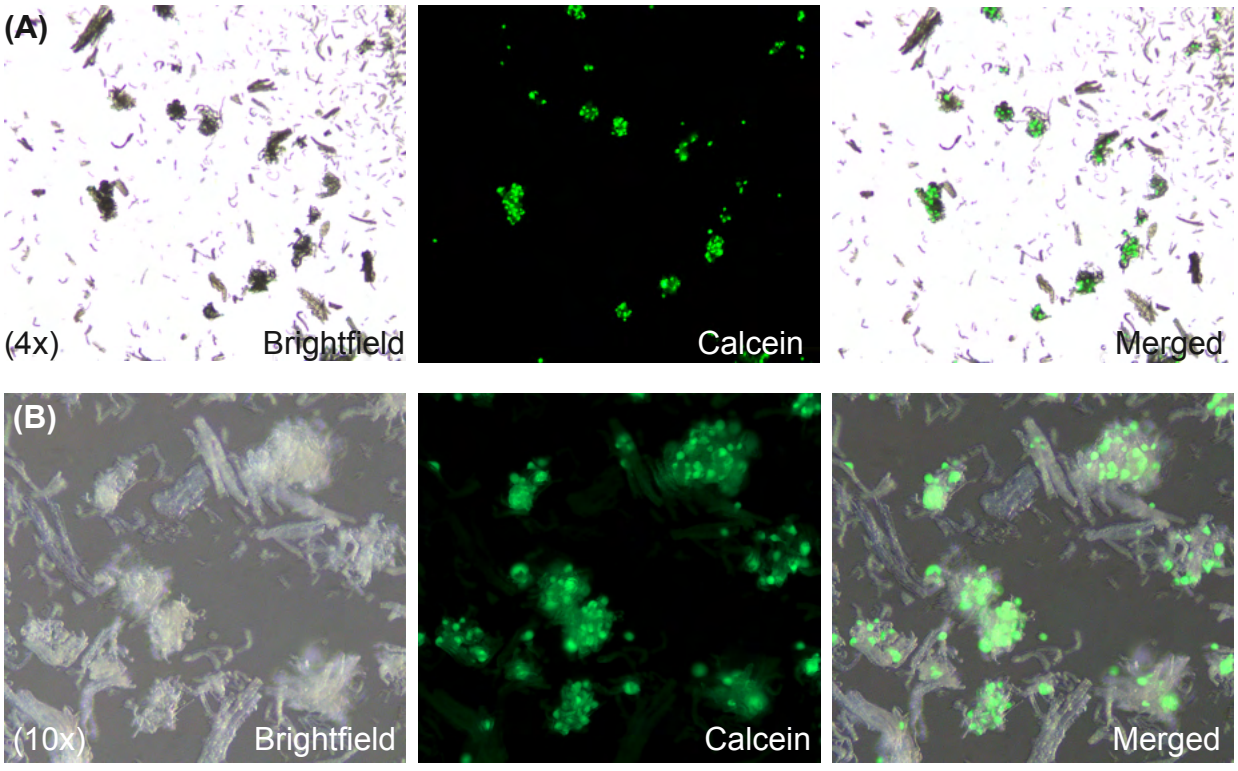



**Figure 5.** Cells adhered onto zein MCs two days post-seeding, and one day after the addition of fresh MCs and media, maintaining a 1 mg/mL loading ratio.

## *Microcarrier appearance*

As MCs are often used in proliferation stages in cell agriculture, they must not significantly interfere with the appearance, taste, or texture of the final meat product. Cell-laden zein microcarriers show an off-white appearance (Figure 4) under light microscopy, which is consistent throughout incubation and cell culture.

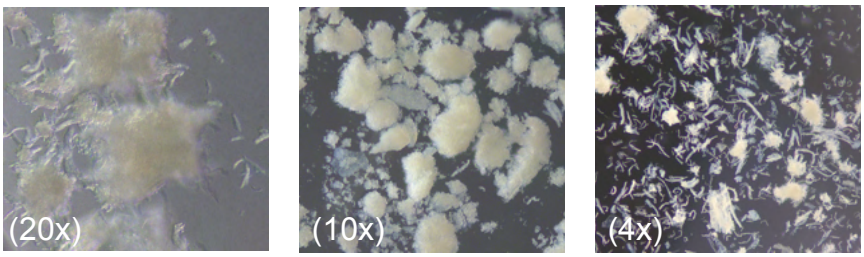



**Figure 6.** Cell-laden zein microcarriers imaged by light microscopy after 2 days of incubation.

MC usage during proliferation will affect meat production, including the formulation and texturizing of end meat products. To understand the extent of the zein MC on a final meat product, unladen zein MCs were incorporated into proprietary vegan meat to see if they change final appearance or texture (Figure 5, A-C). Some zein was visible in the 5% MC version, it did not appreciably alter the mouthfeel of the end product.


**Figure 7.** Zein MCs (without cells) incorporated into Matrix's proprietary vegan meat alternative to understand the effect of their presence on the final appearance and texture of the alternative meat product.

## Materials and Methods

### *Cell Culturing*

C2C12 cells were grown in complete growth media (DMEM GlutaMax High Glucose + 10% FBS + 0.1% penicillin streptomycin). Cells were split once they were nearly 60% confluent; all cells were used under passage 30.

### *Seeding Procedure and Characterization*

Zein microcarriers were seeded with 5 x $10^5$ cells/mL and plated in a 24-well plate for 2 days in 1 mL complete growth media. Following seeding, the microcarriers were transferred to a new 24-well plate with 1 mL/well fresh growth media and allowed to expand for 4 days post seeding.

The seeded microcarriers were imaged following expansion. Cells were imaged under brightfield and after Calcein and/or Phalloidin staining across the duration of the experiments for evidence of cell attachment and growth on the microcarrier.

### *Carrier-to-Carrier Studies*

Zein microcarriers were seeded at a density of 1 or 5 mg/mL in a vented conical tube with 5 x $10^5$ cells/mL in 3 mL for 4 hours in a humidified incubator set to 37 °C, 5% $CO_2$. After 4 hours, the zein microcarriers were stained for evidence of cell attachment using Calcein as an indicator of viable cells. Following the 4 hour seeding, the cell suspension and zein mixture were transferred to a 10 mL Erlenmeyer flask and were agitated on an orbital shaker in the incubator overnight at 110 rpm. Following overnight incubation (14-18 hours), a small aliquot was collected for staining and evidence of cell growth and attachment. Following the overnight suspension, a 1:1 ratio of new unseeded zein microcarrier was added to each flask (i.e. 3 additional mL of zein solution at a concentration of between 1 and 5 mg/mL in complete media), agitated briefly to mix, and allowed to settle in the incubator for 1 hour. Once the 1 hour incubation was complete, the flasks were placed on an orbital shaker set at 110 rpm and spun over 2 days. The seeded microcarriers were imaged 1 day and 2 days post addition of the new microcarrier for evidence of cell attachment.

## Want to work with us?

We offer customized R&D services to the cellular agriculture industry and are experts in finding your ACF, plant-based microcarrier and/or scaffolding solutions to scale your process and Value Proposition for cultivated meat and cellular agriculture.

We also offer **free** samples of a variety of scaffolding products in an iterative design process with your company.

Please contact Marilyn McNamara, Ph.D. at **mmcnamara@matrixfood.tech** to learn more about our R&D services or ordering product samples.

## Confidentiality Statement



## Citations

[1] Jacob Reiss, Samanta Robertson, and Masatoshi Suzuki. "Cell Sources for Cultivated Meat: Applications and Considerations throughout the Production Workflow." *International Journal of Molecular Sciences*, July 2021. DOI: 10.3390/ijms22147513

[2] Vincent Bodiou, Panagiota Motsatsou, and Mark J. Post. "Microcarriers for upscaling cultured meat production." *Frontiers in Nutrition,* February 2020. DOI: 10.3389/fnut.2020.00010

[3] Sanne Verbruggen, Daan Luining, Anon van Essen and Mark J. Post. "Bovine myoblast cell production in a microcarriers-based system." *Cytotechnology,* May 2017. DOI: 10.1007/s10616-017-0101-8