

Tissue Engineering

Solid Freeform Fabrication of Scaffolds

Scaffold-guided tissue regeneration is one enabling methodology in tissue engineering. Highly porous, biodegradable scaffolds in the shape of the target tissue are first seeded with donor cells, signaling molecules, or both. Then the seeded scaffolds are implanted to induce and direct the growth of new tissue as the scaffold degrades. Not only do these scaffolds act as delivery systems for cells and signaling molecules, but also they provide cells with the structural cues required for tissue organization.

The scaffold's external shape and material composition are important, and its internal architecture is critical for proper functioning and optimal performance. The size, shape, orientation, and spatial distribution of the scaffold's internal voids affect cell seeding efficacy, cell attachment, mechanical properties, nutrient transport, and vascular ingrowth. Tissue engineers therefore need manufacturing processes that can build scaffolds precisely according to specified designs.

Computer-aided design and manufacturing (CAD/CAM) processes can be used to precisely build up scaffolds with custom exterior shapes and complex interior features. In particular, solid freeform fabrication (SFF) is one CAD/CAM technique that can quickly and automatically fabricate arbitrarily complex shapes directly from computer models.

SFF processes build shapes by incremental material deposition and fusion of cross-sectional layers.

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A 3-D computer model is first decomposed, or "sliced," into cross-sectional layer representations that are typically 0.5 to 1 mm thick. These representations are similar to cross-sectional images produced by CT scans. To build the object, successive layers are deposited, each fused to the previous one. In order to support the object while it is built up, what are called sacrificial materials may be deposited with each layer and then removed when the object is completed.

Decomposing complex shapes into simpler 2-D layers dramatically simplifies planning the construction of objects as well as building them. This feature has been particularly important in the use of SFF processes in rapid prototyping applications. In surgery, for example, a patient-specific anatomical model for use in planning and rehearsal can be quickly fabricated directly from CT scans of the patient.

Another key advantage of SFF processes is that they provide full access to the interior of objects as they are being built up. Shapes with complex interior architectures can therefore be fabricated. This capability is proving to be important in building scaffolds for tissue engineering, which is being tried by several methods.

Stereolithography forms objects directly from a vat of liquid photocurable polymer by selectively solidifying the polymer. To form the first layer, a laser beam is guided across the surface of a platform, drawing a cross-sectional pattern and forming a solid section. The platform is lowered into the vat, and the next layer is drawn and adhered to the first.

John Halloran at the University of Michigan has used this process to build hydroxyapatite scaffolds with honeycomb-like interiors for

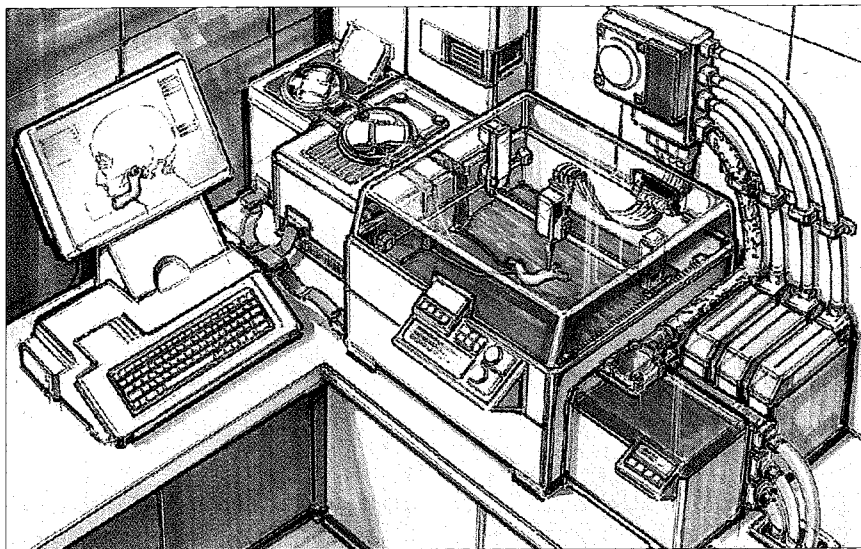
bone repair. Hydroxyapatite powder is first suspended in a liquid acrylate resin. Photopolymerization produces a solid "green" hydroxyapatite part temporarily bound together by the polymer. The "green" part is then sintered at 1500° C to burn out the polymer and fuse the remaining hydroxyapatite powder to produce the final scaffold.

Another SFF process that has been used to build complex hydroxyapatite scaffolds is selective laser sintering. Here, each layer is formed by first spreading powdered material over the current top surface of the scaffold being built up. A CO₂ laser selectively scans the layer to fuse those areas defined by the geometry of the cross section.

After each layer has been deposited, an elevator platform lowers the object by the thickness of the layer, and another layer of powder is deposited and shaped. The powder that is not fused supports the structure as it is being built up. To form hydroxyapatite scaffolds, Joel Barlow of the University of Texas at Austin uses a polymer-coated hydroxyapatite powder such that the polymer is fused to form the "green" scaffold.

Three-dimensional printing is a versatile process for forming a variety of powdered materials into complex shapes. An ink jet printing mechanism scans the powder surface of each layer to form the solid part by selectively injecting a binder into those areas defined by the geometry of the cross section. Michael Cima and Linda Griffith at MIT use three-dimensional printing to fabricate scaffolds composed of polylactide-coglycolide (materials used in sutures) and other biopolymers.

For this application, salt is used as the powder and the polymer, which is dissolved in a volatile solvent, is used as the binder. The sol-



A computer-aided bioreactor that adds cells and signaling molecules while a scaffold is being built might look like this.

vent evaporates upon deposition, leaving a solid block of polymer and salt. Submerging the completed shape in water leaches out the salt, leaving a porous polymer scaffold. Complex arrays of microchannels have been formed by this method to help support angiogenesis.

There are several lamination and extrusion processes for building up structures layer by layer. Tony Mikos at Rice University produces scaffolds by laminating individual cross-sectional layers together, including sheets of poly (L-lactic acid) and copolymers of poly (D,L-lactic-coglycolic acid), with a chloroform solvent binding process. Ahmad Safari at Rutgers University extrudes polymer-hydroxyapatite materials through a heated nozzle to draw out intricate scaffold patterns.

Beyond the capability to manufacture scaffolds with custom shapes and complex internal microstructures, it will be important in next-generation scaffold designs to have complete spatial control over the internal distribution of cells, signaling molecules, and materials. Cells migrate along concentration gradients of growth factors, for example, so the ability to build in such gradients would be beneficial. Gradients of vascular endothelial

growth factors would guide angiogenesis from surrounding vascularized tissue into the interior of the scaffold.

Heterogeneous scaffold designs, such as bone-tendon-muscle constructs, would require a variety of cell types and material compositions. Even a homogeneous scaffold should be seeded with cells throughout, which with current seeding methods is difficult to do in scaffolds more than a few millimeters thick. If the cells do not survive implantation due to the initial lack of a vascular supply, they can still contribute signaling molecules and extracellular matrix components prior to or even after cell death.

SFF has the potential to enable complete spatial control over scaffold composition by incorporating selective material deposition processes and by simultaneously and selectively adding cells and growth factors to the layers as the scaffolds are being built up. Current SFF processes, however, that involve heat or organic solvents preclude the simultaneous addition of cells and sensitive signaling molecules. And, in general, they are implemented in such a way that spatially varying material composition is difficult.

To address these limitations, Carnegie Mellon University's bone

tissue engineering group is developing a SFF approach that assembles prefabricated layers into three-dimensional structures mechanically, without requiring heat or chemicals. Prefabricated cross-sectional layers are stacked up and joined with miniature biodegradable fasteners.

Each prefabricated section can be seeded with cells or growth factors before assembly. Normal tissue growth across the layers, *in vitro* or *in vivo*, fuses the assembly together as the scaffold degrades. The assembly approach permits the manufacture of heterogeneous scaffolds that are composed of various materials with different microstructures, cell types, and growth factors used in different sections.

Another SFF approach, compatible with incorporation of cells and growth factors, uses photo-activated biological hydrogels and proteins. For example, Steve Goodman at the University of Connecticut has demonstrated the fabrication of bovine serum albumin and fibrinogen structures while simultaneously trapping surrogate drug molecules within the structure. Fabrication is done in a solution containing photo-initiators and monomers. A femtosecond laser with an adjustable focal point is focused into the solution to selectively polymerize or cross-link the material, and it can achieve submicrometer spatial resolution.

With the capability to control cellular and signaling molecule distributions in scaffolds, it will not be enough for the CAD models to contain only geometric information. CAD systems that will drive future SFF machines will also need to incorporate general biological information and specific patient history in order to achieve a successful design. The challenge for tissue engineering is to develop the underlying biological knowledge base that will be the foundation for such next-generation machines.

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