

Microarrays for drug screening

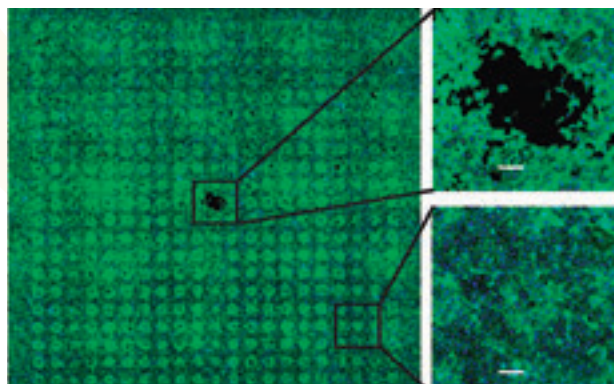


David Sabatini, Brent Stockwell, and Steve Bailey at the Whitehead Institute for Biomedical Research have developed easy-to-use microarrays that can test the effects of small molecules on mammalian cells. The new technology uses microscope slides, which greatly reduce the amount of sample needed, instead of microwell plates (*Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16,144–16,149). “The goal of this project was to develop a higher-throughput screening method that is compatible with mammalian cells,” says Stockwell.

The array is a microscope slide printed with ~200- μm -diam dots of poly-(D),(L)-lactide/glycolide. This polymer slowly and consistently releases a chemical load and partly dissolves in dimethyl sulfoxide (DMSO), the solvent used in most chemical libraries. A commercial arrayer overprints each dot with ~20 nL of a chemical dissolved in DMSO. A layer of cells is poured over the dried slides, and after a given time, the researchers use robotic immunofluorescence microscopy to observe the cells.

“You could have a slide with 5000 chemicals on it just sitting in the freezer. When you’re ready to use it, you just put it in a dish and grow cells on it,” Bailey says. The amount of compound needed per spot is only one-hundredth to one-thousandth of that required for a well-based assay. “So, if you had a microliter of some rare natural product, you would have enough to use it thousands and thousands of times,” says Bailey.

The researchers tested the array by seeding human lung cancer cells on top of dots impregnated with either the cytotoxic compound phenylarsine oxide



Human lung cancer cells were seeded onto an array containing the toxic compound PAO in the center spot. Cell nuclei are stained blue and actin filaments green. Scale bar = 100 μm . (Adapted with permission. Copyright 2004 National Academy of Sciences, U.S.A.)

(PAO) or only DMSO. Cells grew on the control spots but not on the dots releasing PAO. The response was dose- and distance-dependent, and the array slowly released PAO for at least 10 days. Also, the assay worked with several types of cancer cells and toxic compounds in addition to PAO.

To examine a nonlethal phenotype, the researchers printed rapamycin onto polymer dots and incubated the slides with lung cancer cells. Rapamycin inhibits the signaling pathway that triggers the phosphorylation of a ribosomal protein. Staining with a fluorescent antibody showed that the cells on the rapamycin dots had lost their phosphorylation signal. “There’s no reason to think that you couldn’t [use these arrays to] study any signaling event with an antibody-based stain,” Stockwell says.

The researchers then used the array method to screen compounds with unknown cellular effects. They transfected small interfering RNAs (siRNAs) into lung cancer and HeLa cells to inactivate certain oncogenes and tumor suppressors.

When the researchers exposed these cells to 70 synthetic compounds, 4 of the 980 combinations of compound, siRNA, and cell type proved lethal. Well-plate experiments confirmed this effect for one of those combinations.

Bailey says that the next goal is to decrease the diffusion of the chemicals so that the spots do not intermingle. But John Weinstein at the National Cancer Institute says, “The issues of controlling diffusion and of quantitation—other than in a relative sense—may be limitations of the technology fundamentally.” Weinstein also wonders whether various compounds

diffuse out of the polymer at different rates, which would impede comparisons of their effects on a given cell type.

Kit Lam at the University of California, Davis, says that his group and another group developed a cell-based microarray about 10 years ago by immobilizing one-bead one-compound libraries in soft agar together with living cells (*Mol. Diversity* **1996**, *2*, 57–63). “The main difference between Bailey’s library [and ours] is that his is spatially addressable, but ours is not and therefore requires decoding of the positive beads,” Lam says. “However, with our method, we can easily generate and screen an array of hundreds of thousands to millions of different compound-beads in a short time.”

The developers of the slide-based array hope to see their technology commercialized. “A biologist would be very excited about the possibility of buying a microscope slide that carries all 1500 drugs approved in the United States or 5000 biologically active compounds,” Stockwell says. ■

—Linda Sage