Citations

Click into Place

Azide-alkyne click chemistry allows efficient, selective reactions under mild conditions. Until recently, however, a toxic metal catalyst has also been required. With the development of reactive cyclooctynes, click chemistry has become catalyst-free and cell-friendly. Writing in *Nature Materials*, DeForest et al. leverage this advance to develop click hydrogels for cell encapsulation. Click chemistry forms the hydrogel by joining a tetra-azide poly(ethylene glycol) (PEG) to a polypeptide functionalized at each end with a fluorinated cyclooctyne. Because crosslinking occurs in aqueous solution at 37°C, cells can be mixed right in, becoming encapsulated as the reaction progresses. If the peptide sequence of the crosslinker is designed to include a photoreactive allyl ester, this moiety can be a substrate in a subsequent UV-activated thiol-ene click chemistry reaction. Thus, photolithographic techniques can allow patterned placement of thiol-containing reporter or effector molecules within defined areas of a biomaterial platform, providing a powerful experimental system to study cell culture in three dimensions. In addition to probing the biochemical and biomechanical determinants of cell behavior, this dual click chemistry method may also find use in tissue engineering.

DeForest et al. 2009. Sequential click reactions for synthesizing and patterning three-dimensional cell microenvironments. *Nat Mater* 8(8):659-64.

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Mixed Messages

Deriving cell type-specific gene expression profiles using mRNA extracted from whole tissues or organisms does not come easy. One approach is to introduce a fluorescent marker or biochemical tag driven by a cell type-specific promoter, then physically separate the target cells for RNA extraction. While the first step can be relatively painless, the second requires manual dissection, flow cytometry, or laser capture microdissection, each of which has nontrivial drawbacks. Fortunately, Sanz et al. describe a new line of attack in a paper appearing in the *Proceedings of the National Academy of Sciences* where the targeted cells are not physically separated; rather, the ribosomes in cells of interest are selectively tagged to capture the desired transcripts. First, the authors created “RiboTag” mice, which contain a Cre-inducible HA-tagged form of the core ribosomal subunit RPL22. In the absence of Cre recombinase, wild-type RPL22 is expressed, but when RiboTag mice are crossed with a strain in which Cre is under the control of a cell type-specific promoter, target cells in the progeny will produce tagged RPL22 only. The authors first show that the HA-tagged form of RPL22 co-purifies with the polyribosomal fraction in sucrose density gradients, and can be efficiently immunoprecipitated. To find out whether tagged RPL22 can pull out cell type-specific expression profiles from a complex mixture, Sanz et al. performed three different tests,

crossing their RiboTag mice with transgenic strains expressing Cre exclusively within the dopaminergic neurons, medium spiny neurons of the striatum, or Sertoli cells in the testis. In each case, transcripts known to be expressed in the corresponding cell type were enriched to a 5–15-fold increase over input (brain or testis homogenate). Because many Cre-expressing mouse strains already exist, the RiboTag mouse can be harnessed immediately to answer a variety of research questions.

Sanz et al. Cell-type-specific isolation of ribosome-associated mRNA from complex tissues. *Proc Natl Acad Sci* [Epub ahead of print, August 4, 2009; doi: 10.1073/pnas.0907143106].

Leading Edge

Cell migration assays can take several forms. In the popular scratch method, a wound is mechanically created in a monolayer and cells are monitored in their progress across the fissure. Though the technique is easy to perform, interpretation is much more complex, given the number of factors at play, including cell damage, the possible presence of residual matrix molecules on the scratched surface, and variations in the thickness of the wound along its length. Microfluidic devices have been developed to study cell migration under milder, more controlled conditions, but to date no

device allows monitoring of cell migration across a homogeneous surface. In an article published in *Lab on a Chip*, Doran et al. present a microscale multichannel migration device that fills this void. The concept is remarkably simple: cells grow in a main chamber that is attached to 30 microchannels, but surface tension keeps medium from flowing down these passages. Using a port on the far side of the channels, the user can add culture medium, opening up new conduits for cell growth. The progress of the cells down each channel is then followed using light microscopy, and the velocity of the leading edge of cells is calculated. The authors provide evidence showing that the system generates reproducible data consistent with results obtained in other, more complex setups. Researchers interested in exploring the effects of attachment factors or soluble factors on cell behavior in a clean, controlled system might consider migrating to this convenient new device.

Doran et al. 2009. A cell migration device that maintains a defined surface with no cellular damage during wound edge generation. *Lab Chip* 9(16):2364-9,

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