Cell Membrane-Mimetic Culture Surfaces for Stem Cell Expansion

Researchers are emulating the natural environment of blood stem cells to influence their growth and differentiation.

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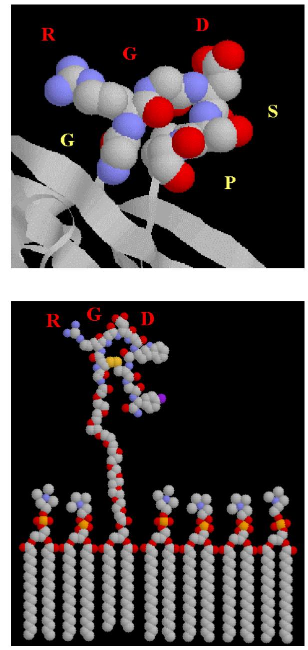
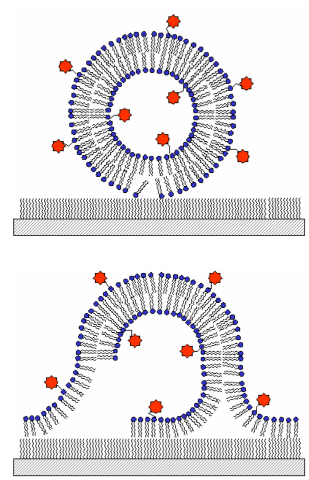
Objective: Hematopoietic (blood) stem cells (HSCs) have shown great promise for applications ranging from gene therapy to cord blood HSC transplants. However, progress in this area has been limited by HSC availability and by the tendency of cultured HSCs to differentiate into cells with restricted potential. This contrasts with sustained HSC expansion *in vivo*, and has led to the hypothesis that a stem cell "niche" supports HSC self-renewal in the bone marrow by providing soluble factors and direct contact to accessory (or stromal) cells and the extracellular matrix that they secrete. The Miller group is attempting to mimic these direct-contact interactions in a defined manner by designing and synthesizing peptide-based ligands for adhesion molecules and receptors on HSCs known to influence their growth and differentiation. To deliver these ligands to the HSCs, they are conjugated to lipid carriers and incorporated into supported lipid-based cell culture surfaces analogous to stromal cell membranes. Using combinations of carrier lipids, the properties of these interactive surfaces can be adjusted to take advantage of receptor mobility and clustering that are associated with ligand engagement *in vivo*.

Approach: Receptor-specific ligands are prepared via solid-phase peptide synthesis. Post synthesis modification (*e.g.,* cyclization) is done on the solid support, followed by attachment of polyethylene glycol (PEG)-tethered lipids. More complex, dimerized peptides are created on the solid phase, followed by tethered-lipid attachment in solution. Peptide-lipids are purified by reverse-phase HPLC and characterized by mass spectrometry. These molecules are incorporated into lipid vesicles (size ~ 100 nm), which are used to create a lipid-monolayer on hydrophobic glass surfaces in a novel cell culture cassette. Specific adhesion to the surface is measured using a normal force assay, while cell differentiation is monitored via flow cytometry.

Results: The Miller group has successfully synthesized linear arginine-glycine-aspartic acid (RGD)- and PHSRN-lipid molecules, the peptoid moieties of which mimic one of the fibronectin (Fn) cell binding domains, and explored their ability to bind to KG-1a cells – a hematopoietic cell line that shares many adhesion molecules and surface markers with HSCs. The PHSRN sequence alone has shown no intrinsic adhesive activity, but it can synergize with RGD in Fn to enhance adhesion to $\alpha 5\beta 1$ integrins. This synergistic effect was reproduced in the lipid-based system, even at very low levels of RGD-lipid (1%) and PHSRN-lipid (0.2%) molecules. The stability of the membrane-mimetic surface and the mobility of incorporated lipid-peptides after several days of cell culture is shown by fluorescence recovery after photobleaching of surfaces incorporating a fluorescent NBD-lipid. The Miller group has also prepared a high-affinity $\alpha 5\beta 1$-specific, cyclic RGD-lipid, which produces levels of adhesion similar to the linear RGD-lipids but at concentrations nearly an order of magnitude lower. Other Fn cell adhesion moieties currently being explored include the $\alpha 5\beta 1$ integrin ligand LDV and several heparin-binding ligands that have been shown to interact with HSCs. The researchers are also attaching a lipid linker to a growth factor peptide-mimic to enhance its influence on HSCs.



RGD as it occurs in fibronectin (top) and a cyclic RGD-lipid targeted to the $\alpha 5\beta 1$ integrin in a lipid monolayer (bottom).



The deposition from vesicles of a lipid monolayer onto a hydrophobic glass surface.