Application Note 003

Cytocaf (Caf1) Polymers Enable Defined Adherent and Suspension Culture of Porcine iPSCs for Cultivated Meat Applications.

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Abstract

Porcine induced pluripotent stem cells (piPSCs) are a valuable model for emerging applications in cultivated meat production, where scalable, xeno-free systems are essential. piPSCs require a supportive extracellular matrix (ECM) to maintain pluripotency and enable expansion. The Cytocaf (Caf1) platform, enables modular, customisable ECM design for defined and animal-free stem cell culture. We demonstrate the use of Cytocaf polymers to support the culture of piPSCs in adherent culture and suspension formats. Cytocaf-ECM coatings supported robust expansion of piPSCs in 2D adherent culture while maintaining pluripotency and enabling tri-lineage differentiation. A custom, porcine-specific variant—pigVTN-Cytocaf—supported piPSC proliferation and pluripotency maintenance. In 3D suspension culture, the non-adhesive Anticlump-Cytocaf variant enabled microcarrier-free, aggregation-based expansion, improving viable cell numbers and reducing clumping. Together, piPSCs and Cytocaf are novel tools to support both research and scalable bioprocessing workflows in the development of next generation cultivated meat technologies.

Introduction

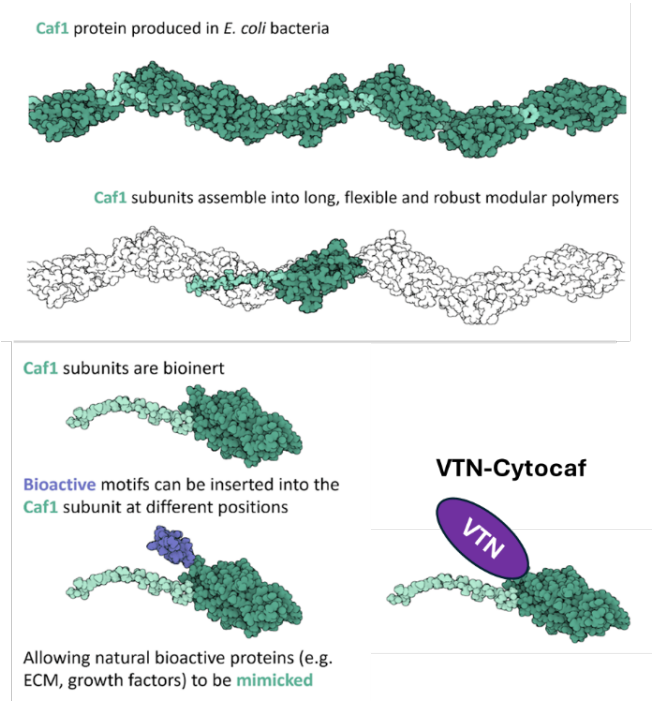

Figure 1: Schematic of Cytocaf Technology. Caf1 can be engineered to express ECM proteins such as vitronectin (VTN).

The culture and expansion of induced pluripotent stem cells (iPSCs) require defined extracellular matrix (ECM) environments that support adhesion, proliferation, and maintenance of pluripotency [1]. Conventional ECM coatings, such as vitronectin and laminin, are widely used but often rely on animal-derived substrates, limiting reproducibility, scalability, and regulatory compliance—especially in food applications such as cultivated meat [2,3].

MarraBio's Cytocaf technology provides a recombinant, modular ECM platform suited to cultivated meat production. Based on a self-assembling bacterial protein polymer, Caf1 can be engineered to mimic the bioactivity of full-length ECM proteins, including vitronectin [4–6] (Figure 1). This enables fully defined, xeno-free culture surfaces and species-specific variants that support animal stem cell lines such as porcine iPSCs (piPSCs), which are strong candidates for cultivated

pork due to their self-renewal and ability to differentiate into muscle and fat.

As cultivated meat advances toward scalable 3D bioprocessing, there is increasing interest in microcarrier-free suspension culture systems [7,8]. These require suspension-adapted cell lines and optimized conditions. Non-adhesive polymers promote controlled aggregation, reducing heterogeneity and process variability while preserving stem cell identity [9–11]. Anticlump-Cytocaf is a useful non-stick formulation that enables suspension growth without microcarriers.

This study evaluates Dragon Bio piPSCs with MarraBio's Cytocaf polymers in both adherent and suspension formats, providing a versatile, food-compatible solution for next-generation cultivated meat manufacturing.

Materials and Methods

Adherent Culture of piPSCs

piPSCs (Dragon Biotechnologies, DB_001) were cultured in Lacey's PluriPlus medium (DB_101, Dragon Biotechnologies) on tissue culture-treated plates coated with either vitronectin (A14700, Thermofisher), VTN-Cytocaf (ZAK-MB002-1, TCSBiosciences), LM-Cytocaf or Low-adherance-Cytocaf (ZAK-MB003-1, TCSBiosciences). piPSCs were seeded at 10,000 cells per 24 well in triplicate for growth curves. Total viable cell counts were measured daily using a NucleoCounter (Chemometec) following Accutase (A1110501, Thermofisher) dissociation. Total viable cell number was plotted over time to access proliferation.

3D Aggregate Suspension Culture

Suspension-adapted piPSCs (Dragon Biotechnologies, DB_001s) were cultured in Lacey's PluriPlus 3D medium (Dragon Biotechnologies, #DB_102). Suspension-adapted piPSCs were seeded at 200k per 6-well in non-treated 6-well plates in Lacey's PluriPlus 3D medium supplemented with 250 µg/mL Anticlump-Cytocaf (ZAK-MB004-1, TCSBiosciences) and 10µM Rock inhibitor (#1254, Tocris). Suspension cultures were maintained on an orbital shaker with a spin speed of 70rpm.

Embryoid Body Differentiation and qPCR

Spontaneous differentiation was induced by aggregating piPSCs into embryoid bodies and culturing in APEL2 basal medium (#05270, StemCellTechnologies) for 7 days. Total RNA was extracted (T2010S, NEB), and cDNA synthesized (4368814, Thermofisher) for qPCR analysis. Custom primetime assays (IDT) were designed for lineage markers including *PAX6* (ectoderm), *T* (mesoderm), and *SOX17* (endoderm). qPCR was performed using a QuantStudio 1 real-time PCR system (Applied Biosystems) and fold change in gene expression was assessed relative to GAPDH housekeeping gene using ΔΔCT method.

Flow Cytometry

Pluripotency marker expression was quantified via flow cytometry on a Aurora 3 laser cytometer (Cytek Biosciences). Cells were stained with antibodies against *OCT4*-PE (#NB100-2379PE, NovusBio), *SOX2-488* (5#3-9811-82, Invitrogen), and *SSEA4-647* (#330408, BioLegend). 1 test of each antibody was used per 1 million cells. Viable cells were selected using eFluor 780 viability dye (#5-0865-14, Thermofisher).

Immunocytochemistry

piPSCs were fixed in 4% paraformaldehyde and stained with the following antibodies: rabbit *OCT4A* (2890S, Cell Signalling Technology, 1:100), Mouse *NANOG* (#4893S, Cell Signalling Technology, 1:100) and nuclear DAPI stain (D1306, Invitrogen, 1:10,000). Fluorescent images were captured using a EVOS fluorescence microscope.

Results

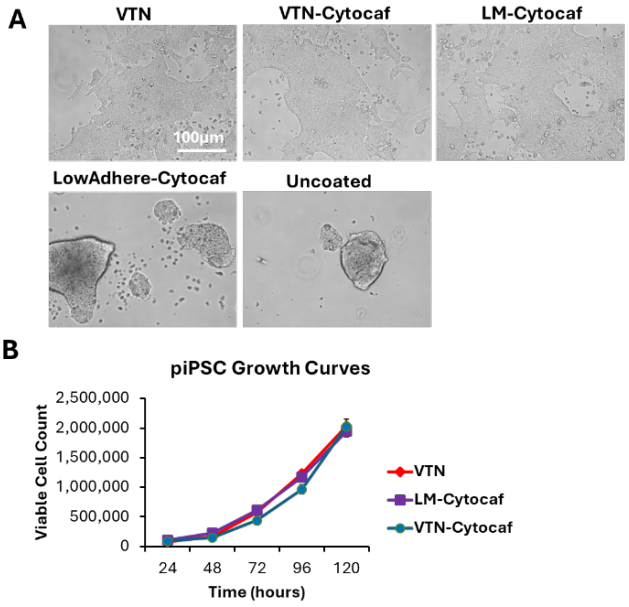

Figure 2: Cytocaf-ECM variants support piPSC expansion in adherent culture. A) Brightfield microscopy images of piPSC morphology on ECM coated vs uncoated control. B) Growth curve showing 5-day proliferation rate on each coating.

Cytocaf ECM Polymers Support piPSC Expansion During Adherent Culture

Porcine iPSCs were generated using non-integrating reprogramming of porcine fibroblasts. piPSCs cultured on VTN-Cytocaf or LM-Cytocaf ECM coatings formed well-defined, compact colonies (Figure 2A), confirming that these recombinant ECMs provide an effective adhesive microenvironment for piPSC attachment. In contrast, cells seeded on LowAdhere-Cytocaf or uncoated tissue culture plastic failed to adhere and remained in suspension, highlighting the non-adhesive nature of certain Caf1 variants.

Growth curve analysis showed robust proliferation of piPSCs on both VTN-Cytocaf and LM-Cytocaf, in comparison to the standard ECM coating vitronectin. Thus, the recombinant Cytocaf-ECM matrices effectively support piPSC expansion under adherent 2D culture conditions.

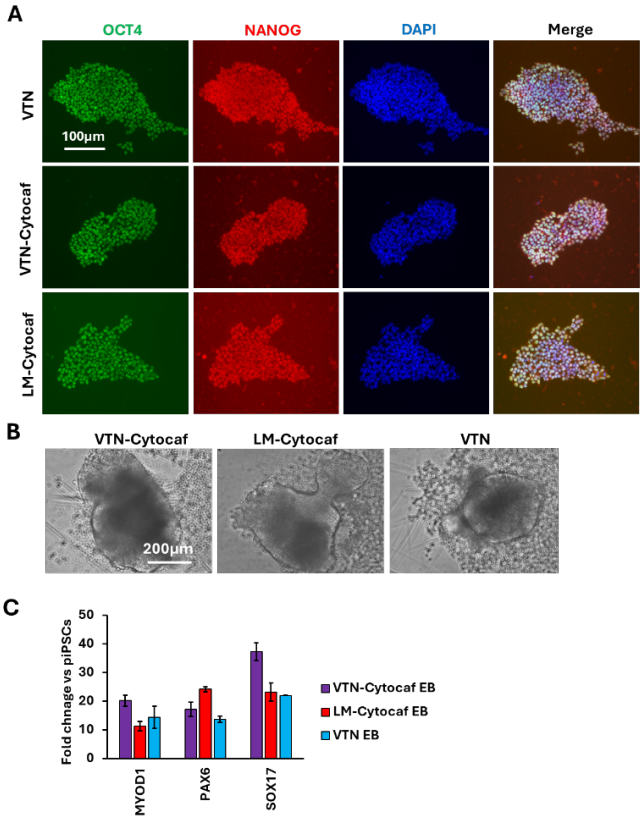

Figure 3. Cytocaf-ECM polymers support pluripotency and tri-lineage differentiation of piPSCs. (A) Immunofluorescence of piPSCs cultured for five passages on VTN-Cytocaf and LAM-Cytocaf showing *OCT4* (green) and NANOG (red) expression; nuclei stained with DAPI (blue). (B) Brightfield images of embryoid bodies after 7-day spontaneous differentiation on Cytocaf ECMs and vitronectin control. (C) qPCR showing upregulation of *MyoD1* (mesoderm), *SOX17* (endoderm), and *PAX6* (ectoderm), confirming tri-lineage differentiation.

Cytocaf ECM Proteins Support Pluripotency and Tri-Lineage Differentiation of piPSCs

We assessed pluripotency and differentiation potential of piPSCs cultured on Cytocaf ECM coatings over five passages. Cells maintained on VTN-Cytocaf and LAM-Cytocaf expressed pluripotency markers *OCT4* and *NANOG*, indicating stable maintenance of the undifferentiated state (Figure 3A).

To evaluate differentiation capacity, embryoid body (EB) formation was induced for 7 days, yielding structures comparable to the vitronectin control (Figure 3B). qPCR analysis showed upregulation of *MYOD1* (mesoderm), *SOX17* (endoderm), and *PAX6* (ectoderm), confirming tri-lineage differentiation potential on Cytocaf ECMs (Figure 3C).

Together, these findings demonstrate that Cytocaf ECM coatings support piPSC pluripotency and differentiation into all three germ layers under defined, xeno-free conditions.

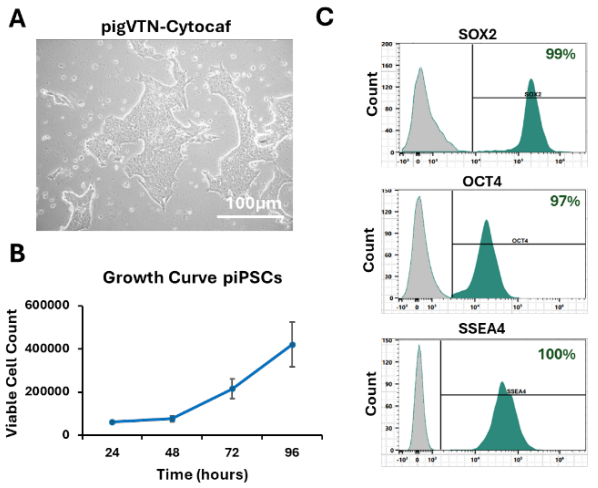

Figure 4: pigVTN-Cytocaf supports proliferation and maintains pluripotency of piPSCs in 2D adherent conditions. A) Brightfield images showing typical colony morphology of piPSCs cultured on pigVTN-Cytocaf. B) Growth curve over 4 days demonstrating robust proliferation of piPSCs on pigVTN-Cytocaf. C) Flow cytometry histograms at passage 5 showing strong expression of pluripotency markers *SOX2*, *OCT4*, and *SSEA4*.

Customised pigVTN-Cytocaf Supports piPSC Proliferation and Maintenance of Pluripotency

To evaluate the performance of a porcine-specific Cytocaf-ECM variant, pigVTN-Cytocaf, piPSCs were cultured under 2D adherent conditions and assessed for morphology, proliferation, and expression of pluripotency markers. piPSCs grown on pigVTN-Cytocaf formed well-defined colonies with typical piPSC morphology (Figure 4A). Cells were maintained on pigVTN for over five passages and exhibited robust proliferation over a 4-day growth period, indicating sustained expansion (Figure 4B). Flow cytometry analysis was performed after 5 passages, and piPSCs retained high expression of pluripotency markers, including nuclear transcription factors *SOX2* (99%) and *OCT4* (97%), as well as the surface antigen *SSEA4* (100%) (Figure 4C).

These findings demonstrate that the porcine ECM mimic pigVTN-Cytocaf provides a defined and supportive microenvironment for piPSC proliferation and maintenance of pluripotency in 2D culture. Importantly, the Cytocaf platform enables customisation with animal-specific ECM proteins to provide a food-compatible alternative to human vitronectin, supporting the development of xeno-free, scalable systems for cultivated meat applications.

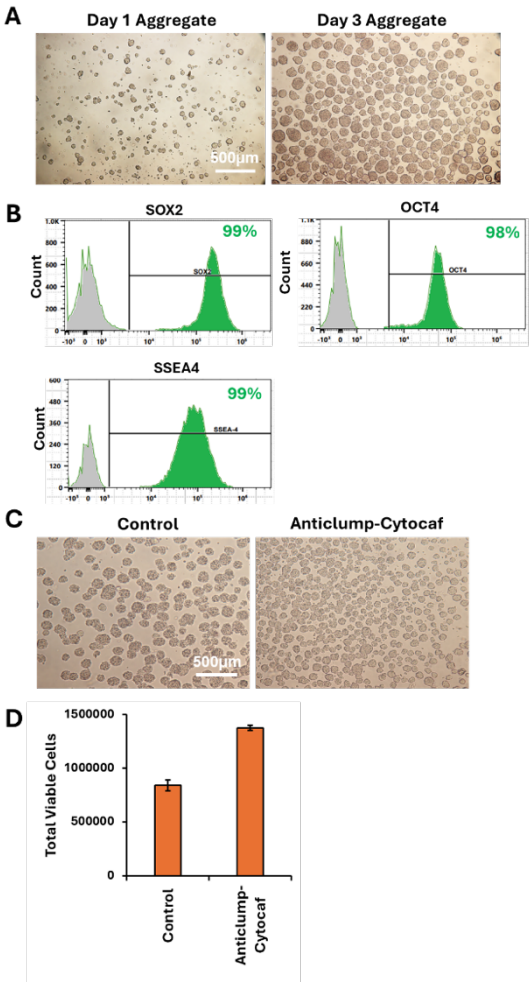

Figure 5. Anticlump-Cytocaf supports piPSC proliferation in 3D suspension culture. (A) Representative images showing aggregate morphology of piPSCs grown in suspension. (B) Flow-cytometry histograms after 10 passages showing strong expression of pluripotency markers *SOX2*, *OCT4*, and *SSEA4*. (C) Brightfield images of aggregates cultured with or without 250 µg/mL Anticlump-Cytocaf; treated cultures form smaller, more uniform aggregates. (D) Quantification of viable cells showing higher yields in Anticlump-Cytocaf-treated versus control conditions.

Anticlump-Cytocaf Increases Viable Cells in 3D Suspension Culture of piPSCs

Porcine iPSCs were adapted to suspension conditions over 10 passages in shake flask culture. Suspension-adapted piPSCs form aggregates, which increase in size over 3 days in culture (Figure 5A). Over 10 passages in suspension, the adapted piPSCs retained expression of key pluripotency markers (99% *SSEA4*, 98% *OCT4*, 99% *SOX2*), confirming their stability and suitability for 3D culture applications (Figure 5B).

To support microcarrier-free suspension culture of piPSCs, we evaluated a non-adhesive variant of Cytocaf, termed Anticlump-Cytocaf. This variant lacks cell-adhesive motifs and functions to reduce excessive aggregate clumping while also acting as a

protective polymer that mitigates shear stress in suspension environments.

In suspension, iPSCs tend to form large aggregates that can impair nutrient diffusion and reduce cell viability. The addition of Anticlump-Cytocaf at 250 µg/mL promoted the formation of smaller, more uniform aggregates (Figure 5C). This reduction in aggregate size correlated with an >60% increase in total number of viable cells after 3 days of suspension culture (Figure 5D).

These findings support a role for Anticlump-Cytocaf as a protective polymer, reducing excessive clumping and supporting healthy piPSC expansion in 3D suspension conditions—an important feature for scalable bioprocessing applications.

Discussion

This study demonstrates the development and application of novel tools for cultivated meat, combining Dragon Bio's piPSCs with MarraBio's Cytocaf platform. piPSCs are suitable for producing cultivated pork because they can self-renew and differentiate into muscle and fat—key components of cultivated meat. We show the versatility of the Cytocaf platform as a recombinant ECM solution that supports piPSC culture in both adherent (2D) and suspension (3D) formats—an essential capability for integrated bioprocesses in cultivated meat production.

In 2D adherent culture, Cytocaf polymers expressing vitronectin or laminin supported robust piPSC attachment, proliferation, and maintenance of pluripotency. piPSCs retained expression of core stem cell markers and demonstrated tri-lineage differentiation, validating the biological compatibility of Cytocaf ECM variants. Importantly, a custom porcine-specific variant offers a species-matched, food-compatible alternative to human-derived ECM proteins.

To address scalability, we developed a suspension-adapted piPSC line that maintained pluripotency over 10+ passages and enabled the functional testing of Anticlump-Cytocaf in 3D culture. The non-adhesive Anticlump-Cytocaf reduced aggregate size and improved cell viability, acting as a protective polymer that mitigates shear stress—facilitating microcarrier-free, high-density expansion with reduced downstream complexity.

The availability of both 2D- and 3D-adapted piPSC lines and the flexibility of the Cytocaf system supports various stages of the cultivated meat pipeline—from early expansion to scalable production. Together, these novel tools provide a defined and species-specific system that enables the creation of scalable, and commercially viable cultured pork products. This work highlights a significant step forward in the development of next generation 2D and 3D cell culture technologies to advance the field of cultivated meat.

Acknowledgements

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