Affinity cell separation with HC hydrogel coated substrates

Application note

Current techniques

Cell isolation by differential adsorption to solid supports has become an important research tool with numerous applications including establishment of pure cell culture lines, diagnosis of cancer or bacterial diseases, and even therapeutic treatments. Affinity ligand-assisted separation holds considerable potential as it takes advantage of the high specificity of receptor-ligand systems; the technique is based on interactions between either a cell surface ligand and its immobilized complementary binding molecule or specific ligand-targeting molecules on the cell surface and immobilized ligands. Depending on the application, the immobilization matrix can be a flat surface, composed of beads or a chromatographic column support.

Limits and pitfalls

With any support material the essential prerequisite for effective and selective affinity cell separation is a bioinert surface, which prevents cells from being adsorbed non-specifically and mimics the natural environment of the cells, i.e. it should not trigger unwanted reactions of the cell metabolism. On the other hand, it must allow a dense immobilization of correctly oriented receptor molecules with retention of their activity. With state of the art coated surfaces, beads or other support materials, the selectivity represents a critical issue which potentially limits the scope of applications. Cells tend to adhere non-specifically to the modified surfaces especially at longer incubation times; this makes careful optimization necessary and may often still yield insufficient separation rates. Another complication results from the fact that unfolding and steric hindrance inactivates 90 – 95% of the immobilized receptor proteins. The typical active fraction of antibodies physiosorbed on a 2D surface is only 0.1 ng / mm². Peptides, carbohydrates, adhesion or growth factors and small molecules are usually difficult to immobilize at all.

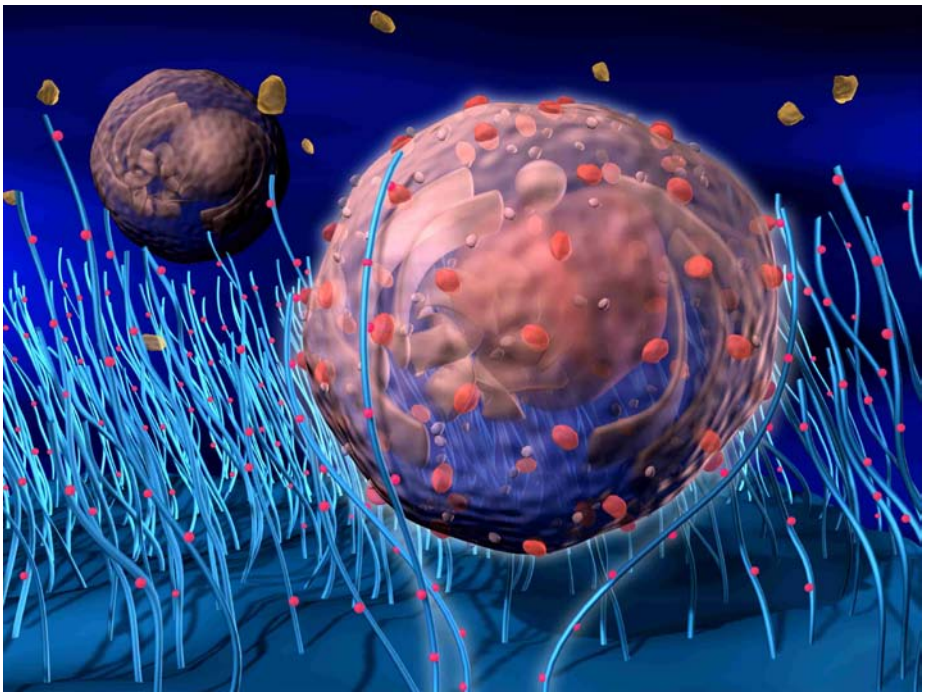

Fig. 1: Cells, bound by seaweed-like structured, ligand derivatized HC hydrogel chains.

HC hydrogels open up new possibilities

Initially developed for enhancing the sensitivity of protein microarrays, the brush like structured HC hydrogel coatings are also an excellent surface for selectively attaching cells to solid supports. The non-modified form is inert, consisting of a highly hydrated, slightly negatively charged polymer matrix that effectively prevents non-specific cell adhesion. When coupled to specific receptor molecules (proteins, peptides, carbohydrates or small molecules), a high density of binding sites is generated which, in contrast to normal crosslinked hydrogel matrices, are sterically accessible for the relatively voluminous cells.

As illustrated in fig. 1, the flexible hydrophilic polymer chains of this 1-2 µm thin layer align themselves smoothly to the cell surface and allow the receptor molecules to attach at multiple sites simultaneously. The result is binding of specifically attached cells which are safely embedded in a cell friendly environment. In contrast to common panning techniques, it is not necessary to carefully optimize the incubation parameters or the reaction time as practically no time dependant non-specific adhesion occurs.

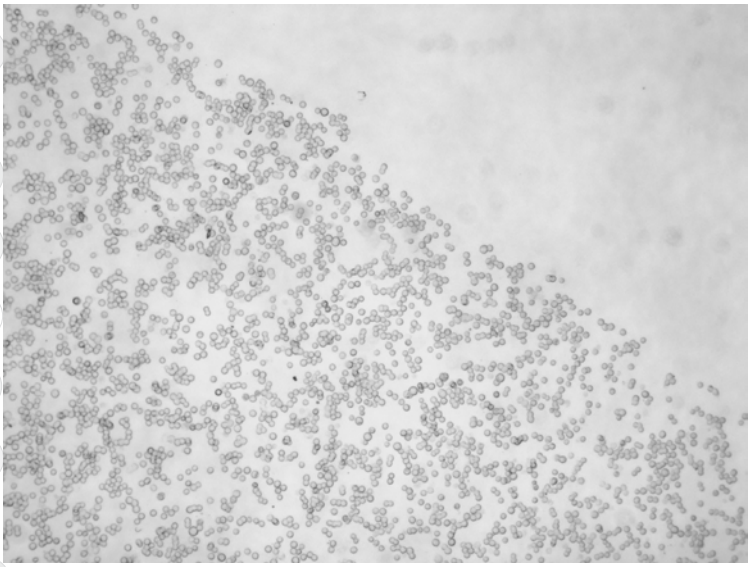

Fig. 2: MDA breast tumour cells (surface antigen EGFR) in human serum were incubated on a partially antibody derivatized HC hydrogel surface.
Left: Anti EGFR IgG functionalized HC hydrogel surface.
Upper right corner: Blank HC hydrogel surface (control).

The immobilization of specific receptor molecules to the HC hydrogel matrix can be done in different ways. The standard method is suitable for most applications; covalent coupling via amino or hydroxyl functionalities to the NHS preactivated hydrogel derivative (product code HCX). NHS mediated immobilisation on HC hydrogels typically yields receptor densities of 20 – 100 ng/mm^2 with a remaining activity of >80%. As the immobilized molecules are covalently bound rather than adsorbed, the immobilization process is equally well suited for proteins, peptides, low MW compounds, nucleic acids and other species as long as they bear amino, (di)sulphide or aldehyde groups. For alternative coupling strategies, streptavidin, protein A, disulphide or hydrazide functionalized coatings are available. The receptor containing buffers can be either applied to the entire surface or spotted onto defined regions. It is thus possible to combine different receptors on one substrate.

Available formats and accessories

HC hydrogel coatings are offered on polished ultra planar glass slides with low autofluorescence (packs à 5 and 50 units); other substrates can be coated upon request. An optional coupling kit contains all necessary buffers and materials for fast and easy immobilization of ligands. For cell culture applications, disposable rectangular plates with lids are available. These standard microplate sized dishes accommodate four slides each and allow separation or growth of cells directly on the HC glass slides as well as fixation, staining and examination inside the plate.

Ordering information

The slides listed below represent a selection of the current program. Please contact our customer support for information on the full product range.

Part #	Description	Units
SL HCX-5 SL HCX-50	HC Hydrogel, activated carboxyl	5 50
SL HHC-5 SL HHC-50	HC Hydrogel, hydrazide derivatized	5 50
SL THC-5 SL THC-50	HC Hydrogel, disulfide (thiol) derivatized	5 50
SL SHC-5 SL SHC-50	Streptavidin immobilized in HC Hydrogel	5 50
SL PAHC-5 SL PAHC-50	Protein A immobilized in HC Hydrogel	5 50
SL BHC-5 SL BHC-50	Biotin immobilized in HC Hydrogel	5 50
BI HCX-5	Immobilization Kit HCX Contains buffers and materials for immobilization on 5 HCX slides	100 ml 10 x spotting buffer 100 ml quenching buffer 5 microdialysis units
BI HCX-50	Immobilization Kit HCX Contains buffers and materials for immobilization on 50 HCX slides	1000 ml 10 x spotting buffer 1000 ml quenching buffer 50 microdialysis units

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