Sero Krystal™ PDL Microplates Enhance Cell Viability, Morphology and Proliferation under Serum-free Conditions

Authors: Ewa Wosiek, Project Support Scientist & Dr. Lindsay Parkes, Senior Project Scientist, Porvair Sciences Ltd.

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Abstract

Researchers grow cells in a serum-free environment to more accurately mimic physiological conditions. This presents additional challenges in cell culture as many cell lines struggle to attach to tissue culture plastics in the absence of serum. This short application note demonstrates that Sero Krystal™ Poly-D-Lysine (PDL) coated microplates overcome these issues and promote superior attachment, growth and proliferation for challenging cell types in serum-free media.

Introduction

Cell cultivation and analysis continues to evolve at rapid pace and the need for high throughput screening (HTS) is increasing, especially for large scale applications such as drug screening and discovery. In recent years multiple cell-based HTS approaches have been utilised in the discovery of potential anti-coronavirus treatment options where a broad spectrum of antiviral drugs have been analysed for inhibition of SARS-CoV-2 replication. In experiments where adherent cell lines are used scientists may consider supplementing their cell growth media with animal-derived serum such as foetal bovine serum (FBS). Serum supplements contains extracellular matrix (ECM) proteins that allow cells to attach to the plastic support of the culture vessel. Although serum is beneficial for cell growth there are risks associated with the addition of sera of animal-origin into cell culture systems. Contamination, environmental and ethical concerns, cost and availability as well as lot-to-lot variation are major drawbacks to this traditional practice. For adherent cells, attachment to the surface of cultureware is an essential prerequisite for proliferation and differentiation. Sero™ Krystal™ microplates coated with PDL are the perfect solution; eliminating the usage of animal-derived serum in cell culture experiments without sacrificing on cell attachment, viability and proliferation.

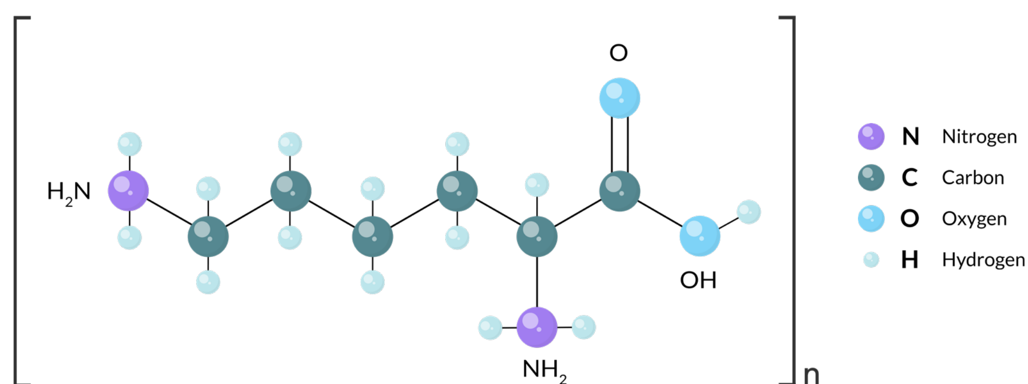

Figure 1. Schematic representation of the molecular structure of Poly-Lysine. Lysine is an amino acid that exists as two enantiomers, L-Lysine and D-Lysine

Poly-lysine is a well-known protein polymer which exists as two enantiomers: Poly-D-Lysine (PDL) and Poly-L-Lysine (PLL) (Fig 1). Both are commonly used as a thin coating for tissue culture ware that supports cell growth, attachment and differentiation of adherent cell lines. Typically, the synthetic and positively charged PDL is the favoured option over PLL for culture ware coating due to its resistance to enzymatic digestion by cellular proteases secreted by certain cell lines (Wiatrak, 2020). Additionally, PDL as an artificial protein does not influence the signalling pathways of the cultured cells therefore it is widely used in cell culture systems.

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Sero Krystal™ PDL coated microplates, available in 96- and 384-well format, are coated with a 70 to 150 kDa PDL polymer via proprietary methods. The coating creates a uniform net positive charge on the surface of the plate wells. This positive surface charge improves electrostatic interactions between the coated surface and the negative charges of the cell membrane (Curtis, 1983), thus improving cell attachment. Sero Krystal™ PDL microplates are ideal for the culture of transfected cell lines, primary neurons, glial cells and fibroblasts, such as the adherent mouse fibroblast cell line L929 and other common adherent cell lines including HEK-293, BHK-21, PC12 and, NSC-34.

L929 is a fibroblast cell line derived from a clone of normal subcutaneous areolar and adipose tissue of a male C3H/An mouse (Heap, 2021). The L929 cell line is commonly used by researchers to study viral transfection and vaccine performance. It is considered to be the gold standard for testing cytotoxic effects (Dumitrașcu, 2022). For this reason, the L929 fibroblast cell line was selected for this study. Morphology, viability and cell growth of the cell line was investigated when cultured in serum-free Dulbecco's Modified Eagles Media (DMEM) on selected PDL coated or uncoated surfaces.

Performance results were compared between the tissue culture treated (TC) uncoated 96-well polystyrene (PS) surface and coated Sero Krystal™ PDL microplates. The morphology and cell growth data of L929 on both surfaces was assessed against data achieved with growth media supplemented with serum on the uncoated TC surface as a control.

Materials and Methods

L929 Cell Stock Culture

Frozen stocks of AATC L929 cell line (Sigma, UK) were prepared at 3 x10^6 cells/ml in freezing media. L929 mouse adherent fibroblast cells in freezing media were thawed and cultured in DMEM (Lonza, Belgium) supplemented with 2mM Glutamine (Lonza, UK), 1% Penicillin/Streptomycin (Sigma, UK) and 10% FBS (HyClone, South America) at 37°C and 5% CO_2. Cells were passaged every 48 hrs.

PDL Plate Selection

Sero Krystal™ clear 96-well PDL coated microplates (Porvair Sciences, UK, Product No. 500269-PDL) were used for the study. As a control, Sero™ Krystal™ uncoated clear 96-well TC treated plates (Porvair Sciences, UK, Product No. 500269-TC) were selected.

Plate Seeding

A monolayer of adherent L929 cells was cultured in a 75 cm^2 flask to confluency in full media supplemented with FBS (HyClone, South America), 1% Penicillin/Streptomycin (Sigma, UK) and 2mM Glutamine (Lonza, UK). Cells were detached with 0.25% Trypsin/EDTA (Sigma, UK). After 5 min of trypsinisation time at 37°C, cells were detached from the surface of the flask and 10 ml of DMEM (with 10% FBS) was added to deactivate the trypsin. The L929 cell suspension was transferred to a 15 ml centrifuge tube and centrifuged at 1,200 rpm for 5 min to collect the cell pellet. The supernatant was discarded and 10 ml of serum-free DMEM was added to the cell pellet and gently mixed in fresh media to form a single cell suspension. Cell counts were performed with Trypan Blue (Biorad, UK) using cell counter slides and a TC20 Automated Cell Counter (Biorad, UK) according to the manufacturer's instructions. 50,000 cells in 100 µl of serum-free DMEM (Lonza, Belgium) supplemented with 2mM Glutamine and 1% Penicillin/Streptomycin (Sigma, UK) were seeded per well onto selected microplates. Cells were incubated for 24 hrs at 37°C with 5% CO_2.

Morphology Test

After 24 hrs incubation in serum and serum-free DMEM (Lonza, Belgium) on selected plates L929 cells were observed using an inverted light microscope and images were taken using a ZOE Fluorescent Cell Imager (Biorad, UK) to assess cell morphology, growth and attachment. A minimum of 10 wells were viewed to ensure images were representative of the whole plate.

Viability Testing

After 24 hrs incubation time in serum-free media, cell counts and viability tests were carried out on randomly selected wells of each plate. Measurement of cell viability was performed using Trypan Blue dye, cell counter slides and TC20 Automated Cell Counter as previously described. Prior to the measurement, media was removed from selected wells and L929 cells were washed with 100 µl of prewarmed PBS (Lonza, UK). Cells were detached with 50 µl 0.25% Trypsin/EDTA solution (Sigma, UK). 50 µl of PBS (Lonza, UK) was added to each well to make up the volume to 100 µl. Cells were gently aspirated up and down to obtain a unicellular suspension. The cell number and percentage viability were recorded from 12 wells and the averages taken.

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Results and Discussion

To demonstrate the performance of Sero Krystal™ PDL microplates in promoting cell attachment, enhancing proliferation and improving viability in serum-free conditions L929 cells were grown under these conditions on standard tissue culture treated vs Sero Krystal™ PDL coated microplates.

As can be seen in Figure 2A L929 cells grown in serum-free conditions exhibit cell clumping and poor cell attachment on the uncoated TC treated plastic surface. Cell morphology is irregular and cells attach only sporadically to the surface of the microplate well. Low cell density is observed on the surface of the TC-treated uncoated microplate with aggregates of poorly differentiated cells. In contrast, cells on the Sero Krystal™ PDL microplate were fully confluent, well differentiated and regularly shaped. L929 cells are evenly spread over the uniformly coated microplate well surface (Fig 2B). L929 cell confluency and attachment in serum-free conditions on Sero Krystal™ PDL microplates is comparable to the cell growth on uncoated TC treated plastic surfaces (Fig 2C) with serum supplemented growth media. Thus, eliminating the risks of contamination, environmental and ethic concerns, cost and availability as well as lot-to-lot variation associated with animal sera.

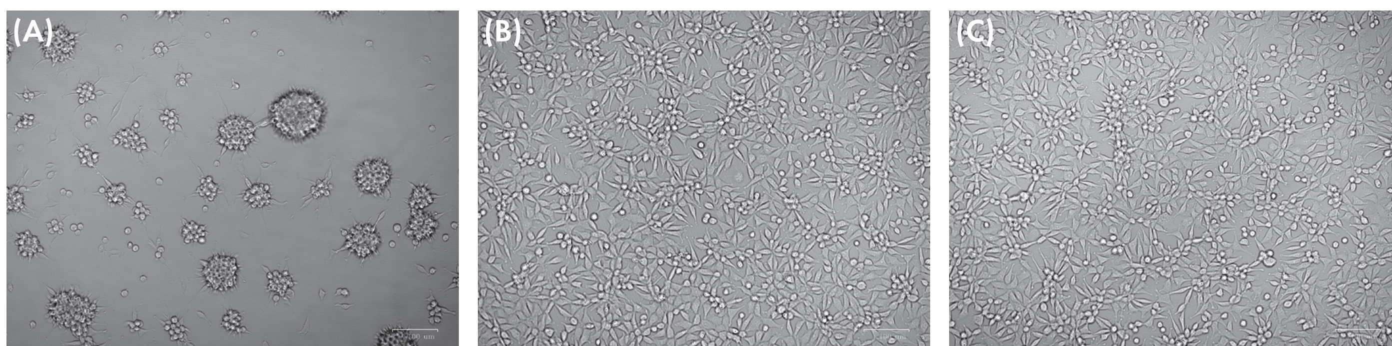

Figure 2. Cell line L929 cultured in serum-free DMEM on TC treated surface (A), Porvair Sciences Sero Krystal™ PDL coated microplate (B) and TC treated surface in DMEM supplemented with FBS (C).

Cell count and viability measurements across selected microplates (see Table 1) demonstrated the highest number of viable cells on the Sero Krystal™ PDL microplate coated plate after 24hrs incubation. These data clearly demonstrate that the Sero Krystal™ PDL coated surface has the best survival properties for the adherent L929 cell line under serum-free conditions. Growth on the Sero Krystal™ PDL microplate increased the viability of the cell line by approximately 50%.

	Uncoated TC treated Microplate	Sero Krystal™ PDL Microplate
Total Cell No. (cells/ml)	70,500	69,900
Live Cells No. (cells/ml)	37,400	69,900
Cell Viability (%)	53%	100%

Table 1. Cell count and viability readings of uncoated TC treated and Sero Krystal™ PDL coated microplates.

Conclusion

This application note highlights the superior performance of optically clear 96-well Sero Krystal™ PDL microplates under serum-free or low-serum conditions. The adherent cell line L929 exhibits enhanced cell attachment, uniform cell morphology, and accelerated growth and proliferation. The results demonstrate that the range of Sero Krystal™ PDL coated microplates is the perfect animal free alternative for serum-free risk-free enhanced cell adhesion of adherent cell lines.

Sero Krystal™ PDL microplates are available in a range of well formats for high-throughput screening, frame colours for assay detection systems and minimum crosstalk for enhanced photometric readings.

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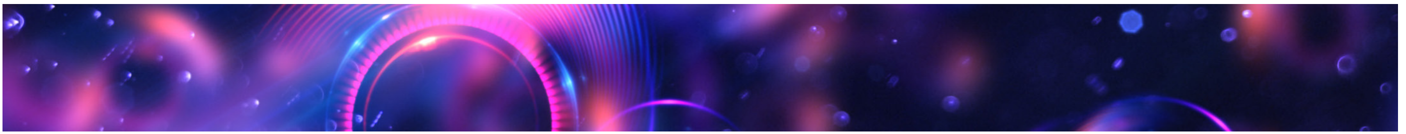

Contact Information

EU/RoW Enquiries: hello@porvairsciences.com

USA Enquiries: info@jgfinneran.com

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