BIOMATERIALS

# Making a muscle

**A new cultivation strategy allows scientists to grow vascularized skeletal muscle capable of successful integration with existing host tissues after transplantation.**

Building muscle from scratch is no mean feat; skeletal muscle is highly organized and requires considerable oxygenation—and thus vascularization—to survive and function. Earlier attempts to cultivate muscle tissue have focused largely on the transplantation of myoblasts, and although some of these efforts have proven partially successful, proper graft vascularization still remains an issue.

When postdoc Shulamit Levenberg first arrived in Robert Langer's lab at the Massachusetts Institute of Technology, she hoped to take advantage of his team's experience with biomaterials and cell culture to develop more effective tissue engineering approaches. "The whole idea was to find… a new way to engineer a tissue with a whole network of blood vessels inside *in vitro*," says Levenberg, now at the Technion Institute in Israel. In a report in *Nature Biotechnology*, Levenberg, Langer and their colleagues describe an important first step—a method for the culture of vascularized, transplantable muscle tissue that could serve as a model for future tissue engineering efforts.

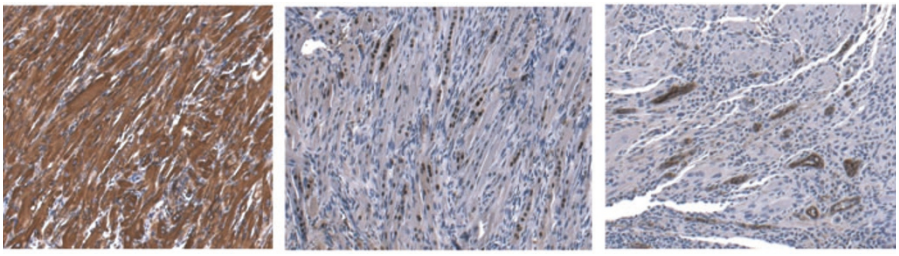

**Figure 1 |** Engineered muscle grafts exhibit organization and vascularization after transplantation into abdominal muscle of nude mice. (a–c) These implants were immunostained for the muscle-specific proteins desmin (a) and myogenin (b), and for an endothelium-specific protein, von Willebrand factor (c).

Their strategy relies on two important innovations. The first involves the use of a three-dimensional polymeric scaffold for mouse myoblast culture. The material is porous, providing space for cells to grow, but also biodegradable—the cells gain more space for growth as the material gradually breaks down; after transplantation, the scaffold will eventually dissolve completely. The second key feature is the coculture of two other cell types alongside the myoblasts: human endothelial cells, which form the foundation for developing vasculature, and mouse embryonic fibroblasts, which support and stimulate vascular formation through growth factor secretion.

This system, which Langer dubs 'triculture', proved highly effective *in vitro*. Within two weeks, the myoblasts had differentiated and started to assemble into

DRUG DISCOVERY

## HASTENED TO DEATH

**Chemical-genetic selection in yeast identifies an inhibitor that targets a spindle-checkpoint kinase resulting in massive chromosome loss and cell death.**

Despite the devastation tumors cause, individual tumor cells are actually quite fragile. They are often unable to correctly attach their chromosomes to the spindle, a structure of microtubules that ensures that the chromosomes are evenly distributed to the cell poles before division. Scientists have tried to take advantage of this Achilles' heel of cancer cells and sought to destabilize their spindle even more. The goal is to inhibit the spindle checkpoints and to increase the speed at which cells with unequally distributed chromosomes divide—a process that eventually kills the cell. Proteins that control checkpoints are therefore interesting targets for drugs, but identifying them remains a challenge. Andrew Murray and his colleagues at Harvard University have developed a system for the identification of small molecules that inhibit spindle checkpoints in yeast and then go on to find their targets, work described in a recent article in *Current Biology*.

The trick was to create a yeast strain that allowed them to perform chemical-genetic selection. Murray explains their objective: "What we are requiring is that the compounds inhibiting the checkpoint confer on cells the ability to proliferate under conditions where they otherwise would not." They combined a linear minichromosome, which activates the spindle checkpoints, with a dominant negative checkpoint inhibitor under the control of a tetracycline promoter. The addition of doxycycline turns off the inhibitor, triggering growth arrest as a result of checkpoint control activation by the minichromosome. The researchers then tested a library of small molecules in the presence of doxycycline and found five compounds that allowed the cells to grow and were thus likely inhibitors of the spindle checkpoint.

One of the compounds chosen for further characterization was named cincreasin, for chromosome instability increasing compound; screens of mutant yeast strains showed that cincreasin leads to rapid chromosome loss and cell death after several cell divisions. Only genes involved in chromosome

the elongated, multinucleate myotubes that are the foundation of muscle tissue; after a month of tri-culture, vessel-like endothelial structures were clearly visible, surrounded by fibroblast-derived smooth muscle cells. To assess the extent to which these differentiated constructs could integrate into host tissue, Langer's group implanted their cultures into different muscular contexts in immunodeficient rodents. In all cases, the grafts continued to grow and differentiate, effectively incorporating into the host (**Fig. 1**). "They looked very connected with the host tissue," says Levenberg, "and there were some areas where you could see almost no border between the implanted muscle and the host muscle. Our muscle is still not really a fully organized muscle… but we saw long fibers that were aligned in the mice."

Notably, the grafts were well vascularized, and in many cases the newly-formed vascular tissue appeared to be functionally incorporated into the host; when recipient mice were injected with labeled lectin via the tail vein, roughly 40% of the implant-derived blood vessels in the graft were found to be perfused with the lectin. The group also noted that tri-culture–derived implants had a marked and significant increase in implant survival and functional vascularization relative to myoblast-only implants.

The authors were impressed with the level of integration demonstrated by these implants, and hope to optimize their system to improve graft organization and functionality. Most importantly, however, they express hope that this system will offer a foundation for the development of similar engineered implants for other complex, highly vascularized tissues, such as the liver and the lung.

**Michael Eisenstein**

**RESEARCH PAPERS**
Levenberg, S. *et al.* Engineering vascularized skeletal muscle tissue. *Nat. Biotechnol.*; published online 19 June, 2005.



segregation turned out to be sensitive to cincreasin, and among these, the scientists identified the gene encoding Mps1, a kinase known to be involved in checkpoint regulation, as a *bona fide* target of cincreasin.

In Murray's opinion, it is the identification of the target Mps1, rather than the compound cincreasin, that is the most exciting outcome of their selection and screens. He says: "If you were someone interested in any cancer therapy,… you would be completely uninterested in the structure of cincreasin. What you would care about is the fact that Mps1 has been identified as a target whose inhibition compromises the spindle checkpoint." Other kinases involved in this checkpoint have been targeted in the past and are now at the stage of clinical trials. Murray reckons that if a pharmaceutical company is interested in pursuing their discovery, they could use their selection scheme and find more effective small molecule inhibitors of Mps1, which in conjunction with other inhibitors of the spindle checkpoint may prove to be a very powerful way of killing certain tumors.

**Nicole Rusk**

**RESEARCH PAPERS**
Dorer, R.K. *et al.* A small-molecule inhibitor of Mps1 blocks the spindle-checkpoint response to a lack of tension on mitotic chromosomes. *Curr. Biol.* **15**, 1070–1076 (2005).

## NEWS IN BRIEF

GENOMICS

### Genome-scale identification of nucleosome positions in *Saccharomyces cerevisiae*

Yuan *et al.* describe a microarray-based strategy for the high-resolution identification of nucleosomal and linker stretches of DNA, and apply their strategy to the analysis of half a megabase of *S. cerevisiae* genomic sequence. They find that the majority of yeast nucleosomes are well-ordered, and that nucleosomes tend to be absent from active promoter regions.

Yuan, G.-C. *et al. Science*; published online 16 June, 2005.

CELL BIOLOGY

### Use of human tissue to assess the oncogenic activity of melanoma-associated mutations

Interspecies differences can pose an obstacle to the reproduction of human disease pathology in mice. Chudnovsky *et al.* demonstrate a new strategy for modeling melanoma, transplanting grafts of human skin cells that have been genetically modified to express oncogenes with melanoma-associated mutations into immunodeficient mice.

Chudnovsky, Y. *et al. Nat. Genet.* **37**, 745–749 (2005).

RNA INTERFERENCE

### Dissecting RNA interference pathway with small molecules

Several details of the RNA interference (RNAi) process remain poorly understood. Chiu *et al.* performed a chemical library screen in an effort to identify compounds specifically capable of blocking ATP-dependent steps of RNAi. They identify and characterize two such molecules, compounds that could prove useful for future mechanistic studies.

Chiu, Y.-L. *et al. Chem. Biol.* **12**, 643–648 (2005).

PROTEIN BIOCHEMISTRY

### DNA shuffling as a tool for protein crystallization

For many proteins, crystallization is not a straightforward process—especially in cases where no structural information is available as a starting point. Keenan *et al.* use DNA shuffling as the basis of a high-throughput screen for the generation and identification of protein variants with changes that permit crystallization while still conserving protein solubility and function.

Keenan, R.J. *et al. Proc. Natl. Acad. Sci. USA* **102**, 8887–8892 (2005).

CELL BIOLOGY

### Quantification of the cellular uptake of cell-penetrating peptides by MALDI-TOF mass spectrometry

The uptake of cell-penetrating peptides (CPPs) is typically quantified indirectly, via reporters. Burlina *et al.* describe a new, direct approach, wherein cells are treated with an affinity-tagged CPP; the cytoplasmic CPP can then be isolated with magnetic beads, and absolute uptake can be quantified via mass spectrometric analysis versus an isotopically labeled internal standard.

Burlina, F. *et al. Angew. Chem. Int. Ed. Engl.* **44**, 4244–4247 (2005).