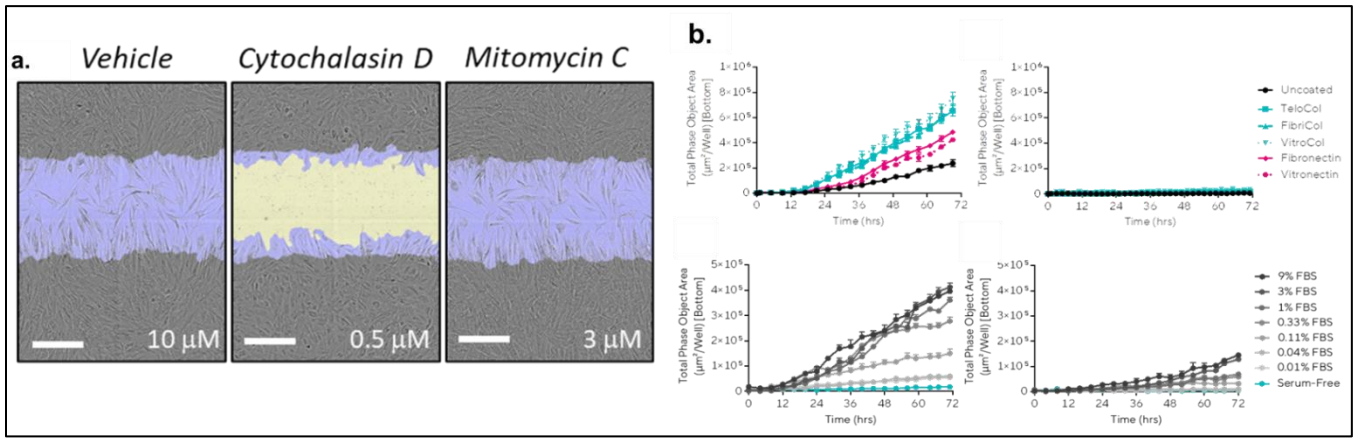
Xeno-Free ECM Coatings Promote Chemotaxis Migration of Human Fibroblasts

Chemotaxis is crucial for wound healing, guiding fibroblasts to injury sites in response to biochemical signals. This study demonstrates that Advanced BioMatrix's xeno-free VitroCol® Collagen I solution enhances fibroblast migration and chemotaxis in response to fetal bovine serum (FBS) gradients. We compared fibroblast movement on VitroCol®-coated and uncoated plates, confirming that VitroCol® provides a stable surface that supports directed migration. Additionally, fibroblasts maintained expected functional behaviors under pharmacological treatments, establishing VitroCol® as a reliable xeno-free ECM coating for in vitro chemotaxis and wound healing models.

Materials	Catalog Number	Final concentration
VitroCol® (xeno-free)	5007	100 µg/mL

The VitroCol® Collagen I solution significantly enhances fibroblast migration in response to chemotactic gradients, offering a stable and biologically relevant platform for wound healing research. Its composition is free from animal-derived contaminants, making it ideal for functional studies and drug testing applications.



Xeno-free VitroCol® supports NHDF migration under drug treatment and improves NHDF chemotaxis in response to FBS.

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Abstract

Chemotaxis, the directed migration of cells in response to chemical gradients, is a critical process in wound healing and tissue regeneration. This study investigates the impact of xeno-free VitroCol® collagen type I solution on chemotaxis and migration of normal human dermal fibroblasts (NHDFs). Using Incucyte® live-cell imaging, we compared cell migration rates on VitroCol®-coated and uncoated plates in response to fetal bovine serum (FBS) gradients. Results showed significantly enhanced fibroblast migration on VitroCol®-coated surfaces, confirming its ability to replicate physiological signaling environments. Additionally, VitroCol® maintained fibroblast functionality under pharmacological treatments, demonstrating its reliability for functional studies. These findings establish VitroCol® as a superior xeno-free ECM coating, advancing in vitro models for wound healing and chemotaxis research.

Materials	Supplier	Cat. Number
VitroCol®	Advanced BioMatrix	5007

Cell line
Normal human dermal fibroblasts (NHDF)

Introduction

Chemotaxis refers to the directed migration of cells along a chemical gradient and is essential for wound healing, guiding fibroblasts to injury sites to facilitate tissue repair.[1] Traditional models for studying chemotaxis often rely on animal-derived ECM products, which can introduce contaminants and lack translational relevance.[2,3] Xeno-free ECM coatings, such as Advanced BioMatrix's VitroCol® collagen, can provide a reliable alternative, supporting human cell studies without these limitations.

This study examined the efficacy of VitroCol® in enhancing fibroblast chemotaxis in response to fetal bovine serum (FBS). By comparing VitroCol®-coated and uncoated plates, we demonstrated the superior performance of xeno-free ECM coatings in promoting stable cell attachment and effective migration, offering improved models for wound healing research.

Results

Xeno-Free VitroCol® Maintains Functional Behavior of NHDF In Response to Drug Treatment

To evaluate whether xeno-free coatings preserve cellular functionality, chemical inhibitors dissolved in DMSO were applied to NHDFs cultured on VitroCol®-coated plates after wounding with the Incucyte® 96-Well Woundmaker Tool. Two inhibitors were tested: Cytochalasin D, which inhibits actin polymerization and affects cell migration,[4] and Mitomycin C, which prevents DNA transcription into RNA, thereby inhibiting cell proliferation.[5] After 12 hours of treatment, representative phase images showed distinct effects on NHDF wound healing, as anticipated (Fig 1). Wounds in both the vehicle control and Mitomycin C conditions were fully closed, whereas the wound remained unhealed with Cytochalasin D at the tested concentration (Fig 1A). Quantification of relative wound density for Cytochalasin D revealed no significant differences across concentrations, indicating that NHDFs migrated into the wound rather than proliferating to close it (Fig 1B). Conversely, higher concentrations of Mitomycin C significantly impaired cell migration, demonstrating that VitroCol® effectively supports expected functional cellular behaviors under these conditions (Fig 3C).

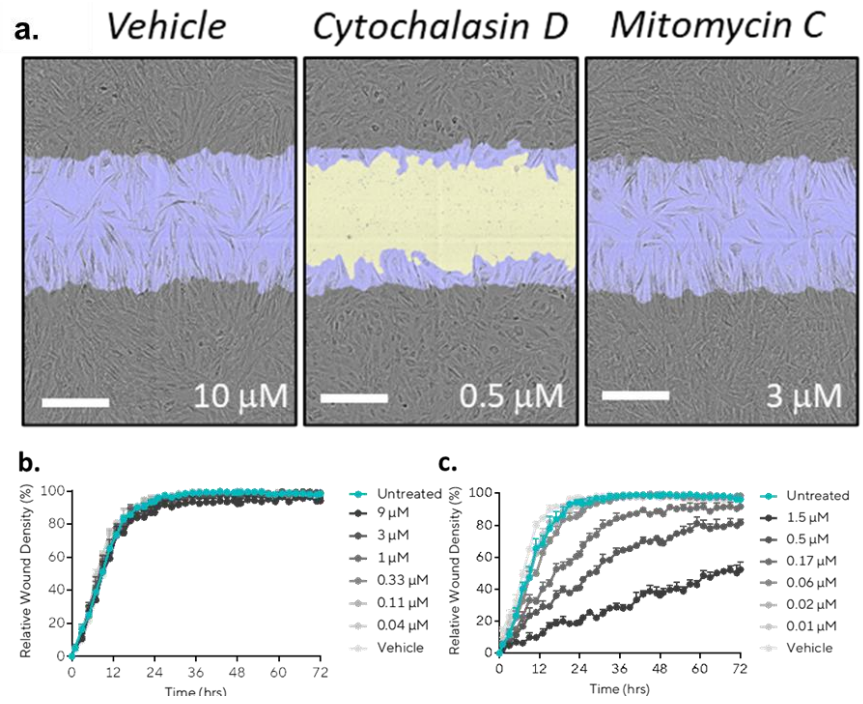

Figure 1. Xeno-free VitroCol® supports functional behavior of NHDF in response to drug treatment. (A) NHDFs were seeded near confluency on 96-well culture plates coated with

VitroCol® solution. Cell layers were wounded, rinsed, and fresh media was added containing various concentrations of a drug dissolved with a vehicle (DMSO). (A) Shown are representative images of the wound 12 hours after the initial scratch at one concentration of the respective drug treatment with initial scratch (light blue, and wound closure (yellow). Scale bars = 500 µm. The relative wound density of (B) Cytochalasin D or (C) Mitomycin C treatment was quantified over time using an Incucyte SX5 Live-Cell Analysis System.

Xeno-Free Vitrocol® Improved FBS-Driven Chemotaxis Migration of NHDF

In vivo, wound healing is a complex process driven not only by the physical disruption of cell layers but also by various chemical signals.[6] To investigate how xeno-free coatings support wound healing in response to chemical stimuli, we examined fibroblast migration in the presence of FBS using Incucyte® Clearview 96-Well Chemotaxis Plates. A selection of Advanced BioMatrix (ABM) biomaterials was applied to coat the plates, after which NHDFs were seeded into the insert wells, and FBS was added as a chemoattractant to the reservoir wells.

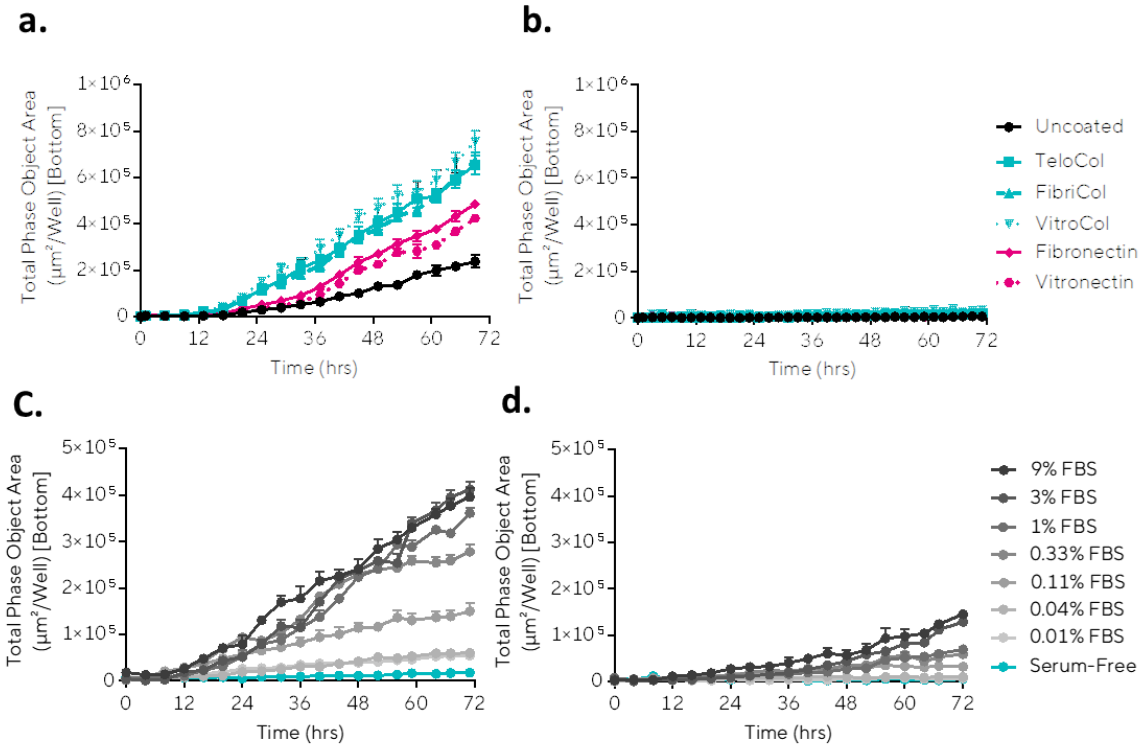

Figure 2. Xeno-free VitroCol® supports chemotaxis migration of NHDF in response to fetal bovine serum. NHDFs were seeded at 1000 cells/well on uncoated or coated wells in the top chamber of an Incucyte® Clearview 96-Well Chemotaxis Plate. Media containing a

chemotactic agent (FBS) was added to the bottom chamber, and cell migration through pores in the membrane between each chamber was monitored using an Incucyte SX5 Live-Cell Analysis System. Cells were masked, and the total phase area of the bottom chamber was quantified over 96 hours for different ABM matrix coatings, with respect to both 10% FBS (A) and serum-free media (B). Similarly, chemotaxis migration in response to various concentrations of FBS was quantified using separate VitroCol®-coated (C) or uncoated plates seeded with NHDFs (D).

Cells were observed migrating through the membrane pores from the insert well toward the FBS gradient in the reservoir well. Quantification of the bottom masked phase area over time showed significantly increased NHDF migration in response to 10% FBS for all coating conditions compared to the uncoated plates (Fig 2A). Consistent with results from the scratch wound assay, collagen I coatings facilitated faster migration compared to other coatings. Additionally, NHDFs exhibited minimal to no migration in serum-free conditions (0% FBS), confirming that FBS acted as the primary driver of migration rather than the coating itself (Fig 2B).

Conclusion

This study demonstrates the effectiveness of xeno-free VitroCol® collagen I solution in enhancing fibroblast migration and chemotaxis. VitroCol®-coated plates supported stable NHDF attachment and significantly improved migration rates in response to FBS gradients compared to uncoated surfaces. Additionally, VitroCol® maintained expected fibroblast behaviors under pharmacological treatments, validating its reliability for functional studies. These findings underscore the value of xeno-free ECM coatings like VitroCol® in advancing wound healing research by providing a biologically relevant and translationally applicable platform, offering a superior alternative to traditional animal-derived ECM products.

Materials and Methods

Cell Culture

Normal human dermal fibroblasts (NHDFs) were maintained at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS. Cells were grown to 90–95% confluency and passaged using TrypLE™ at a seeding density of approximately 7000 cells/cm². The culture medium was refreshed every 3 days, and cells from passages 4 to 8 were used for cultures.

VitroCol® Coating Preparation

The VitroCol® stock solution was diluted to a concentration of 100 µg/mL and applied to Incucyte® Imagelock 96-well Plates (Sartorius, Cat. No. 4806) at 50 µL per well. The plates were air-dried in a biosafety cabinet (BSC) for 2 hours at room temperature to create monolayer coatings. Afterward, the coatings were rinsed once with DPBS and allowed to dry completely in the BSC.

Scratch Wound Healing Assay

NHDFs were harvested using TrypLE™ and resuspended in fresh DMEM supplemented with 10% FBS. Cells were counted and seeded onto coated plates at a density of 15,000 cells per well. The plates were incubated overnight to promote cell adhesion. The plate lids were removed, and wounds were created in all wells simultaneously using the Incucyte® 96-Well Woundmaker Tool (Sartorius, Cat. No. 4563). After wounding, the media was aspirated, and the cells were washed twice with 100 µL of culture media. Subsequently, 100 µL Cytochalasin D or Mitomycin C at various concentrations (in DMSO as vehicle) reconstituted in fresh media was added to each well, and the plates were incubated for 12 hours before being placed in the Incucyte® SX5 Live Cell Analysis System for image acquisition and analysis (Fig 3).

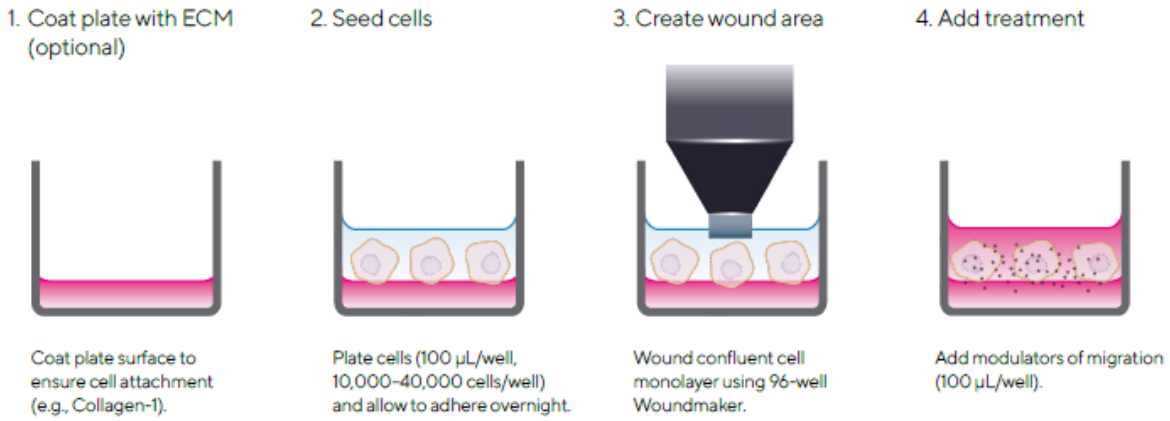

Figure 3. Schematic of Scratch Wound Migration Assay

Chemotaxis Migration Assay

Incucyte® Clearview 96-Well Chemotaxis Plates (Cat No. 4452) were coated with 100 µg/mL of VitroCol®, TeloCol®, FibriCol®, Fibronectin, or Vitronectin from Advance BioMatrix. One type of

solution was added to both, the insert well (20 µL) and reservoir well (150 µL) and left in a cell culture hood to adhere for 2 hours at room temperature. Plates were then rinsed 1X with serum-free DMEM and left to dry completely (~45 mins). NHDFs were prepared using a similar rinsing/trypsinization procedure as described above using serum-free culture media. The harvested cells were added (60 µL) to the previously coated plates at a density of 1,000 cells/well and allowed to settle at ambient temperature for 15 mins. 200 µL of DMEM was added to each reservoir well with various concentrations of FBS before placing in an Incucyte® SX5 Live Cell Analysis System for image acquisition and analysis.

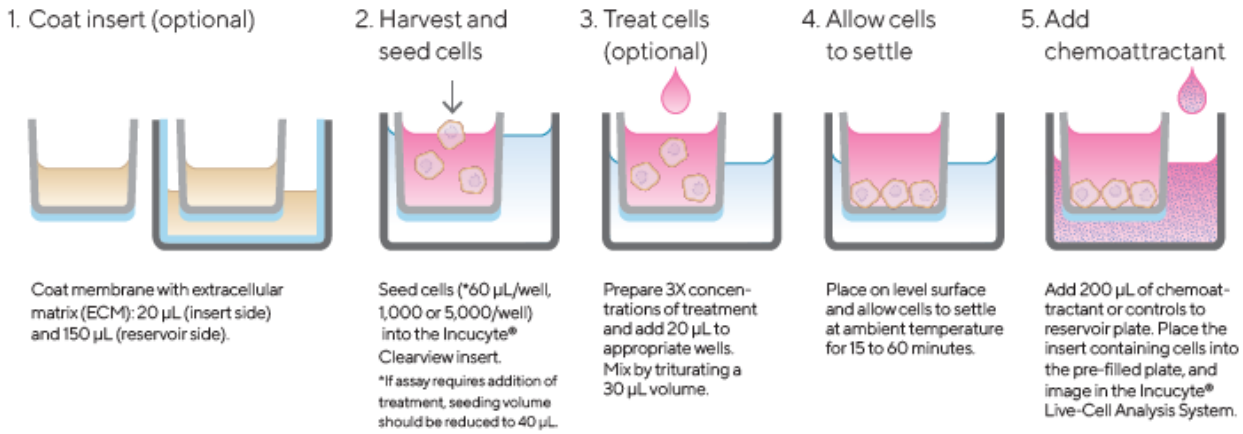

Figure 4. Schematic of Chemotaxis Migration Assay

Imaging and Data Analysis

HD Phase images were acquired every 2 hours using the Incucyte® system and analyzed (n=4) using the Incucyte® Scratch Wound Analysis Software Module (Cat. No. 9600-0012) and the Incucyte® Chemotaxis Analysis Software Module (Cat. No. 9600-0015) for each respective assay. Statistical analyses were performed using the Incucyte® Software and reported as mean ± standard error of mean (SEM). Data was then exported, and graphs were produced using GraphPad Prism software.

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