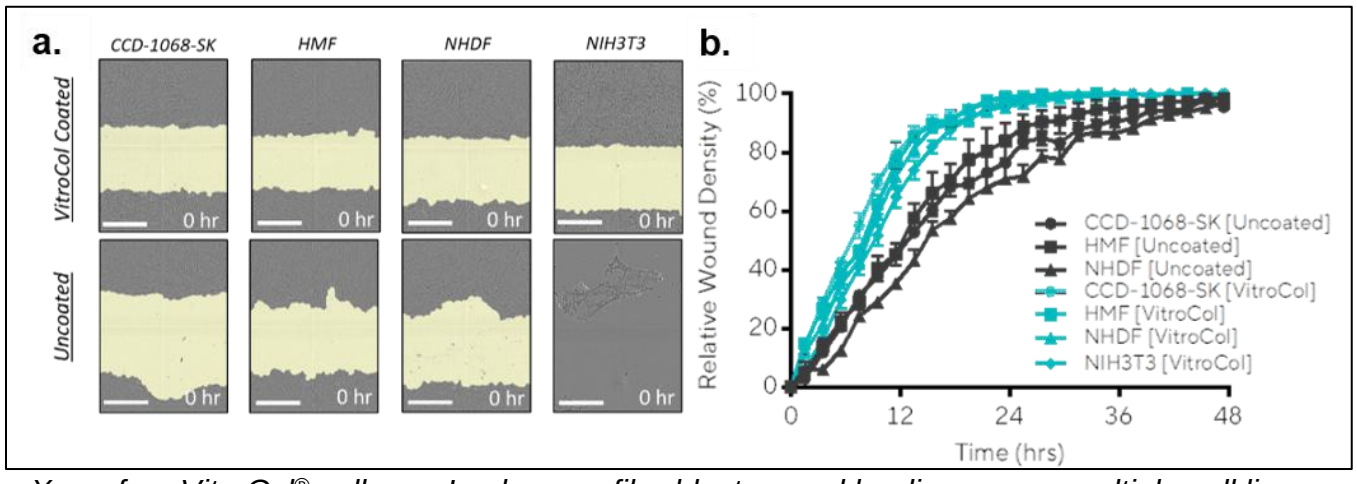
Xeno-Free VitroCol® Collagen I Solution Supports Wound Healing Across Multiple Lines of Fibroblasts

Fibroblast migration is essential for wound healing. This study demonstrates that Advanced BioMatrix's xeno-free VitroCol® Collagen I solution enhances fibroblast migration and wound closure across multiple fibroblast lines, including NHDFs, CCD-1068-SK, HMFs, and NIH-3T3, using the Scratch Wound Assay. Compared to uncoated plates, VitroCol® provides a stable and uniform surface, supporting cell attachment and consistent migration, particularly for NIH-3T3 cells, which failed to adhere to uncoated plates. These results confirm VitroCol® as a biologically relevant, xeno-free alternative to traditional ECM coatings, improving the reproducibility of wound healing assays.

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

Our VitroCol® Collagen I solution supports superior fibroblast migration across multiple fibroblast lines compared to the uncoated plates. Its xeno-free composition ensures a biologically relevant platform for wound healing research, eliminating animal-derived contaminants and improving assay reproducibility.



Xeno-free VitroCol® collagen I enhances fibroblast wound healing across multiple cell lines.

Xeno-Free VitroCol® Collagen I Solution Supports Wound Healing Across Multiple Lines of Fibroblasts

Abstract

This study evaluates the effectiveness of the xeno-free VitroCol® collagen I solution in promoting fibroblast migration and wound healing across multiple fibroblast cell lines, including NHDFs, CCD-1068-SK, HMFs, and NIH-3T3. By using the Incucyte® Scratch Wound Assay, we demonstrate that VitroCol® significantly enhances fibroblast migration and wound closure when compared to uncoated plates. VitroCol® provided a stable and uniform surface for fibroblast attachment and migration. This was particularly evident in NIH-3T3 cells, which failed to adhere to uncoated plates and were washed away after wounding. The results confirm that VitroCol® supports faster migration and more consistent wound closure across various fibroblast lines, offering a biologically relevant, xeno-free alternative to traditional animal-derived ECM products. These findings highlight the potential of xeno-free coatings to improve the accuracy and reproducibility of wound healing models and emphasize their importance in human cell-based research.

Materials	Supplier	Cat. Number
VitroCol®	Advanced BioMatrix	5007

Cell lines
CCD-1068-SK
Human mammary fibroblasts (HMF)
Normal human dermal fibroblasts (NHDF)
NIH-3T3 human fibroblasts

Introduction

Wound healing is a vital process involving multiple stages including hemostasis, inflammation, proliferation, and remodeling, where various cell types and proteins work together to repair damaged tissue.[1,2] The transition from inflammation to proliferation is crucial, with fibroblasts

migrating to create the extracellular matrix (ECM) that supports new tissue growth. This phase is highly sensitive, and improper healing can lead to chronic wounds.[3,4,5]

The ECM is a complex network of macromolecules, including structural proteins like collagen and elastin, proteoglycans, glycoproteins, ions, and growth factors, that provides physical scaffolding and biochemical cues essential for cellular functions such as wound healing.[6,7] The ECM is crucial in biological research, with applications like coating flasks or creating hydrogels to replicate its roles in supporting cell adhesion, proliferation, migration, differentiation, and intercellular communication [8,9]. However, many ECM products are derived from animals, introducing potential xeno-contaminants. The contaminants can alter cellular behavior, leading to inaccurate results, and raise immunological concerns, limiting the applicability of these materials in translational research.[10,11]

At Advanced BioMatrix (ABM), we provide a selection of high purity, quality proteins and biomatrices that are free from animal-derived contaminants and researchers are able to construct xeno-free matrices for cell culture. In this white paper, we prepared xeno-free ECM coatings using ABM's VitroCol® type I collagen (Cat. No. 5007) to evaluate its performance to support human fibroblast growth, migration and wound healing capability.

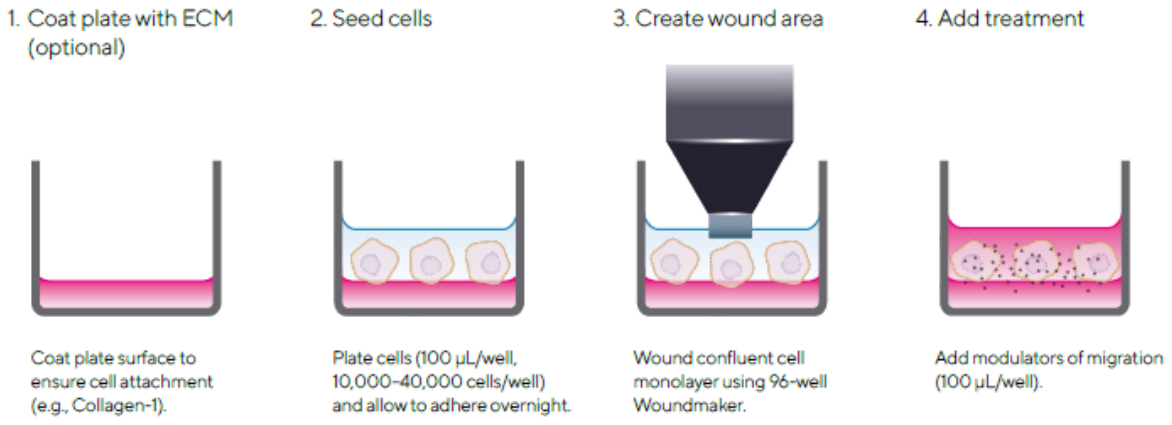


Figure 1. Schematic of Scratch Wound Migration Assay

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Results

Four fibroblast cell lines (CCD-1068-SK, HMFs, and NIH3T3) were cultured on VitroCol® coated plates to explore the potential of the xeno-free coatings for promoting fibroblasts wound healing capability. As shown in Fig 2A, the phase contrast images from coated and uncoated wells displayed successful attachment of the different fibroblasts at 0 hour. However, the wounds created using the Incucyte® 96-Well Woundmaker Tool were not consistent across different cell lines, with uncoated plates having jagged, inconsistent scratches for CCD-1068-SK, HMF, and NHDF, or nearly all cells removed for NIH-3T3.

Quantification of relative wound density revealed significantly higher migration rates on VitroCol®-coated plates compared to uncoated conditions across all tested fibroblast lines (Fig 2B). Notably, the NIH3T3 cells in the uncoated condition were unable to adhere properly, resulting in their detachment and rendering migration data unquantifiable. These findings underscore the effectiveness of VitroCol® in providing a stable substrate that enhances cell attachment and supports consistent fibroblast migration, highlighting the critical role of xeno-free coatings in advancing wound healing research.

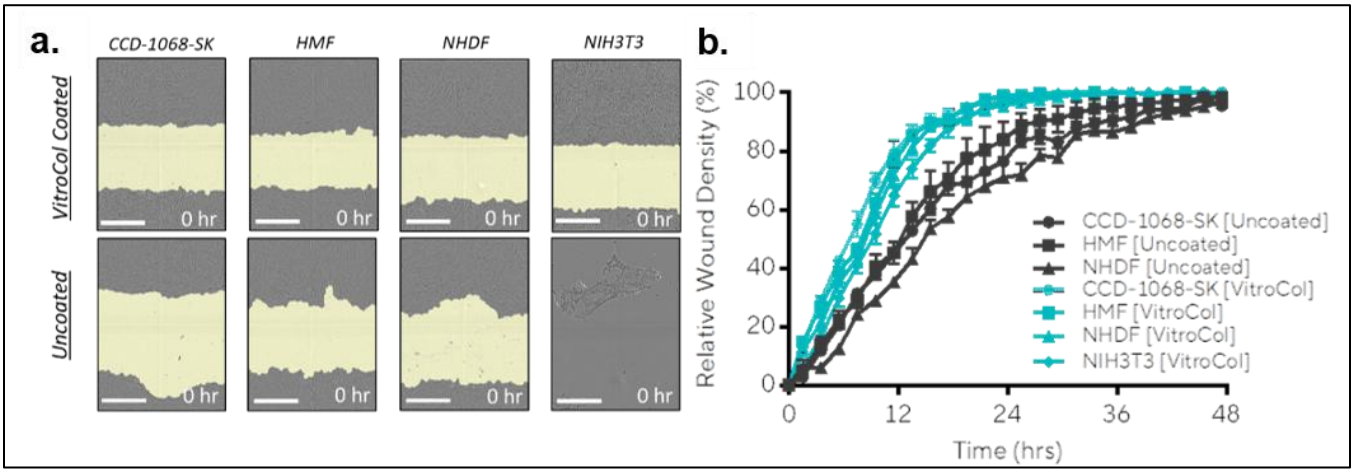

Figure 2. Xeno-free VitroCol® collagen-I solution promotes wound healing across multiple types of fibroblasts. Separate 96-well culture plates were coated with VitroCol® collagen-1 solution and seeded at respective densities to create confluent layers of CCD-1068-SK, HMF, NHDF, or NIH-3T3. Cells were then wounded and imaged using an Incucyte SX5 Live-Cell Analysis System. (A) Representative images after the initial scratch (yellow) at 0 hour are shown for both VitroCol® Coated Plates and similarly prepared uncoated culture plates. Scale bar = 500 µm. (B) The relative wound density was quantified over time and overlayed to compare both coating conditions across all cell types (data represent mean ± SD).

Conclusion

This study demonstrates the significant advantages of VitroCol® xeno-free collagen I coatings in promoting fibroblast attachment, migration, and wound healing. Across four fibroblast cell lines (CCD-1068-SK, HMF, NHDF, and NIH3T3), VitroCol® consistently supported higher migration rates compared to uncoated plates, as evidenced by quantification of relative wound density. These findings highlight the critical role of xeno-free coatings like VitroCol® in providing a stable, biologically relevant substrate for wound healing research.

Materials and Methods

Coating Preparation

VitroCol® stock solution was diluted to 100 µg/mL and added to the Incucyte® Imagelock 96-well Plates (Satorius, Cat. No. 4806). The 50 µL solution was added to each well and the plate was air-dried in a biosafety cabinet (BSC) for 2 hours at room temperature to form the monolayer coatings. Later, the coatings were rinsed with DPBS once and dried completely in the BSC.

Cell Culture

CCD-1068-SK, human mammary fibroblasts (HMF), normal human dermal fibroblasts (NHDF), and NIH-3T3 were cultured at 37°C and 5% CO_2 in Dulbecco's Modified Eagle Media (DMEM) with 10% fetal bovine serum (FBS) in cell culture flasks. Cells were cultured until 90% confluency and subcultured at 7000 cells/cm^2 for passaging. Culture media was replaced every 3 days, and cells between passage 4 to 8 were collected for experiments.

Scratch Wound Migration Assay

All cells were harvested using TrypLE and resuspended in fresh DMEM with 10% FBS. Cells were counted and added to the previously coated plates at a density of 15,000 cells/well for the CCD-1068-SK, HMF, NHDF and 20,000 cells/well for the NIH-3T3. The plates were transferred to an incubator and left overnight to promote cell adhesion. The plate lid was then removed and an Incucyte® 96-Well Woundmaker Tool (Satorius, Cat. No. 4563) was applied to create wounds in the wells. After wounding, the media was removed, and cells were rinsed twice with 100 µL of culture media. Next, 100 µL of fresh media was added to each well, and the plates were placed into the Incucyte® SX5 Live Cell Analysis System for image capture and analysis (Fig 1).

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