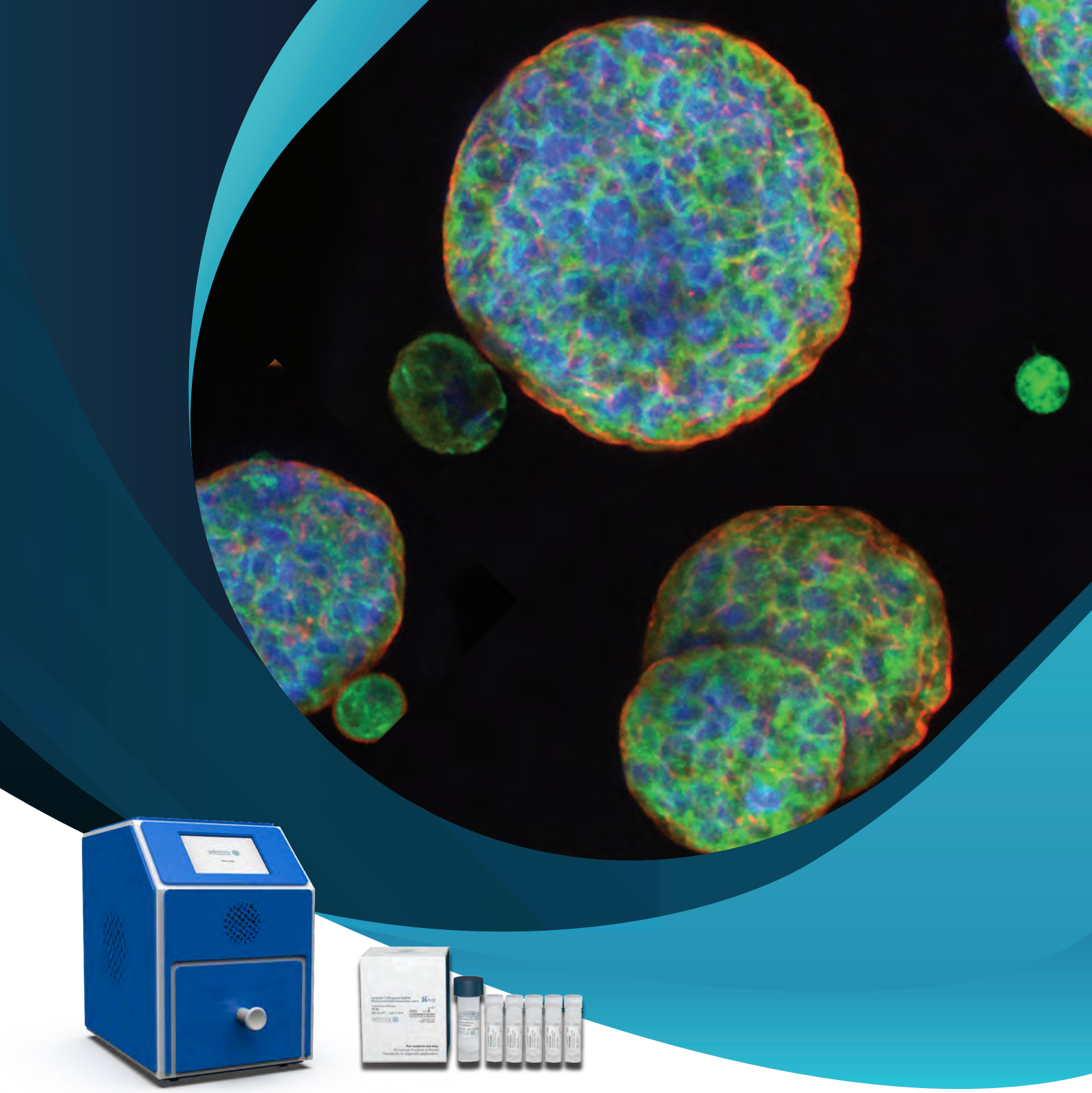


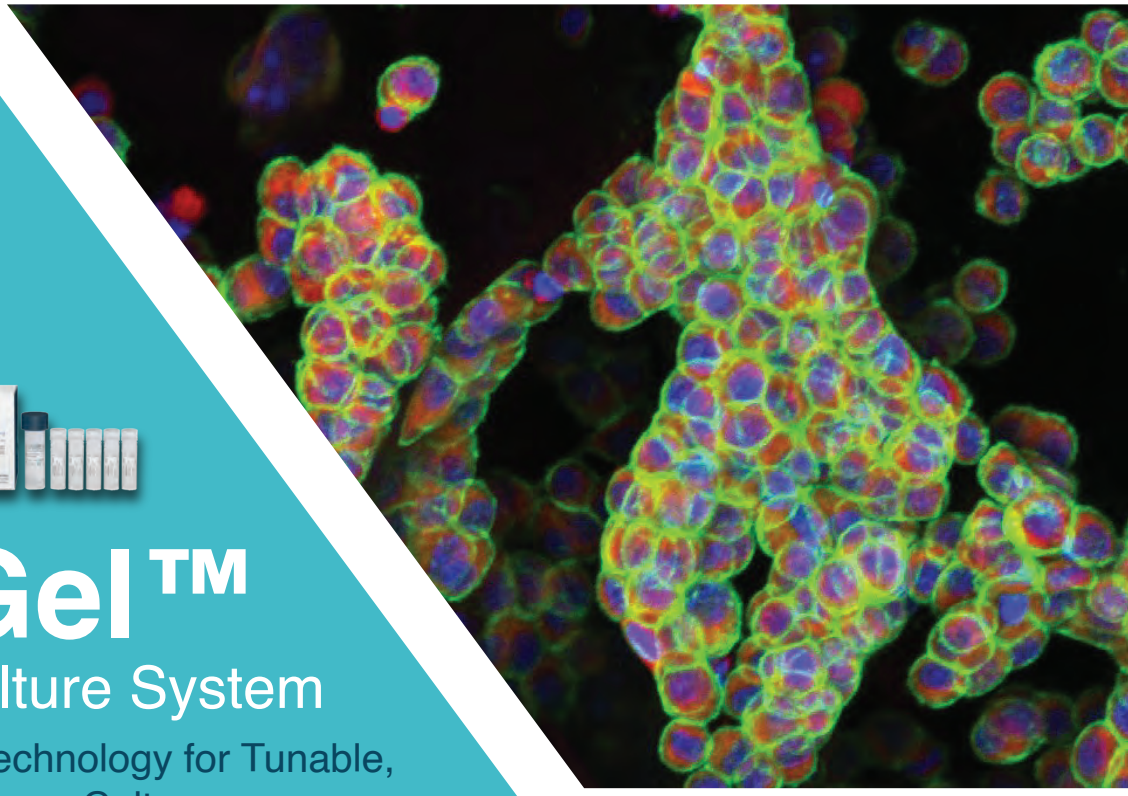
LunaGel™ 3D Tissue Culture

A Comprehensive Comparison To Matrigel®

Enhanced Tunability, Reproducibility, and Biological Relevance for Advanced Research Applications

gelomics
3D cell culture technologies

gelomics
3D cell culture technologies



LunaGel™

3D Tissue Culture System

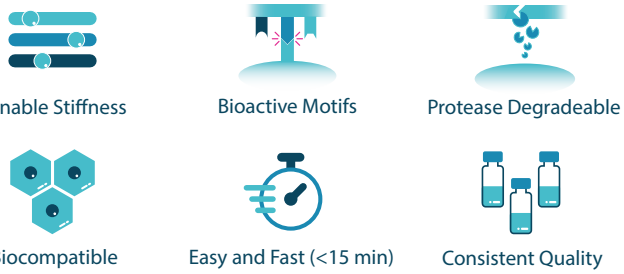
Photocrosslinking Technology for Tunable, Reproducible 3D Tissue Culture

INTRODUCTION

This white paper provides a comprehensive comparison between Gelomics' LunaGel™ 3D Tissue Culture System and Matrigel®, a commonly used basement membrane extract for 3D cell cultures. While Matrigel™ has been widely adopted due to its extracellular matrix (ECM) protein content, it presents several challenges in terms of batch variability, undefined composition, lack of mechanical tunability, and scalability. These limitations hinder its ability to create reproducible and physiologically relevant models, especially for advanced applications in cancer research and drug development.

In contrast, LunaGel™ offers a semisynthetic alternative, engineered from pharmaceutical grade raw materials, which enables precise control over ECM stiffness and provides reproducible, consistent results across batches. This tunability makes it a highly versatile platform for a wide range of 3D cell culture

This white paper outlines how LunaGel™ overcomes the limitations of Matrigel® and establishes itself as a more advanced, user-friendly solution for research in fields such as cancer biology, drug development, and tissue engineering.



LUNAGEL VS. MATRIGEL

The comparison between LunaGel™ and traditional basement membrane extracts highlights several key differences in performance and usability. LunaGel™ is a semisynthetic, controlled ECM platform, offering tunable stiffness and high consistency across batches. It contains no growth factors, allowing researchers to customize conditions. With a quick 15-minute protocol and handling at room temperature, LunaGel™ is both easy to use and highly scalable, making it ideal for a wide range of research applications. In contrast, basement membrane extracts are derived from mouse sarcomas and have an undefined composition, which varies by batch. They offer very limited control over mechanical properties, making them less adaptable for diverse tissue environments. These extracts suffer from high batch variability, contain uncontrolled growth factors, and require 12 hours of thawing followed by 45 minutes of gelation. Additionally, they must be handled on ice, and their scalability is limited compared to LunaGel™.

	LunaGel™ Extracellular Matrices	Matrigel® Basement Membrane Extract
Source	Semisynthetic	Natural, from mouse sarcomas
Composition	Controlled	Undefined, Varies by Batch
Mechanical Properties	Tunable Stiffness	Uncontrolled
Reproducibility	Highly Consistent	High Batch Variability
Growth Factors	None	Uncontrolled, Variable
Protocol Duration	15 min	12 h thawing + 45 min
Handling	Room Temperature	On Ice
Scalability	Highly Scalable	Limited Scalability

CONTROLLING STIFFNESS

The mechanical stiffness of the ECM is a critical factor that influences a wide range of cellular behaviors, including migration, proliferation, differentiation, and epithelial-to-mesenchymal transition (EMT). Being able to adjust ECM stiffness allows researchers to more accurately replicate the physiological or pathological conditions of various tissues, making it a key consideration in 3D cell culture systems.

Matrigel® relies on collagen fibrillogenesis, which results in hypo-physiological stiffness once gelled. This process, while reliable for basic 3D cell cultures, is highly sensitive to environmental conditions such as temperature, pH, ionic strength, and biochemical composition. As a result, Matrigel® offers limited control over mechanical properties, severely limiting its ability to model different tissue types or study mechanical influences on cell behaviour. Moreover, its stiffness can vary between batches and is typically substantially lower than native tissues, reducing experimental reproducibility and recreating challenges when trying to replicate specific in vivo conditions.

LunaGel™ provides unprecedented control over ECM stiffness, thanks to its photocrosslinking technology. By adjusting the duration of light exposure, researchers can fine-tune the stiffness to match the mechanical properties of different tissues—ranging from soft tissues like brain and lung (as low as 0.1 kPa) to stiff tissues such as cancerous breast tissue (up to 25 kPa). This tunability covers a much wider range than Matrigel® and other competitor products, for which stiffness is limited to <1 kPa, which is unphysiological for most tissues.

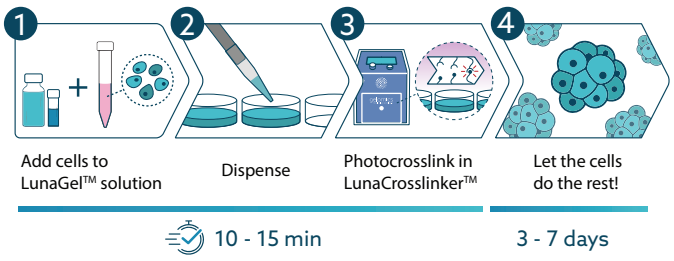


Figure 1: Simple workflow. Cells are added to the LunaGel™ solution, dispensed, and photocrosslinked using the LunaCrosslinker™ for 0-8 minutes to create highly controlled 3D Tissue Cultures.

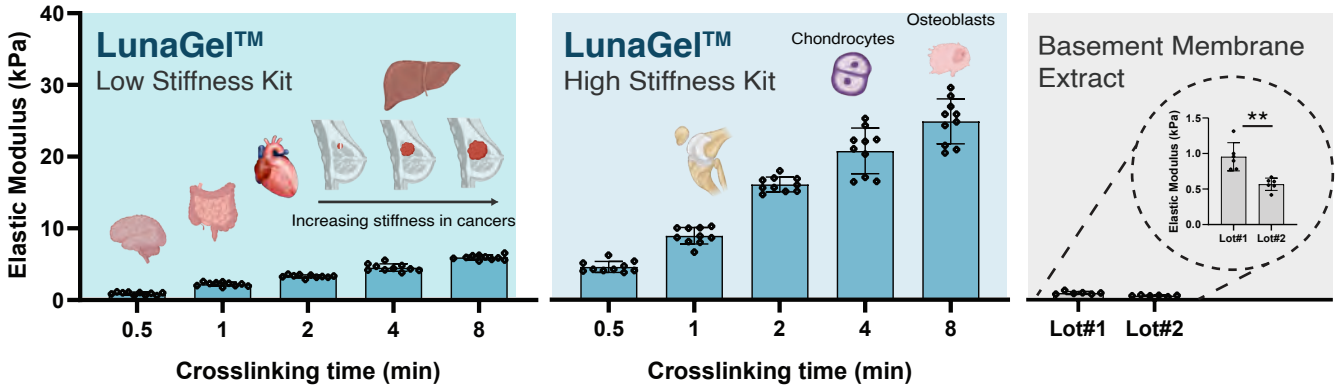


Figure 2: LunaGel ECMs enable mechanical tuning to replicate physiological and pathophysiological tissue microenvironments. Elastic modulus measurements of LunaGel™ Low Stiffness Kits (left), LunaGel™ High Stiffness Kit (middle), and Basement Membrane Extract (Matrigel®, right) across different crosslinking times (0.5 to 8 minutes). LunaGel™ demonstrates tunable mechanical properties, with stiffness increasing in a time-dependent manner, allowing researchers to tailor the stiffness to physiological levels relevant for various tissue types. In contrast, Matrigel® shows very low stiffness (<1 kPa) and high variability between batches (Lot #1 vs Lot #2). This highlights LunaGel's advantage in providing reproducible and tunable stiffness compared to Matrigel, which is both too soft and inconsistent, limiting its applicability for many mechanobiological studies.

LunaGel™ offers low and high stiffness kits, allowing users to easily create 3D models of both healthy and diseased tissue environments (Figure 2). The ability to control ECM stiffness is particularly beneficial in fields such as cancer research and tissue engineering, where cell behaviour is strongly influenced by mechanical cues - as demonstrated below.

LunaGel™ ECMs are the ideal substrate to study Epithelial-to-Mesenchymal Transition (EMT) processes in vitro. Figure 3 demonstrates that MDA-MB-231 breast cancer cell phenotypes are highly regulated by ECM stiffness. After 7 days of culture on LunaGel™ substrates replicating breast issue in different states of pathology (ranging from healthy tissue to late-stage cancer), cell invasiveness increased as a function of ECM stiffness, corroborating clinical data suggesting higher occurrence of metastasis in breast cancer with higher tissue stiffness. A shift towards more migratory cell morphologies associated with metastasis (magnified inserts) was observed with increasing LunaGel™ ECM stiffness. **Critically, this key biological process—so consequential for cancer metastasis and patient survival—cannot be effectively studied using Matrigel® and BMEs, which lack the ability to replicate the pathological stiffness required to model advanced cancer environments.**

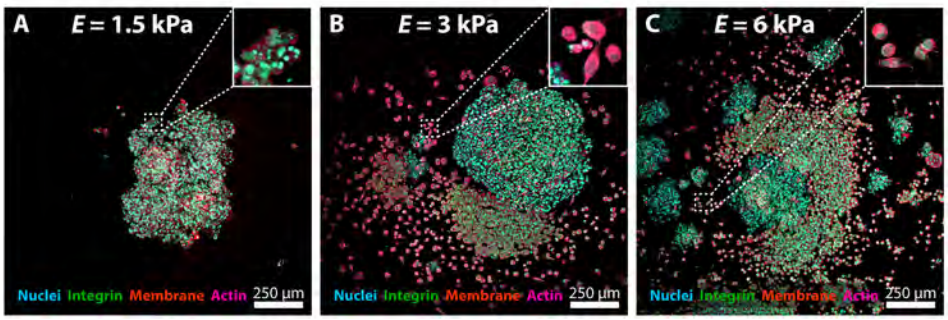


Figure 3: MDA-MB-231 breast cancer cell invasiveness increases with ECM stiffness. MDA-MB-231 spheroids were cultured on LunaGel™ Low Stiffness ECM with elastic moduli of (A) 1.5 kPa, (B) 3 kPa, and (C) 6 kPa to mimic the mechanical properties of healthy breast tissue, as well as breast tissue with early stage and late stage cancer, respectively, for 7 days.

LUNAGEL™ SUPPORTS CONSISTENT SPHEROID MORPHOLOGY AND ENHANCED CELL GROWTH COMPARED TO MATRIGEL®

Spheroid Morphology: Representative confocal images of MCF-7 breast cancer spheroids cultured in Matrigel® and LunaGel™ for 7 and 14 days are shown in Figure 4A. MCF-7 cells in Matrigel® display inconsistent morphology with variable spheroid sizes and irregular shapes. By day 14, the spheroids become large and disorganized, suggesting poor structural integrity and potential overgrowth. In contrast, cells grown in LunaGel™ show consistent, well-formed spheroids with a defined, regular structure at both day 7 and day 14. The uniformity of spheroid size and morphology observed in LunaGel™ suggests better control over the 3D cell culture environment, supporting more reliable experimental outcomes.

Metabolic Activity: The bar graph in Figure 4B (left) quantifies the metabolic activity of MCF-7 cells cultured

in Matrigel® and LunaGel™ at day 1 and day 7. Both BME lots show significant variability in metabolic activity, particularly at day 7. LunaGel™ demonstrates significantly higher and more consistent metabolic activity at day 7 compared to Matrigel® ($p < 0.01$), indicating superior cell viability and potentially enhanced cellular proliferation or metabolic function in this gel matrix. In Figure 4B (right), DNA content is used as a proxy for cell proliferation. By day 7, LunaGel™ exhibit significantly higher DNA content compared to Matrigel® ($p < 0.001$), further supporting the conclusion that LunaGel™ provides a more conducive environment for cell growth and proliferation. These data suggest that LunaGel™ offers a more stable and reproducible environment for MCF-7 spheroid formation, metabolic activity, and growth compared to Matrigel®, in which spheroids exhibit variability in both morphology and metabolic performance across different lots.

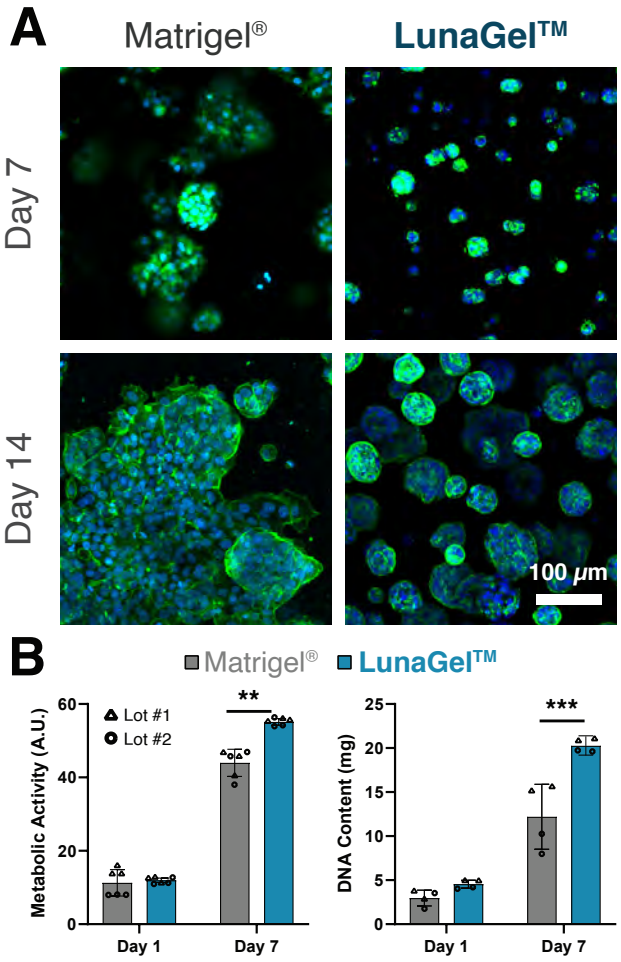
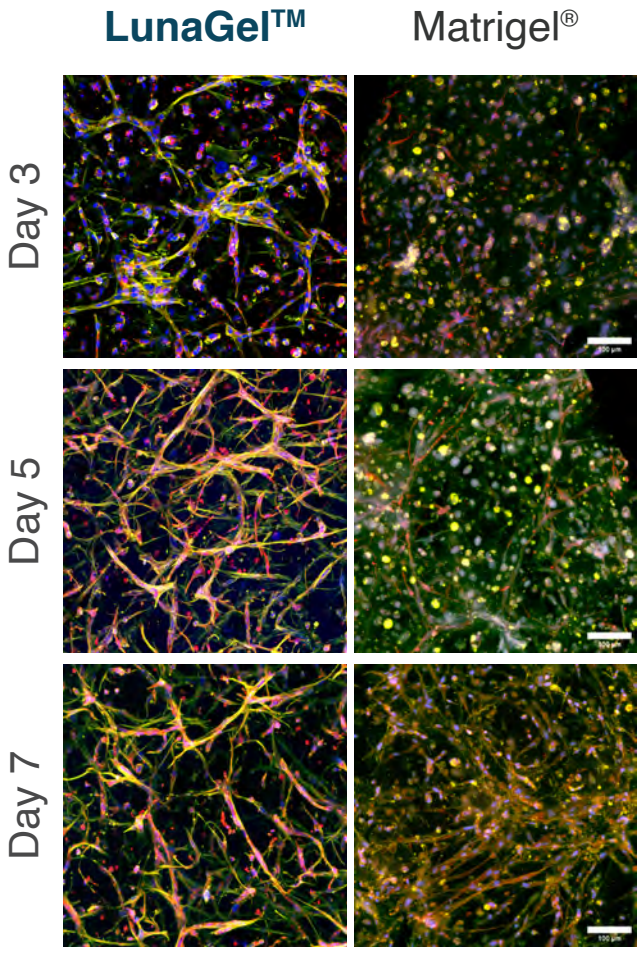


Figure 4 (right): LunaGel™ outperforms Matrigel® in Supporting MCF-7 Spheroid Formation, Metabolic Activity, and Proliferation. (A) Confocal images of MCF-7 spheroids cultured in Matrigel® and LunaGel™ for 7 and 14 days. LunaGel™ supports consistent, well-formed spheroids, while Matrigel® results in inconsistent spheroid morphology. (B) Quantification of metabolic activity (left) and DNA content (right) at days 1 and 7. LunaGel™ shows significantly higher and more consistent metabolic activity and cell proliferation compared to Matrigel® (mean ± SD, $p < 0.01$, $^*p < 0.001$).



LUNAGEL™ SUPPORTS ENHANCED CAPILLARY-LIKE NETWORK FORMATION AND MATURATION IN HUVEC/MSC CO-CULTURES COMPARED TO MATRIGEL®

HUVEC (Human Umbelical Vein Endothelial Cell) and MSC (bone-derived human Mesenchymal Stromal Cell) co-cultures were assessed for capillary-like network formation and maturation over a 7-day period in LunaGel™ and Matrigel® (Figure 5). In LunaGel™, cells formed well-organized, robust capillary-like networks as early as day 3, which matured over time,

Figure 5 (left): LunaGel™ supports superior capillary-like network formation and maturation compared to Matrigel®. Representative confocal images of HUVEC/MSC co-cultures in Matrigel® and LunaGel™ on days 3, 5, and 7. In LunaGel™, well-formed capillary-like networks stained for CD31 (red) and actin (green) develop early and mature over time, while in Matrigel®, networks are less organized and sparse. Nuclei are stained with DAPI (blue). Scale bar: 100 µm.

showing clear structural development by day 7. These networks stained positively for CD31 (red), indicating endothelial differentiation, and actin (green), representing cytoskeletal organization, with nuclei visualized by DAPI (blue). The capillary-like structures in LunaGel™ displayed increased branching and stability from day 3 to day 7, demonstrating LunaGel™'s ability to support prolonged culture and network maturation.

In contrast, cells cultured in Matrigel® formed less defined and more disorganized networks, with significantly lower branching and less pronounced CD31 and actin expression. Over time, the networks in Matrigel® remained sparse and lacked the clear maturation observed in LunaGel™. This indicates that LunaGel™ provides a more stable and supportive environment for endothelial cell differentiation and capillary network development, making it superior for applications in angiogenesis and vascular tissue engineering.

CONCLUSION

LunaGel™ represents a significant advancement over traditional basement membrane extracts like Matrigel®, offering superior tunability, reproducibility, and scalability for 3D tissue culture applications. By enabling precise control over ECM stiffness, LunaGel™ allows researchers to more accurately model a variety of physiological and pathological conditions, from soft brain tissues to stiff cancerous environments. In contrast, Matrigel®'s batch variability, undefined composition, and limited mechanical properties restrict its applicability, especially for advanced studies in cancer research, drug development, and tissue engineering. LunaGel™ not only ensures consistent and reproducible experimental results but also simplifies workflows, with faster setup times and easy handling at room temperature. Its semi-synthetic nature eliminates the uncontrolled factors found in natural extracts, providing a highly customizable platform that is better suited for modern research challenges. Whether for basic research or preclinical drug testing, LunaGel™ offers an optimized solution for creating biologically relevant 3D tissue models.

As the demand for more predictive, reproducible, and ethical alternatives to animal testing grows, LunaGel™ is a versatile, cutting-edge technology that addresses the limitations of current 3D culture methods, helping to accelerate discoveries and improve outcomes across multiple fields of research.