

Surface innovation optimising cell adhesion

by Dr Joseph Granchelli

The value and contribution of surface chemistries to the success of *in vitro* cell culture are emphasised in this article, and the critical issues associated with the development of such technologies, as well as the relevant developments up to date, are discussed and reviewed.

As an important tool for the study of cell biology, the production of proteins and the discovery and characterisation of new drugs, *in vitro* cell biology has uses within many academic, clinical and life science laboratories, as well as applications in pharmaceutical R&D.

Cell-based assays are widely recognised as relevant *in vitro* models, and techniques such as high content analysis (HCA) now form a key approach to drug discovery research. As such, the quality and consistency of *in vitro* cell culture is of paramount importance in order to obtain the most accurate and reproducible results possible. Experimental success or failure will depend on numerous variables that require careful control, including the techniques and protocols used, cell lineage, growth conditions, media formulation and culture surfaces. In adherent cell culture, the surface used can have a significant impact on cell viability, the consistency and purity of a culture, and consequently on the reproducibility and accuracy of the obtained results. When culturing cells, the aim is to maintain their physiology to be representative of the *in vivo* model, and engineering mechanical attachment to artificial cell surfaces via normal cellular processes can help to achieve this. Here we discuss the principles of cell adhesion, along with the basic technologies used to optimise surfaces for improved adhesion and expansion in addition to the effects of various surface compositions on cell growth. A review of more

recent technological developments of surface chemistries will also look at the preferences of more fastidious cell types and the differential use of culture surfaces for different applications.

Cell interaction and communication

Cells respond to external stimuli such as nutrients, growth factors, cytokines, O₂ [1], pH and mechanical/shear stress, through cell surface interactions and the activation of internal signalling pathways (classified as outside-in interactions). In contrast, inside-out intracellular signal transductions allow the cell to remodel its external microenvironment and to upregulate or change the structure of cell surface receptors. This remodelling is often required for growth, survival, intracellular adhesion or migration. Integrins are one group of cell surface receptors that are critical for such processes and consequently normal cell function. Their transmembrane heterodimeric structure allows them to act as mediators between the cells and molecules within the external microenvironment and the internal signaling pathways – they are essentially communicators.

Influential extracellular matrix (ECM) components

In vivo, cells adhere to biological substrates via the basal lamina, a tissue-specific, self-assembling, acellular glycoprotein

matrix, which is secreted predominantly by the cells themselves. The most proximal part of the basal lamina, the extracellular matrix (ECM) contains a combination of different glycoproteins (approximately 45), including laminin, collagen, perlecan and nidogen. Modifications to the cell surface, (i.e. the up- or down-regulation of integrins), are commonly required for cellular events such as growth, migration, or apoptosis, which through complex signalling pathways induce a rapid reconfiguration of the ECM. Furthermore, the binding affinities of glycoproteins allow homologous and heterologous interactions resulting in the formation of a complex network of molecules, with numerous integrin binding sites for cell attachment. One unit of the cell surface integrin molecule confers binding specificity, while the other confers binding affinity to the corresponding ECM component. This binding initiates conformational changes of the integrin, activating complex signaling pathways for communication to the intra-cellular components. As a result, significant effects on cell physiology are observed [2].

Consequently, cellular interaction and adhesion are essential contributors to cell survival. When deprived of the correct attachment signal via their integrin receptors, catastrophic effects on the cell culture population as a whole may result. Even if they adapt to survive in suspension, their physiology and morphology may have greatly

altered. Cells are likely to divide into sub-populations with distorted physiology, or be stimulated to undergo significant cellular events such as differentiation or apoptosis. The result is a heterogeneous population which has moved away from its true phenotype and may exhibit a varied response to controlled experimental stimuli. In order to maintain cell physiology and phenotype *in vitro*, the provision of culture conditions specific for that cell type can reduce or eliminate these potential changes. Normal cell attachment and growth can be facilitated through the application of an appropriate artificial culturing surface.

In vitro substrates

Novel culture surfaces must be capable of facilitating rapid re-attachment to quickly re-establish normal cell function and recovery from any integrin disruption that may have occurred as a result of the enzymatic treatment used for cell dissociation and passaging [3]. However, the materials used need to be structurally similar to the glycoprotein molecules of the ECM. Many scientists have used coatings for their culture vessels, for example in-house manufactured rat tail collagen isolates [4]. Maintaining a consistent level of purity and coating uniformity across all culture flasks is not easy and the use of in-house preparations containing animal derivatives can provide a potential source of contamination. Thus, manufacturers have worked to develop surfaces using both natural glycoproteins and man-made alternatives. Many of these alternatives such as polystyrene, PETG, thermanox and permanox are thermoplastic polymers, which are easy to mould and manufacture, and are very uniform. However, they are hydrophobic in nature, so will exhibit a very different surface topology from the ECM. With no functional oxygen or nitrogen groups, even more hydrophilic surfaces such as polycarbonate and glass

require some form of surface modification to further facilitate cell attachment and growth.

Common surface modifications Energetic oxidation

This method enables the creation of small hydrophilic regions on the polymer surface, which can stabilise the protein's conformational changes that are essential for binding. There are a variety of treatments that can be used to do this, such as corona discharge, plasma treatments, or UV [5] and gamma irradiation, all of which effectively bombard the surface with ions, electrons or photons. The result is a subtle difference between the chemical groups formed. This bombardment breaks some of the bonds in the polymer chains as well as the gaseous material surrounding them, providing readily available free-radicals on the culture surface. With the ability to quench nearby molecules, the distribution of chemical groups will become slightly hydrophilic to facilitate the protein unfolding required to expose the hydrophobic domains for binding [Figure 1]. However, without the coating of animal-derived substrates, the cells must use proteins for integrin binding from the surrounding media. Ready-made cell culture media commonly contains animal serum, which includes fibronectin and vitronectin. These will self-

assemble in critical concentrations and bind to the artificial substrate, providing a ligand-rich surface for cell binding [6, 7]. Each surface will exhibit different affinities for each of the soluble proteins and the conformation and orientation will vary, demonstrating a unique binding pattern that is more suited to some cell lines than others [8]. Consequently, other surface modifications have been developed in order to further improve on the surfaces available.

Passive adsorption

Passive adsorption is one surface modification that involves coating the polymer surface with naturally occurring, self-assembling protein molecules from the ECM (i.e. collagen) [3]. During passive adsorption, binding occurs when the ligand collides with the surface by random thermal motion in the correct orientation with enough energy to interact non-covalently. The binding mechanism uses hydrophobic interactions between the polymer chains and the protein hydrophobic domains, and the proteins will bind to the polymers in a random orientation. The proteins used are typically coated in an aqueous solution and the hydrophobic regions are internally positioned for polymer binding. As such, protein chains must partially unfold in order to melt into the polymer surface during binding. This conformational change may have significant

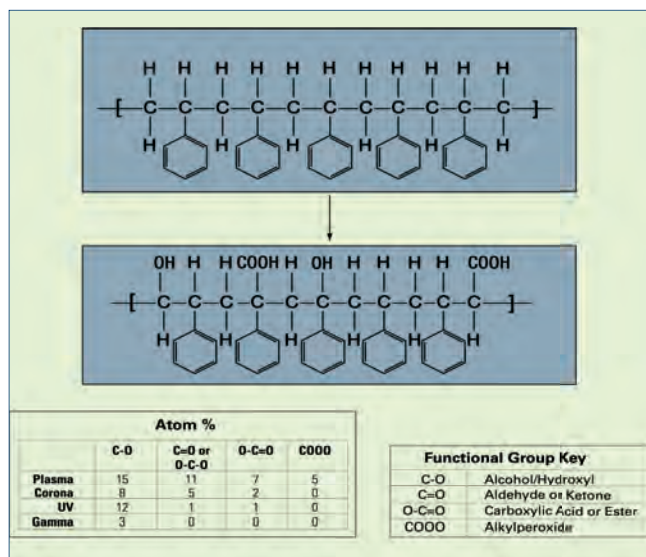


Figure 1. Energetic oxidation of polystyrene – the original surface is hydrophobic but the oxidised surface has both hydroxyl and carboxyl functional groups making it more hydrophilic. The table demonstrates the different surface configuration using the selection of oxidative treatments.

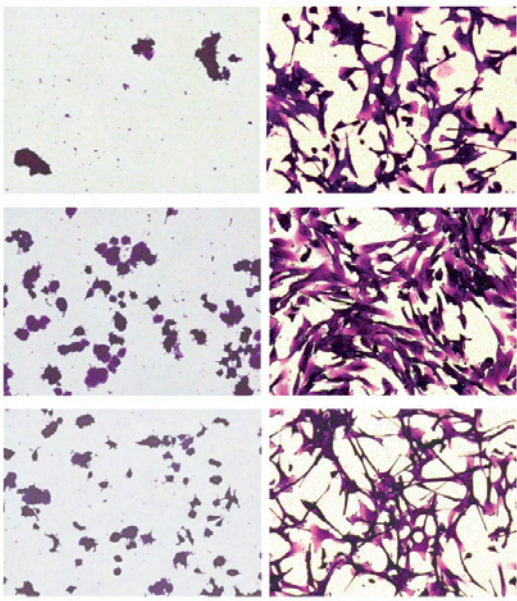


Figure 2. Preferential growth of PC-12 and MDCK cells on different culture surfaces.

negative implications, such as: the activation or deactivation of critical functionality; masking an important recognition domain; or incorrect refolding can cause denaturation, blocking any future cell adhesion events [2].

The main driver for using passive adsorption is to promote normal cell adhesion with molecules that are naturally occurring in the ECM. Although some more sensitive cell lines may require a biological coating, many may just demonstrate better function and proliferation [Figure 2]. However, the degree of experimental success can be dependent upon cell type. There is no standard rule that applies and each set-up may require further optimisation. Surfaces modified using this method can be more costly to produce than alternatives and while these products can be certified aseptic, they are not terminally sterile (to FDA standards), since the methods required for this can alter the biological activity of the protein-coated surface. Passive adsorption can also demand a binding process that is not energetically favorable. Proteins in aqueous solutions do not always readily unfold

their tertiary structure to expose the hydrophobic domains which are required for binding [2].

Fine-tuning

Further optimisation is often essential if the self-assembling matrix is not suitable for a specific, and maybe more sensitive, cell type to adhere. For example, a cell line will naturally adapt, over a number of passages, to a less sophisticated surface and reduce its requirement for serum. Where there are no serum-derived proteins to aid cell attachment, it is thought that the cells themselves will temporarily produce attachment factors until they are able to produce their own ECM. This is commonly known as serum

reduction [Figure 3], but again the degree to which this is possible and successfully implemented is often dependent on cell and surface type [Figure 4]. Serum reduction can be of particular value within bioproduction, where the use of serum necessitates downstream protein filtration steps. Adventitious agents can be introduced at a cost of \$0.80 to \$1.00, which can accrue significantly over time [8]. Thermo Scientific Nunclon Delta is an example of a surface that can be used for such purposes.

A novel surface modification

The covalent grafting of polymers is one rather new and exciting surface modification, which is useful for attaching molecules that do not efficiently adhere through passive adsorption, such as proteins which do not exhibit highly hydrophobic regions. The Thermo Scientific Nunc Immobilizer cell line is one example of this, as it uses a group that reacts with polystyrene, an anthroquinone group which is coupled to a spacer arm. Various chemical functionalities are attached to the other end, allowing the covalent coupling of biomolecules. For example, the reactive

group of Immobilizer amino will covalently couple primary amines, while Immobilizer streptavidin will couple biotinylated molecules.

Stimulus-sensitive surfaces can modify or change their chemistry in response to external stimuli, and can provide significant benefits for cell growth, dissociation and passaging. One such surface, the Thermo Scientific Nunc UpCell, utilises the covalent modification of polystyrene to poly-n-isopropylacrylamide (NIPAM), to provide a material which can modify its chemical conformation upon temperature change. This enables the simple control of cell adhesion: at raised temperatures NIPAM becomes more hydrophobic and interacts more readily with proteins; whereas at lower temperatures numerous hydrophilic groups are exposed to prevent protein interaction. Furthermore, enzymatic methods such as trypsinisation, which can cleave important surface molecules, can be avoided during cell dissociation and passaging. The structure of the basal lamina and cell surface receptors are conserved, allowing whole sheets of cells to be removed. This method has proved especially important for tissue engineering

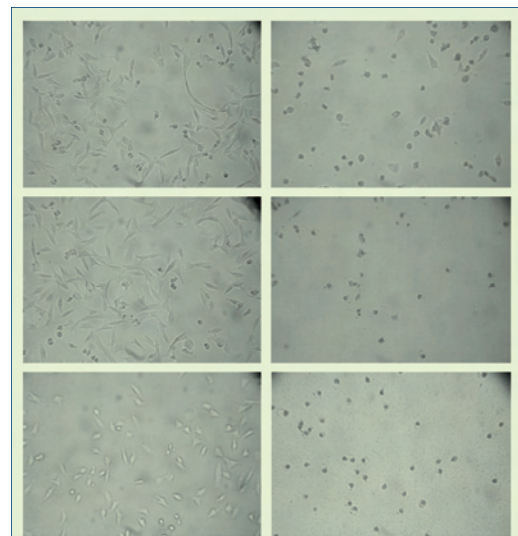


Figure 3. Cell line Hel-299 demonstrates differential adaptation response to serum reduction with Corona and N₂O plasma oxidised surfaces.

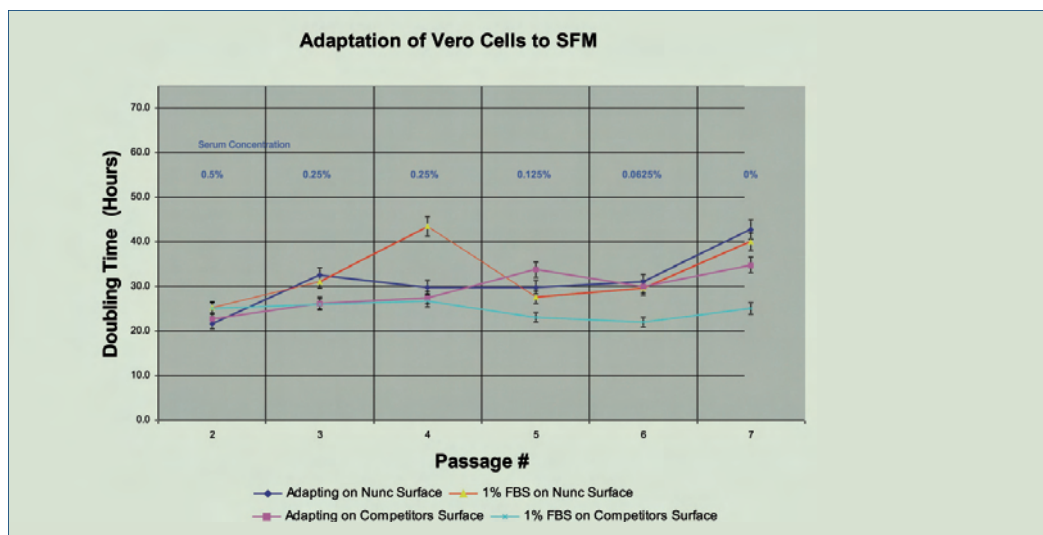


Figure 4.
Vero cells adaptation to serum free media with Corona and N₂O plasma oxidised surfaces.

research applications. For example the Nunc UpCell has been used to provide a feasible and effective method for treating heart failure [9,10] as well as for a range of corneal regenerative therapies [11,12]. Problems related to the intramyocardial injection of cells, including cell loss and limited graft area can be overcome; and bioengineered human corneal endothelial cell sheets can be produced *in vitro* to retain native morphology and functional characteristics.

Conversely, for the cultivation of single cells and cell clusters in suspension, there are surfaces available that have been designed not only to prevent cell attachment, but to also allow the growth of cells that are sensitive to unwanted activation and differentiation signals arising from cell adhesion. The Nunc HydroCell Surface is a thin layer of a covalently-immobilised, super hydrophilic polymer, which enables a more homogenous suspension culture. Higher cell yields and cell-secreted protein production can be achieved across many cell culture applications, including those involving monocytes/macrophages and stem cells. Furthermore, the adsorption of culture medium-derived proteins and cell-secreted proteins to the surface is minimal.

The future

Today, cell culture is widely used and extremely valued across numerous clinical and discovery applications.

Cell-based research is constantly providing the industry with new and exciting discoveries and novel *in vitro* models for further study. As such, it is a constantly evolving discipline which poses new questions on an ongoing basis. The requirements for culture optimisation are also evolving as the types of cells used are expanding and experimental procedures developing in complexity. To meet these demands, scientists and manufacturers alike understand that variables such as the surface, media and the cells themselves all require optimisation to produce strong adhesion, independent of the chemistry used. While it is possible to design surfaces which are good for the majority of cells, more difficult cell types require more advanced modifications. Partnering with companies such as Thermo Fisher Scientific can provide extensive application expertise and practical advice to jointly aid surface selection and development. Furthermore, phenotypic stability and experimental consistency can be significantly improved.

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