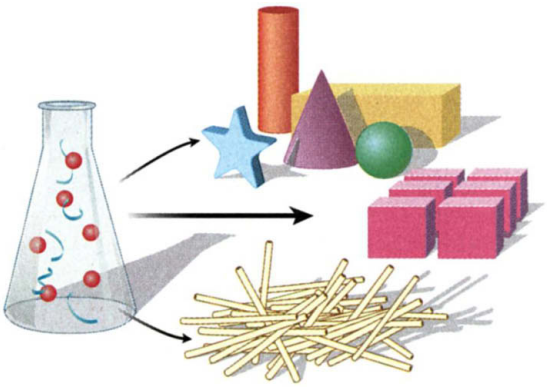
tions as the reductant, in the presence of a polymer directing agent [poly(vinyl pyrrolidone), PVP] (*1*). Highly crystalline silver nanocubes with well-defined {100} faces are made in high yield if all parameters are optimal. If the reaction temperature is too high or too low, irregularly shaped nanoparticles are produced. If the silver nitrate initial concentration is too low, nanowires are the major product. If the polymer concentration is too high, crystallographically defective nanoparticles are produced. Without polymer, crystallographically defective nanoparticles exposing the more stable {111} surface are produced.



Multiple products. Precursors (**left**) can react to form a variety of nanoparticle shapes (**right**), depending on the reaction conditions.

This sensitivity to conditions implies that the silver nanocube reaction is under kinetic rather than thermodynamic control. Consequently, the timing of the reaction controls the product's dimensions. Shorter reaction times at optimal "cube" conditions reduce the nanocube edge lengths to ~70 nm; longer reaction times lead to nanocubes with edges of up to ~175 nm, in a well-controlled manner. The production of smaller nanocubes (down to edges of 50 nm) is not as well controlled under these conditions.

When the silver nanocubes are treated with a gold salt, an oxidation-reduction reaction ensues. The adsorbed gold salt is reduced to gold metal, with concomitant oxidation of the silver to silver ions. In this reaction, the silver nanocubes serve as a sacrificial hard template to make hollow crystalline gold nanoboxes, whose dimensions are controlled by the size of the silver template. The square facets of the gold nanoboxes mirror the crystallographic facets of the original silver nanocubes.

A major challenge for nanotechnology is the rational assembly of individual nanoscale elements into working devices. To develop a reasonable linkage strategy, detailed knowledge of the positions and identities of the surface atoms of the nanoscale object is required. Sun and Xia (*1*) provide a clear picture of which crystal faces are available for future assembly for their nanocubes and nanoboxes. However, it is still a major challenge to predict what capping agent or combination of capping agents in solution will generate a specific desired shape and size of nanocrystal. Also, the microscopic images of Sun and Xia cannot show where the capping agent is physically located on their cubes.

Future experiments using these cubes for optical sensing, catalysis, or nanoelectronics will require a more thorough understanding of what ions or molecules may be adsorbed to the surface. Here is an opportunity for colleagues from many disciplines—colloid chemists, physicists, geologists, biomineralization experts, surface scientists, and materials scientists—all of which share common interests and complementary views of inorganic crystal growth modified by organic molecules.

Potential device applications of inorganic nanomaterials need not be limited to single metals. Metal alloys with size-dependent magnetic properties have been fabricated into supported nanoparticles for information storage that could lead to recording densities an order of magnitude larger than are currently available (*16*). Still, the basics of making materials on the nanoscale is a work in progress. Sun and Xia are helping to pioneer methods to do so.

References

1. Y. Sun, Y. Xia, *Science* **298**, 2176 (2002).
2. M. A. El-Sayed, *Acc. Chem. Res.* **34**, 257 (2001).
3. A. P. Alivisatos, *Science* **271**, 933 (1996).
4. M. Valden, X. Lai, D. W. Goodman, *Science* **281**, 1647 (1998).
5. K. Dick, T. Dhanasekaran, Z. Zhang, D. Meisel, *J. Am. Chem. Soc.* **124**, 2312 (2002).
6. R. Jin *et al.*, *Science* **294**, 1901 (2001).
7. C. J. Murphy, N. R. Jana, *Adv. Mater.* **14**, 80 (2002).
8. K. Kneipp *et al.*, *Chem. Rev.* **99**, 2957 (1999).
9. L. He *et al.*, *J. Am. Chem. Soc.* **122**, 9071 (2000).
10. R. Elghanian *et al.*, *Science* **277**, 1078 (1997).
11. V. F. Puntes, K. M. Krishnan, A. P. Alivisatos, *Science* **291**, 2115 (2001).
12. T. S. Ahmadi *et al.*, *Science* **272**, 1924 (1996).
13. S. R. Nicewarner-Peña *et al.*, *Science* **294**, 137 (2001).
14. A. Filankembo, M. P. Pileni, *J. Phys. Chem. B* **104**, 5865 (2000).
15. C. J. Johnson *et al.*, *J. Mater. Chem.* **12**, 1765 (2002).
16. S. Sun *et al.*, *Science* **287**, 1989 (2000).

PERSPECTIVES: DEVELOPMENT

Fishing Out a New Heart

Ian C. Scott and Didier Y. R. Stainier

A Promethean goal of modern biomedicine is to repair damaged organs. Stem cells, which have the potential to form a wide variety of different cell types, are widely regarded as being essential to this endeavor. However, the results of recent studies call into question the plasticity of adult stem cells, and there are ethical quandaries associated with deriving pluripotent embryonic stem cells from human embryos. An alternative approach is to stimulate the damaged organ to regenerate or heal itself. Poss *et al.* (*1*) adopt this strategy on page 2188 of this issue with their demonstration that the heart of the adult zebrafish *Danio rerio* is capable of regeneration. As the zebrafish has become one of the preferred genetic models of vertebrate development, this finding should open up exciting new avenues for studying cardiac regeneration.

Mammals, including humans, exhibit only a few examples of regeneration, most notably the ability of liver hepatocytes to regenerate damaged liver tissue. In contrast, broader regenerative capacities have been described in animals such as the salamander, newt, hydra, and flatworm—indeed, flatworms are able to generate an entirely new animal from a small piece of tissue. More recently, regeneration of fins, spinal cord, and retina has been documented in the zebrafish (*2*, *3*). Poss and colleagues set out to establish whether the adult zebrafish heart also has the capacity for regeneration. They performed a simple surgery in which the apex of the ventricle, representing roughly 20% of its volume, was removed. Mouse hearts subjected to similar damage induced by freezing do not regenerate, but instead form scar tissue (*4*). In contrast, the authors found that although initial fibrin deposits did form at the wound site in zebrafish hearts, further scarring and collagen deposition, characteristic of damaged mammalian hearts, did not occur. Instead, cardiomyocytes, the specialized muscle cells of the heart, infiltrated the injured area and sealed off the wound. Remarkably, 60 days after surgery, the zebrafish hearts appeared roughly normal both histologically and based on ex-

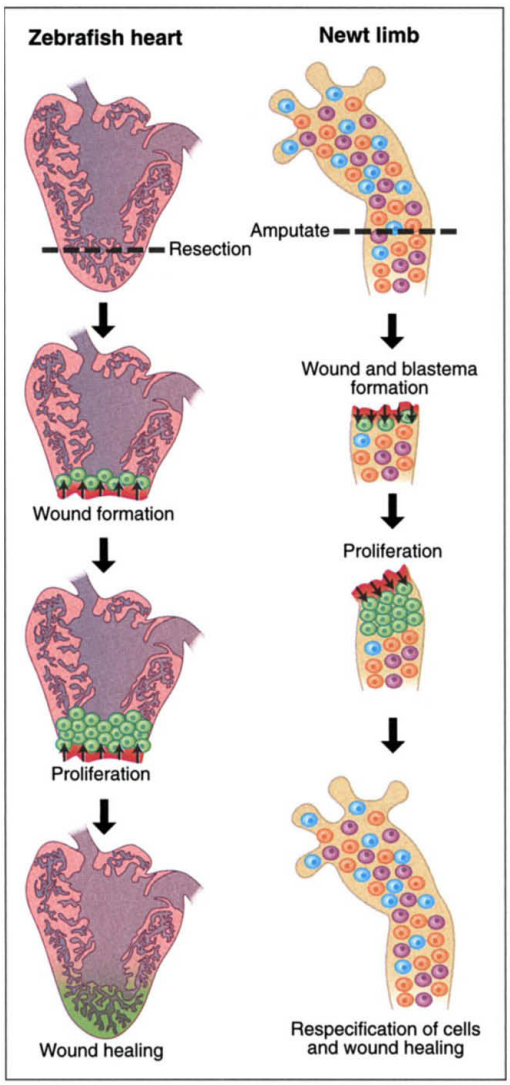
The authors are in the Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143, USA. E-mail: ianjr88@itsa.ucsf.edu, didier_stainier@biochem.ucsf.edu

CREDIT: PRESTON MORRIGHAN/SCIENCE

amination of heartbeat. In effect, the excised area had been replaced by functional cardiomyocytes.

Poss *et al.* next investigated the mechanism underlying this regeneration. The normal response of mammalian hearts to injury or hypoxia is hypertrophy—the growth of cardiomyocytes without cell division. Indeed, although there is evidence that a stem cell–like population may exist in the adult mammalian heart, cell division in this organ is rare (*5*). Bromodeoxyuridine (BrdU), a chemical that is only incorporated into cells undergoing de novo DNA synthesis, was injected into fish at various time points following surgery. Cardiomyocytes from uninjured hearts rarely incorporated BrdU, even after prolonged exposure. However, there was a marked increase in BrdU incorporation following injury, which peaked at 14 days after surgery and was primarily localized to the outermost layer of the myocardium adjacent to the wound area. To identify the fate of these proliferating cells, the authors administered short pulses of BrdU and determined its localization several weeks later. Poss *et al.* found that the labeled cells had assumed a deeper position in the inner layer of the myocardium. These data imply that regeneration resulted from proliferation of cardiomyocytes adjacent to the area of injury. The cardiomyocytes were then displaced inwards to add to the newly formed compact myocardium. In support of this model, the authors demonstrate that regeneration failed to occur in fish harboring a temperature-sensitive mutation in *mps1*, a gene encoding a mitotic checkpoint kinase required for cell proliferation and regeneration of the zebrafish fin (*6*).

Regeneration of the zebrafish heart appears at face value to differ from that described in other tissues, including the newt limb and zebrafish fin (see the figure). In these cases, differentiated cells (including muscle, cartilage, and skin), adjacent to the wound site, first dedifferentiate to form a blastema or mass of pluripotent cells, which then gives rise to a fully formed and patterned limb or fin. Does this imply that the mechanisms underlying heart regeneration are different from those of other organs? The process described by Poss *et al.* does share features with limb and fin regeneration. In all cases, the regenerative response is localized to a region immediately adjacent to the site of injury. Furthermore, there is proliferation of terminally differentiated cells (functional cardiomyocytes, skeletal myotubes, and so forth), which normally do not proliferate. In the case of heart and fin regeneration, this cellular proliferation is dependent on genes such as *mps1*. The zebrafish myocardium is a relatively simple structure, composed of one major cell type, so the initial step of dedifferentiation observed during limb regeneration may not be required for cardiac regeneration. However, in the Poss *et al.* study, the expression of genes associated with myocardial differentiation was not examined to determine if these cells underwent any form of "dedifferentiation." Interestingly, heart regeneration in the newt similarly proceeds by proliferation of cardiomyocytes, apparently in the absence of noticeable dedifferentiation (*7*).



Regenerating hearts and limbs. (Left) Following surgical removal of a portion of the zebrafish ventricle, cardiomyocytes (green cells) adjacent to the wound site (bright red) undergo proliferation, presumably due to signals (arrows) emanating from the wound. **(Right)** In contrast to the heart, the newt limb is composed of various differentiated cell types (indicated by cells of different colors). Following injury, cells adjacent to the wound epithelium dedifferentiate to form a blastema (green). These cells proliferate and subsequently differentiate to re-form a properly patterned limb.

The intriguing question, of course, is whether this model can be exploited to imbue the human heart with a capacity for regeneration. A number of studies using cultured murine C2C12 cell myotubes suggest that these terminally differentiated cells can be coaxed into regenerative-like pathways (*8*–*10*). Thus, it appears that mammalian cells maintain the pathways required to respond to the proper "proregeneration" signals. The feasibility of mammalian regeneration is best illustrated by studies on the MRL mouse, which has a striking capacity for regenerative wound healing (*4*). Much like zebrafish, these mice can also heal damage to the heart induced by freezing. This trait has been found to map to at least six genetic loci, and it will be fascinating to learn the identity of the relevant genes.

Poss and colleagues have presented an exciting new model for the study of regeneration. Of course, determining the genes that underlie this process is the next goal. A strength of the zebrafish as a vertebrate model is that genetic screens to identify genes involved in, for example, fin regeneration can be accomplished with relative ease. Although screens for genes regulating fin regeneration have been successful (*6*), it would be laborious to carry out a heart regeneration genetic screen because a large number of surgeries would have to be performed. Perhaps an alternative method of inducing limited and reproducible heart damage (for example, by chemical ablation or transgenic expression of a toxin) can be devised. Complementary approaches, including finding genes differentially expressed in regenerating versus normal hearts, should also prove informative. Ultimately, it will be of greatest interest to determine whether similar genes underlie the regenerative capacity of different tissues, or whether there are genes that are specific to regeneration of the heart.

References

1. K. D. Poss *et al.*, *Science* **298**, 2188 (2002).
2. S. L. Johnson, J. A. Weston, *Genetics* **141**, 1583 (1995).
3. T. Becker *et al.*, *J. Comp. Neurol.* **377**, 577 (1997).
4. J. M. Leferovich *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 9830 (2001).
5. M. H. Soonpaa, L. J. Field, *Am. J. Physiol* **272**, H220 (1997).
6. K. D. Poss *et al.*, *Development* **129**, 5141 (2002).
7. J. O. Oberpriller *et al.*, *Ann. N.Y. Acad. Sci.* **752**, 30 (1995).
8. S. J. Odelberg *et al.*, *Cell* **103**, 1099 (2000).
9. C. J. McGann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 13699 (2001).
10. G. R. Rosania *et al.*, *Nature Biotechnol.* **18**, 304 (2000).

CREDIT: KATHARINE SUTLIFF/*SCIENCE*