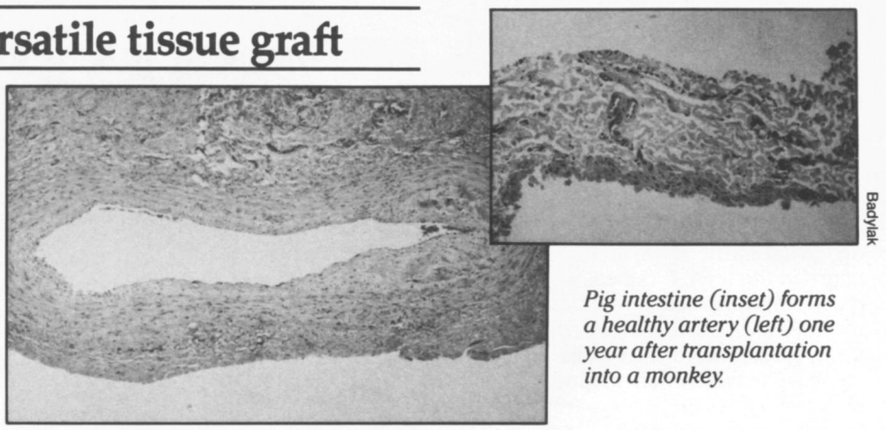
Pig intestine yields versatile tissue graft



Pig intestine (inset) forms a healthy artery (left) one year after transplantation into a monkey.

First, there were chitlins. Then, butchers started packaging sausage in the strong but thin casing of pig intestine. Now, tissue engineers are fashioning the proverbial silk purse out of a sow's gut by using a diaphanous inner layer of pig intestine to make tissue grafts for replacing worn-out blood vessels, ligaments and bladders. Their aim: an off-the-shelf graft that can serve as spare parts for any patient, regardless of tissue type.

Veterinarian-physician Stephen F. Badylak and his colleagues at Purdue University in West Lafayette, Ind., have isolated a tissue film the thickness of two human hairs that they can transplant successfully into many other species, including dogs and monkeys. Badylak told a symposium on tissue engineering last week that his team has transplanted the material into more than 600 animals of various species without any signs of immune rejection. The session was part of the Keystone (Colo.) Symposia on Molecular and Cellular Biology.

The researchers call the material SIS, for small intestinal mucosa. Much like the film of jelly between two layers of a jellyroll cake, SIS lies between the outer, muscular intestinal surface and the layer of finger-like villi lining the intestine's inner wall. It consists mainly of collagen, the connective tissue that holds most organs together.

Badylak and his colleagues have a cheap and plentiful supply of SIS from their local slaughterhouse. "It's very similar to what they use for sausage casings," he says. After meticulously scraping off the inner and outer intestinal layers, the researchers place the resulting SIS in a bottle of antibiotic-laced saline solution and store it in a refrigerator, where it keeps for more than a month.

Because of SIS' ability to resist blood clots, the Purdue group first used it in dogs to replace the aorta, the largest artery in most animals. Remarkably, they found that the opaque-white material dissolved over two months and was replaced by the dogs' own blood vessel tissue, a phenomenon that tissue engineers call remodeling. Several of the dogs have lived four or five years since their transplants and have aortas indistinguishable from those of nongrafted dogs, Badylak says.

Following the success of the aorta transplants, Badylak's group tested SIS in dogs as a replacement for the vena cava, the largest vein in most animals' bodies. Unlike arteries, which carry high-pressure blood away from the heart, veins carry blood under lower pressure toward the heart and do not require a layer of smooth muscle to give them extra strength. The SIS vena cava graft adapted to form muscle-less vein tissue, Badylak's group discovered.

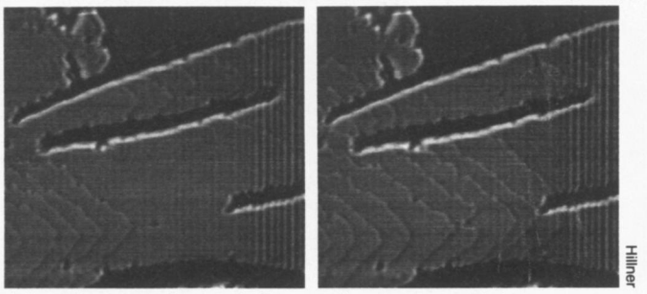
Encouraged by SIS' potential, Badylak's team then used the material to replace canine knee ligaments and Achilles tendons. Within weeks, the SIS became fully developed ligament and tendon tissue, they found. Moreover, a tunnel drilled in the dogs' leg bones to accommodate the knee ligaments closed around and fused with the new tissue.

The group hopes to test SIS grafts in humans "within a couple of years," Badylak says. He predicts that orthopedic applications will come first, because of the lack of an adequate number of donor ligaments and tendons from humans. He and his co-workers recently began testing injections of minced SIS as a means to shore up the muscles of leaky bladders. They also plan to see if SIS will promote wound healing when used as a skin graft. However, Badylak says, "I don't want to oversell this material ... we still don't know how it works."

Eugene Bell, a biologist at the Massachusetts Institute of Technology in Cambridge, calls SIS "extremely promising" as a universal tissue graft. "We are just beginning to probe the regenerative capacities of the human organism," he asserts. Synthetic materials have been "singularly ineffective" as tissue replacements, he says, because they do not serve as templates for the host to regenerate his or her own tissue and are sometimes subject to immune-system attack.

— C. Ezzell

Calcite on the edge of growth, dissolution



These images, obtained 33 seconds apart, reveal the formation and spread of thin layers to produce a pattern of V-shaped steps (lower left) on a calcite surface. Each image represents an area 1 micron wide.

Whether incorporated into seashells or deposited as limestone, marble or chalk, the mineral calcite participates in a variety of biological and geologic processes. To help elucidate how calcite fulfills its varied roles, researchers have developed a new technique for observing hitherto hidden details of the way calcite crystallizes and dissolves.

Physics graduate student Paul E. Hillner of the University of California, Santa Barbara, geoscientist Andrew J. Gratz of the Lawrence Livermore (Calif.) National Laboratory and their co-workers use an atomic force microscope to observe the step-by-step addition or removal of calcium and carbonate ions at a calcite crystal surface. Although they can't detect individual atoms, they can clearly see the apparent movement of edges as ions settle into layers to produce characteristic patterns of steps.

These findings represent "extremely important first observations [by atomic force microscopy] of in situ crystal growth ... of a mineral," comments Richard J. Reeder of the State University of New York at Stony Brook, who has also studied calcite growth.

Hillner and his colleagues describe their work in the April GEOLOGY.

The researchers track changes in surface features during crystal growth by passing a concentrated solution of calcium carbonate dissolved in water across the surface of a calcite sample. By making the solution highly alkaline, they slow the deposition process sufficiently to allow time for repeatedly scanning the surface to detect any changes.

"Our growth rate is so small that it would take us years to grow a reasonably

sized crystal," Hillner notes. The researchers can control the rate of crystallization or dissolution by slightly varying the solution concentration.

The resulting images reveal surprisingly complex processes taking place as ions shuttle between solution and crystal surface. Layers grow by the formation of broad steps spiraling outward from surface outcroppings. These steps, each only 0.3 nanometer high, give the crystal surface a distinctive, terraced appearance. In contrast, dissolution produces sharply etched pits.

"Calcite grows by adding material right at a step," Gratz says. The researchers find scant evidence of ions landing on a flat surface, then diffusing to their final resting places at edges.

And there's much more to see. "It's its own little world," Hillner says. "There's so much going on, it's hard to figure out what to attack next." Hillner and Gratz are now putting together an apparatus that would allow them to study calcite growth and dissolution at elevated temperatures.

"Carbonate minerals exhibit a rich surface chemistry that creates hundreds of crystalline forms in nature," they conclude. "It may now be possible to relate each form not only to the natural environment in which it grows, but also to its detailed growth mechanism."

– I. Peterson

Nuclear site flooding hazard dismissed

Federal plans to store high-level nuclear waste inside a Nevada mountain gained a boost this week when a panel of eminent geoscientists dismissed a controversial theory that the proposed site faces a risk of flooding from rising groundwater.

The prospective site of the repository, Yucca Mountain, lies about 150 kilometers northwest of Las Vegas. The Department of Energy (DOE) is assessing whether the site is suitable for storing roughly 70,000 tons of spent fuel rods for nuclear power plants.

The groundwater issue is critical. The planned repository is designed to remain dry and must be built in rock far above the water table. Should hot fluids rise into the repository during the 10,000 years that the waste will remain harmful, they could carry radioactive material into the environment.

In the late 1980s, DOE geologist Jerry S. Szymanski reported that rising fluids presented a very real risk. Plans for the repository call for excavating the storage rooms about 200 to 400 meters above the current water table, but Szymanski concluded that groundwater had reached much higher levels in the past. He proposed that extensive mineral deposits found near the surface were formed when tectonic stresses from earthquakes or other geological events forced warm water to rise and then precipitate dissolved minerals in cracks and pores in the volcanic rock of the area.

Most scientists working on the project have dismissed this theory, but a few vocal supporters have backed Szymanski's hypothesis, prompting the DOE to ask for an outside investigation from the National Academy of Sciences.

After two years of study, a 17-member panel convened by the academy concluded that "there is no compelling evidence for the repetitive flooding of the environment by expulsion of groundwater," says chairman C. Barry Raleigh, of the University of Hawaii in Honolulu. Instead, the evidence strongly supports the idea that the near-surface mineral deposits resulted from percolating rainwater, which carried soil minerals down into rock fractures.

The panel based its conclusions primarily on field observations and isotopic investigations of elements found in the mineral deposits. Federal and university scientists had previously reported that the isotopic evidence supports the percolating rainwater theory (SN: 10/26/91, p.262).

Panel members stressed that they did not judge the much broader question of whether Yucca Mountain would make a suitable site. They pinpointed other concerns about the region that the Energy Department must now address.

Szymanski, who released his own 600-page report this week, stands by his theory. Not surprised by the new report, he accused the panel of "blowing smoke."

Federal scientists involved in the project say they hope to get beyond the Szymanski controversy and start addressing other pressing questions about Yucca Mountain. "A lot of time has gone into going places and collecting data that at least in hindsight could have been used more effectively elsewhere," says Joseph F. Whelan of the U.S. Geological Survey in Denver. *– R. Monastersky*

When refrigerator fare turns foul

Think back to the last time you cleaned out your refrigerator. If you're like many people, it's not a pretty sight.

Two new studies now implicate bacteria-contaminated food as the source of many cases of listeriosis, a rare, but potentially lethal, illness that can strike pregnant women, the elderly and people with damaged immune systems. *Listeria monocytogenes* can cause flu-like symptoms, blood poisoning, complications of pregnancy and stillbirths. In severe cases, it can lead to meningitis, an infection of the membranes surrounding the brain and spinal cord.

During the last decade, scientific teams traced several listeriosis epidemics to widespread ingestion of foods fouled with the bacterium, found in soil, animals and vegetation. While epidemiologists blamed these large outbreaks on soft cheeses, pasteurized milk (SN: 3/2/85, p.141) and the cabbage in coleslaw, the cause of sporadic listeriosis cases remained mysterious.

Anne Schuchat and Robert W. Pinner of the Atlanta-based Centers for Disease Control (CDC) and their colleagues took a closer look at these infrequent cases. In the first study, the team identified listeriosis patients in parts of California, Tennessee and Georgia, and in the entire state of Oklahoma.

With those reports, as well as population estimates from the U.S. Bureau of the Census, the team calculated the annual incidence of listeriosis in the regions studied as 7.4 cases per million persons. Nationwide, the statistics translate to about 1,850 infections and 425 deaths every year, the researchers estimate.

Next, the team focused on 165 listeriosis patients and 376 healthy controls. A statistical analysis revealed that compared with controls, patients were 2.6 times more likely to have eaten a soft cheese, such as feta or some types of Mexican cheese, and 1.6 times as likely to have bought foods from a delicatessen counter. Eating such foods accounted for 32 percent of the listeriosis cases, the team reports in the April 15 JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION.

In the second study, reported in the same journal, the team collected food from the refrigerators of 123 listeriosis patients. Foods such as lunch meat, cheese and leftovers were packed in sterile bags and shipped on ice to CDC headquarters.

Although they can't prove that any individual food caused a patient's illness, *L. monocytogenes* grew from at least one edible item taken from 79 (64 percent) of the refrigerators sampled. Furthermore, 26 of the 79 refrigerators (33 percent) had food samples that contained the same strain of *L. monocytogenes* that had infected the patient.

Most people with a healthy immune system don't have to worry about listeriosis, Schuchat says. However, she suggests that pregnant women, the elderly and people with damaged immune systems might want to avoid deli-counter foods and certain soft cheeses. In addition, she says, all Americans should adopt safe food-handling practices, such as washing raw vegetables; fully cooking beef, poultry and pork; and reheating leftovers until they are steaming hot.

– K.A. Fackelmann