mature, in concert with the recipient's native angiogenesis and neoinnervation. For level 4 (solid) organs, the vascularity requirements are substantial, and native tissue angiogenesis is not sufficient. The engineering strategies for tissues vary according to their complexity level.

## STRATEGIES FOR TISSUE ENGINEERING

The basic principles of tissue engineering involve the use of the relevant cell populations, where the cell biology is well understood and the cells can be reproducibly retrieved and expanded, and the use of optimized biomaterials and scaffold designs. Cell seeding can be performed using various techniques, including static or flow-based systems that use bioreactors.

Most techniques for the engineering of tissues fall under one of five strategies (Fig. 92e-1):

1. Scaffolds can be used alone, without cells, and implanted, where they depend on native cell migration onto the scaffold from the adjacent tissue for regeneration. The first use of decellularized scaffolds for tissue regeneration was for urethral reconstruction. These techniques are most optimal when the size of the defect is relatively small, usually <0.5 cm from each tissue edge. Larger defects tend to heal by scarring, due to the deposition of fibroblasts, and eventual fibrosis. Scaffolds alone have also been used for other applications, including for wound coverage, soft tissue coverage after joint surgery, urogynecologic applications for sling surgery, and as materials for hernia repair.
2. A more recent strategy in tissue engineering involves the use of proteins, cytokines, genes, or small molecules that induce in situ tissue regeneration, either alone or with the use of scaffolds. For example, gene transcription factors used in the mouse pancreas led to tissue regeneration. Surgically implanted decellularized heart valve scaffolds, coated with proteins that attract vascular stem cells, led to the creation of in situ cell-seeded functional heart valves in sheep. Drugs that induce muscle regeneration are being tested clinically. Small molecules that induce tissue regeneration are currently under investigation for multiple applications, including growth of skin and hair and for musculoskeletal applications.
3. The most common strategy for the engineering of tissues uses scaffolds seeded with cells. The most direct and established type of tissue engineering uses flat scaffolds, either artificial or naturally derived, that are seeded with cells and used for the replacement or repair of flat tissue structures. The flat scaffolds can also be sized and molded at the time of surgical implantation, or they can be shaped prior to cell seeding, for example, for tubular organs such as blood vessels or nontubular hollow tissues such as bladders. Bioreactors are often used to expose the cell–scaffold construct to mechanical forces, such as, stress, strain, and pulsatile flow that aid in the normal development of the cells into tissues (Video 92e-1, engineered heart valve in a pulsatile bioreactor showing the valves opening and closing). This strategy is the most common method used for tissue regeneration to date, and tissues and organs, such as skin, blood vessels, urethras, tracheas, vaginas, and bladders, have been engineered and implanted in patients using these techniques.
4. The fourth strategy in tissue engineering is applicable for solid organs, where discarded organs are exposed to mild detergents and are decellularized, leaving behind a three-dimensional scaffold that preserves its vascular tree. The scaffold can then be reseeded with the patient's own expanded vascular and tissue-specific cells. This strategy was used initially to create solid phallic structures in rabbits that were functional and able to produce offspring. Similar strategies were also used to recellularize miniature heart, liver, and kidney structures, with limited functionality to date, but with an established proof of concept (Video 92e-2, a dye is injected through the portal artery of a decellularized liver showing an intact vascular tree). These techniques are currently under investigation and have not been used clinically to date.
5. The fifth strategy for tissue engineering involves the use of bioprinting. These technologies arose through the use of modified desktop inkjet printers over a decade ago. The inkjet cartridges were filled with a cell–hydrogel combination instead of ink. A rudimentary three-dimensional elevator was lowered each time the cartridge deposited the cells and hydrogel, thus building miniature solid structures, such as two-chambered heart organoids, one layer at a time. More sophisticated bioprinters have now been built that have additional computer-aided design (CAD) and three-dimensional printing technologies. The information to print the organ can be personalized using the patient's own imaging studies that help to define the size and shape of the particular tissue (Video 92e-3, a modified inkjet printer shows the three-dimensional construction of a two-chambered heart and how the structure beats with the cardiomyocytes in synchrony). Bioprinting is a tool that allows a scale-up option for the production of engineered tissues. Its use is still experimental and has not been applied clinically to date.

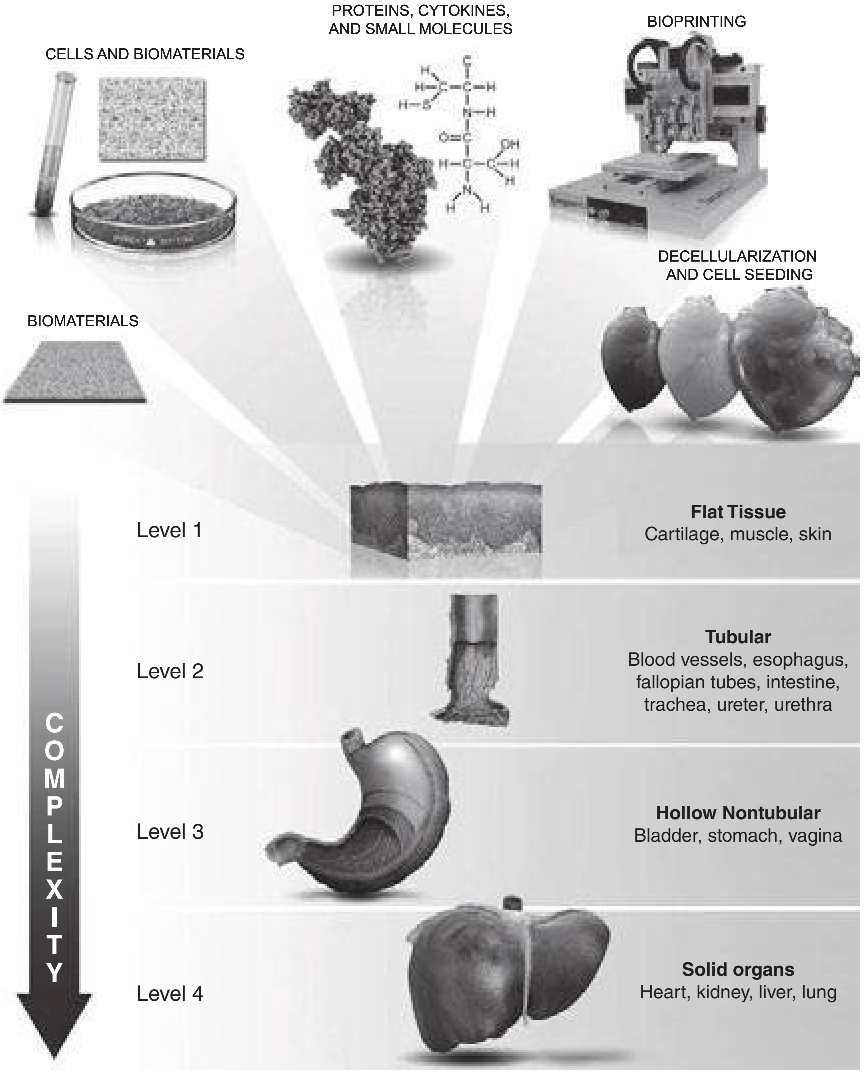

**FIGURE 92e-1** Strategies for tissue and organ engineering.

## FUTURE DIRECTIONS

A number of engineered tissues, including architecturally flat, tubular, and hollow nontubular organs,