Annals of Biomedical Engineering, Vol. 33, No. 10, October 2005 (© 2005) pp. 1333–1343
DOI: 10.1007/s10439-005-6744-1

Computer-Aided Tissue Engineering of a Human Vertebral Body

M. A. WETTERGREEN,[1] B. S. BUCKLEN,[1] W. SUN,[2] and M. A. K. LIEBSCHNER[1]

[1]Department of Bioengineering, Rice University, Houston, TX 77251, and [2]Department of Mechanical Engineering and Mechanics, Drexel University, Philadelphia, PA 19104

(Received 24 November 2004; accepted 21 June 2005)

Abstract—Tissue engineering is developing into a less speculative science involving the careful interplay of numerous design parameters and multidisciplinary professionals. Problem solving abilities and state of the art research tools are required to develop solutions for a wide variety of clinical issues. One area of particular interest is orthopedic biomechanics, a field that is responsible for the treatment of over 700,000 vertebral fractures in the United States alone last year. Engineers are currently lacking the technology and knowledge required to govern the subsistence of cells *in vivo*, let alone the knowledge to create a functional tissue replacement for a whole organ. Despite this, advances in computer-aided tissue engineering are continually growing. Using a combinatory approach to scaffold design, patient-specific implants may be constructed. Computer-aided design, optimization of geometry using voxel finite element models or other optimization routines, creation of a library of architectures with specific material properties, rapid prototyping, and determination of a defect site using imaging modalities highlight the current availability of design resources. This study proposes a novel methodology from start to finish which could, in the future, be used to design a tissue-engineered construct for the replacement of an entire vertebral body.

Keywords—Tissue engineering, Vertebral replacement, Computer-aided design, Imaging modalities, Rapid prototyping, Scaffold engineering.

INTRODUCTION

Tissue engineering aims to restore tissue function through the incorporation of biological materials such as cells, growth factors, and biopolymers. This approach is atypical of current reparative treatments, which focus mainly on drugs that encourage the body to battle disease on its own or to replace a damaged area using grafting. With more than 700,000 vertebral fractures occurring each year and limited longevity of spinal fixation devices, alternatives to metal implants capable of restoring joint function are desperately needed.[45] A given vertebral body (VB) segment experiences various forms of mechanical loading including compression, lateral bending, torsion, and flexion–extension, and thus structural design demands of a replacement will need to be mechanically robust. In this study, we propose a general methodology incorporating noninvasive imaging with computer-aided tissue engineering (CATE) to create patient-specific tissue constructs and to enumerate potential methods for the reconstruction of an entire human VB. We include a brief detailing of the concepts governing this process, current state of the art research, as well as current limitations.

The advent of noninvasive imaging allows information to be gathered about a specific location in the body without causing damage usually incurred by biopsies. The combination of computer-aided design (CAD) with imaging techniques has recently been applied to surgical guides and the design of defect-specific constructs.[10,26,67] Generation of computer models based on defect sites helps to plan complex surgeries where geometric boundaries or features may be obscured.[9] The unity of imaging modalities and computer-based design affords the potential to create a functionally viable tissue and is the basis for CATE. CATE is useful for inexpensively exploring design strategies and for providing personalized engineered solutions (from design to manufacture). Its current disadvantage is, likewise, the amount of patient and health care provider resources expended to bring these solutions to fruition. The three important concepts encompassing the use of CATE are tissue modeling, tissue informatics, and scaffold design and manufacturing. The first step of the process involves obtaining a three-dimensional (3D) model of the tissue, either by extraction from imaging modalities or with CAD generation of a tissue model.[22] Tissue informatics concerns characterizing native tissue properties using the tissue model or through the use of finite element models or assays that characterize the biochemical environment, such as gene analysis or microarrays.[68] However, tissue informatics in its broadest definition defines compiling information about each tissue from organ to subcellular level but is most specifically referred to when analyzing the type and interaction of genes and proteins within tissues. The final step in the process is the design of a scaffold based on both the required location and the treatment type.[53] Interplay between the three

Address correspondence to Michael Liebschner, Department of Bioengineering, Rice University, 6100 Main Street, Houston, TX 77005. Electronic mail: liebschner@rice.edu.

disciplines may yield a functional scaffold without ever breaking the skin before surgery.

A description of the method for design of a VB will be followed by the preliminary work of creating its pieces, or building blocks, which is limited by current technology. Although the method that we detail here is not presently translatable into a clinical application, several aspects of this treatment are already heavily in use, such as material property extraction from imaging and the rudimentary printing of structures using rapid prototyping.[3] With the improvement of computing power, solid freeform fabrication resolution, and a comprehensive knowledge of 3D constructs, we may hope to generate an engineered tissue in the near future. The advantage of this proposed methodology is the specific development of individual steps that may be used to generate any organ, regardless of anatomical site.

CONCEPTUAL MATERIALS AND METHODS

Generation of Bone Geometry

Creation of a defect-specific implant begins with obtaining a 3D model of the organ, defect site, and microstructure. One option would be to invasively remove the organ of choice, evaluate its tissue and mechanical properties, and design an implant based on these demands. Because this strategy creates a serious tissue trauma while the implant is designed and fabricated, it is an improbable option. An additional problem with invasive technologies is that they require the patient to be rendered unconscious unnecessarily, which can facilitate complications due to anesthesia allergic reactions. An alternative method is to use nondestructive imaging modalities to evaluate bone properties via assessment of bone density with dual energy X-ray absorptiometry (DEXA),[8,17,25] quantitative computed tomography (QCT),[7,21,32,36] magnetic resonance imaging,[3,63] or quantitative ultrasound.[43] These noninvasive imaging methods for obtaining tissue information may be completed in real time and are beneficial for use in surgical planning, implant design, and defect healing. The type of information readily obtained from one or more of these techniques is density and global structural parameters like intertrabecular distance. Few limitations apply to the use of these technologies aside from minimal radiation damage and resolution concerns.[23,37] Low resolution of 2D imaging techniques such as DEXA is unfit for a fabrication process that yields control over the 3D properties of the replacement material. For these processes, the use of QCT would be the most advantageous manner to noninvasively determine the appropriate properties sought for a human VB.

Estimation of Material Properties from QCT or X-Rays

Quantitative computed tomography is one of the more powerful imaging modalities, specifically for obtaining information about dense structures, such as a VB. The technique has an attainable resolution of 1.0 mm, however, with the caveat of DNA damage due to radiation exposure.[4,40,66] Density prediction using QCT is relatively straightforward and has been proven with the use of phantoms for calibration. Additionally, QCT is useful not only for the generation of tissue model properties but also for measuring the reduction in bone mineral density of cortical and trabecular bone separately due to osteoporosis or other diseases. The results are digital which ease the generation of tissue models.[30] Numerous programs exist to extract raw CT data and translate it into 3D models such as Analyze or Mimics. The methodology of the programs can be simplified to three steps: reconstruction, segmentation, and volume creation. In the first part, the raw projection data are reconstructed into 3D density data. Next, segmentation of the image is completed to generate surface geometry. During this process, information such as density, porosity, and bone mineral volume can be obtained. In the third and final step, a volume of the material is created that can be manipulated using CAD programs.

Determination of bone mechanical properties is one of the main advantages of CT imaging. The regional bone stiffness is calculated by first converting the CT absorbance into Hounsfield units. By incorporating a phantom of several compositions of a material with known mineral density in the QCT scan, a linear regression curve is established between the given CT Hounsfield units and bone density. By knowing the calculated bone density in conjunction with previously obtained *in vitro* relationships, it is then possible to calculate the modulus of elasticity and subsequently the remaining material properties.[28,61]

When recreating a whole bone where submillimeter resolution is not of great import, it may be possible to forego the use of CT scans. Multiple X-rays taken at different angles, in conjunction with an algebraic reconstruction technique (ART), can be used instead in the interest of lowered costs and accessibility. ARTs were originally used in crystallography,[15] but attempts have been made to adapt its use to medical applications where a 3D image is desirable but CT is not available.[16,44] Similar to CT reconstruction, the number of projections limits the resolution of the final picture. However, in cases such as the proposed treatment where the geometry can be estimated or evaluated through different means, ART is an effective means of reconstructing the regional density of the bone with only three to five projections.[58]

Design of Building Blocks

Computer-generated 3D models of tissue can be altered and modified through CAD processes. Several groups have previously proposed the use of simplified shapes to approximate a complex architecture based on these model groups. Imaging techniques and CAD processes have been

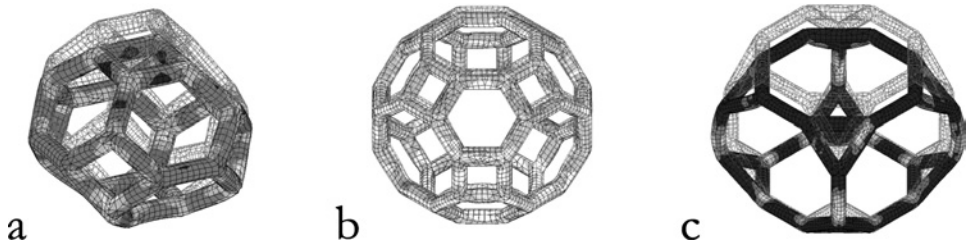

FIGURE 1. Illustrations of results of prescribed displacement on three polyhedra for the determination of structural properties. Adapted from polyhedra previously used by Ref. 65. Connecting link (torus) not shown for clarity.

used to generate geometry that is the same as the tissue replacement.[12,55] The effect of the architecture of simple polyhedra on modulus and stiffness has been explored in 3D.[18,65] An entire discipline within materials research, cellular solids, focuses solely on the determination of the mechanical performance of simplified shapes such as honeycombs for their use in composite solids.[14] On the basis of the success of previous studies, we propose the creation of a library of shapes that can be used as building blocks to generate replacement materials from the tissue level up to the apparent level. Determination of the material properties of each building block creates a library with comprehensive knowledge about each building block's porosity material characteristics and deformation patterns. The shapes in the library can then be assembled (similar to legos) to create a composite structure that is a 3D representation of the global tissue.

Generation of this library of unit shapes requires the use of numerous CAD file databases and the use of finite element analysis (FEA) for cellular solid property characterization. We propose the smallest microstructures generated confined within a 27.0 mm^3 volume, a product of the superposition of three 1.0 mm resolution QCT layers in three orthogonal directions. These microstructures also represent the smallest tissue volume that can currently be built using rapid prototyping systems. Elementary shapes such as beams, cylinders, and spheres may be arranged within these confines producing well-defined isotropy or anisotropy as needed. Quantification of the material properties of these cubes can be completed with the aid of FEA. Subjecting the building blocks to a prescribed displacement (Fig. 1) allows the calculation of force, stiffness, and finally a stress-strain diagram for each shape. This quantification is due to the structural organization of the material solely and is material independent, assuming the same material is used for every unit cell.

Combining the shapes into a composite structure requires merging them with some preventative measure to reduce edge effects. The efficacy of generating building blocks based on mechanical demands has been shown. However, lack of a common interface will distort finite element results (Fig. 2A).[52] size We propose a common interface between the building blocks in the form of a torus. Each side of the cube would contain a torus sliced through its long axis (Fig. 2B). By matching two adjacent cubes, the torus halves are joined together (Fig. 2C). We believe the torus is a good structural choice because of its rounded and subsequent reduced stress concentrations.

The inherent mechanical properties of the building blocks can be used to mimic the material properties of the VB. With the creation of a number of building blocks with separate stiffnesses determined from the tissue level model, a library can be compiled ranging from 100 MPa to 2 GPa, a range of values encompassing both bone and implant stiffnesses. Choosing a building block size of 27.0 mm^3 each, with total VB dimensions of 48.0 mm $\times$ 24.0 mm $\times$ 27.0 mm, implies that there will be around 1008 total building blocks which should be sufficient to approximate a VB.[27] At the border of the VB is the cortical bone that has a much greater stiffness and lower porosity than the trabecular bone contained in the center of the VB. Building blocks contained in these outer extremities would have a

FIGURE 2. Illustration of the need for a common interface between building blocks. (a) Example of two polyhedra lacking a common interface. Notice that at the interface between the two polyhedra, no material interaction occurs. (b) A torus used for the common interface between building blocks. (c) Example of the matching between two dissimilar polyhedra containing a common interface of a split torus.

higher material volume and a much lower porosity to approximate the cortical shell. Wrapping a nonporous shell around the border of the VB would improve the mechanical stability and also prevent any fluid leakage, as well as reduce mechanical discontinuities due to edge effects.

Optimizing the Microarchitecture Based on Mechanotransduction Principles

Generation of building blocks from CAD and subsequent evaluation of apparent stiffness with FEA will not properly account for the biological integration of the global construct. Other exogenous parameters, such as local chemical moieties and pH, will certainly affect its postsurgical success. Of the parameters readily accessible to the designer, intraunit-cell material orientation (or internal microarchitecture) is paramount. Thus, there is a need to optimize materials that do not already exemplify both ideal biological and mechanical properties. For example, scalability of the construct's size will have implications on its cell–substrate interactions due to the fixed size of a given cell, but not on its mechanical properties that depend only on relative dimensions. Recent advancement in the design and manufacture of scaffolds using continuum-based voxel models, homogenization theory, rapid prototyping, or casting techniques have provided the means for very specifically designed scaffolds. Much research has been conducted into the use of repeated microstructures or building blocks to delineate between apparent and tissue level properties in trabecular bone. To our knowledge, little work has been conducted in the area of scaffold design based on mechanotransduction principles—principles that investigate how cellular and biological responses are induced from mechanical stimuli.[1,60]

Previous studies exploring the effect of geometry on material properties have been able to demonstrate in 2D and 3D an improvement in mechanical quantities such as bending and porosity. Using simultaneous nonlinear optimization, it has been shown that various material properties may be altered according to predefined constraints like minimum pore size.[18] The rearrangement of material in a structure may result in a stronger architecture or higher effective stiffness. Indeed, this is similar to the rearrangement of bone *in vivo*. To this end, Ruimerman *et al*. have created an iteratively based program that simulates bone remodeling based on the mechanotransduction stimuli derived from osteocytes.[46] Still others have approached remodeling from a cellular automata standpoint using simple rules acting on a large problem set.[60]

Since the initial steps in bone tissue formation include adherence of bone cells onto a synthetic(biodegradable template, differentiation in the case of mesenchymal stem cells, and the production of osteoid, it becomes obvious that the surface environment will largely affect the success or failure of a scaffold. Surface characteristics are complex, however, and describe energies involved in protein–integrin binding as well as mechanical deformation applied from the surrounding locality. As a first step toward understanding the effect of surface activity, we propose a hypothesis based on mechanical characteristics alone: tissue growth will be accentuated through a uniform surface energy distribution. Justifiably, extremely large energies will encourage crack propagation and discourage cellular attachment either from migrating or from seeded cells. A nonuniform driving force (ultimately resulting in a uniform end state) as a mechanical objective has been examined on the whole bone level as well.[1,46] As follows, voxel models are created of previous scaffold microstructures, and the geometry altered based on finite element results to distribute the material in such a way to eliminate peak stresses, strains, or strain energy densities (Fig. 3). We believe that these types of optimized shapes will play a major role in the improvement of topology and internal architecture and will definitely contribute to the determination of mechanical stimuli involved in bone growth and cell–scaffold interactions.

The use of microstructural units is advantageous because it supplies biological scalability while maintaining constant mechanical properties. Though it is true that apparent mechanical properties will differ from those on the microstructural scale, any shift in the overall size of the construct, while keeping relative dimensions consistent, should not affect its overall mechanical response. However, size increases or decreases will affect the cellular response to the scaffold (since the ECM and cells do not have the ability to be proportionately scaled). In this way, fine tuning of the scaffold's biological response may be conducted simply by altering its size. Certainly, there exits a bandwidth in which scaling will not prove biologically favorable, most likely, when an interconnected pore structure is not possible. Yet, by mixing and matching different microstructural units, increasing some and decreasing or skewing others, a net biological advantage may be achieved.

Arrangement of Building Blocks into Composite Structure

Following their construction and the optimization of building blocks to match specific stiffness values, the building blocks need to be arranged appropriately in the VB based on the determined material demands. Using a modulus map generated from the imaging modalities, a building block can be placed in the location of each corresponding stiffness value. Previous studies evaluating the strength and density of lumbar vertebral trabecular bone have demonstrated a difference based on anatomical site for both quantities. Mechanical testing was completed on sectioned lumbar vertebral trabecular bone, which showed an increased strength in the central posterior portion of the VB that corresponded to stress maximums for *in vivo* loading.[27] QCT has been used to show that density differences of the VB were also localized to this area.[38] From the 3D map of

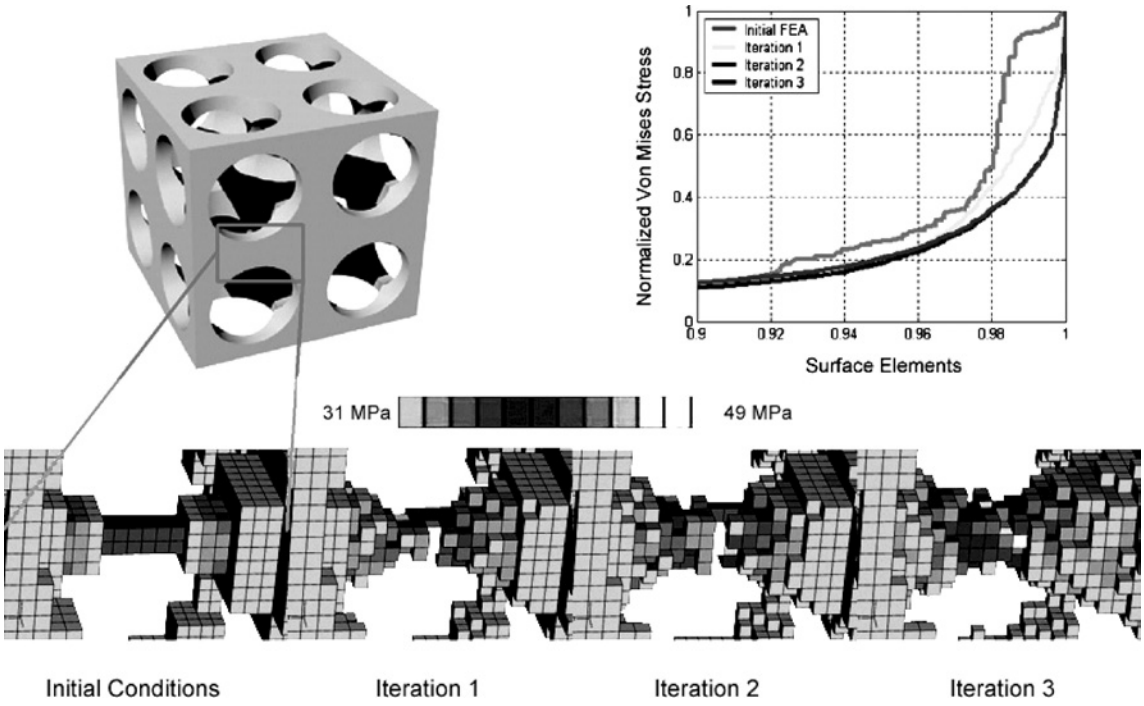

FIGURE 3. Voxel-based microstructural unit initially and after one iteration. Finite element results were tabulated under unit compressive displacement (into page) and a fully constrained opposite face. Note that material reinforces areas of high strain energy (the objective function in this case) and are taken from areas of low energy to narrow the energy profile.

stiffness values for the VB obtained from literature values or imaging modalities, a building block would be selected from the library which approximates the estimated property. Using CAD, the cubes can be arranged in series or parallel resulting in a layered reconstruction of the bone (Fig. 4).[52,54]

Following the construction of the array of building blocks in the proper location, a general approximation of a VB exists. However, the global shape requires a continuous boundary. To accomplish this, Boolean or cutting functions that are innate to all CAD programs can be used. A simple analogy of this process is making cookies using a cookie cutter. As illustrated in Fig. 4, a surface with a complex border is placed over the complex shape, represented here by a cube. The excess material that overlaps outside of the border is then removed, leaving the former object with the complex shape that defined the border of the surface.

Arrangement of the posterior elements proves to be a much more daunting problem based on location and function. While the posterior elements are responsible for (30% of the total load transfer of the VB,[2,69] it is important to include them both for the protection of the spinal cord but also consistency of meshing with adjacent vertebrae. Since the posterior elements are to a significant degree simply cortical bone, these elements can be approximated as a thick shell with a hollow channel running through the center.[24] The complex global shape of the posterior elements can be obtained using the previous imaging techniques, and its manufacturability may be dependent on a piecewise Boolean of largely fluctuating surfaces (Fig. 5).

Fabrication Techniques

Creation of a tissue level model of a VB using building block structures is followed by fabrication of the construct. Because the complex structure is near to the resolution of the microarchitecture of bone, precise manufacturing methods must be used to generate the models. Several rapid prototyping processes, such as stereolithography and fused deposition modeling (FDM), are now able to generate an entire bone model including complex microarchitecture at or near the size of individual trabeculae.[55,70] Table 1 highlights

TABLE 1. Achievable resolution of currently available rapid prototyping systems.

Fabrication method	Print resolution
Stereolithography[11]	0.0762 mm *x,y* 0.0508 mm *z*
Fused deposition modeling[51]	0.013 mm *z* 0.0762 mm *x,y*
3D plotting[31]	0.05 mm *x,y,z*-direction
Sciperio[49]	0.05 mm *x,y,z*-direction
Therics[59]	Not published

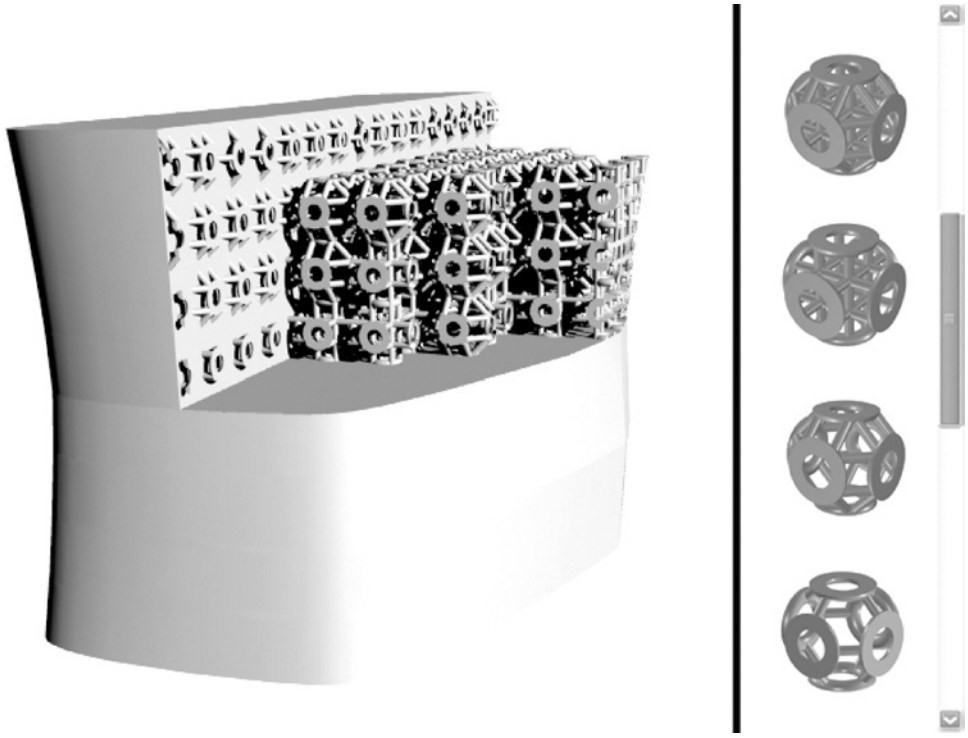

FIGURE 4. Using a library of building blocks to generate a global shape. The library at the right can be used in any location that is required to approximate the material demands of the vertebral body. The global shape approximates a vertebral body following the assignment of the building blocks to their respective locations.

the achievable resolution of relevant rapid prototyping systems.

Stereolithography utilizes a precision laser driven on a plotter which crosslinks a polymer in predetermined arrangements. At the time this study is in press, stereolithography cannot attain the resolution of FDM, but laser technology will eventually surpass all other processes in achievable resolution. One distinct advantage of stereolithography over other methods is the ability to fabricate structures using photocrosslinkable polymers. Poly(propylene fumarate) was previously used with stereolithography to produce simple structures,[6] which is the first step toward generating entire scaffolds out of an implantable biomaterial. Complex structures are currently a problem for stereolithography because the process uses only one material to build objects and lacks adequate support structure to generate complex architectures or structures with oblique angles.

The ability to print a scaffold using the implantable material is indeed an attractive promise, one which other rapid prototyping systems, besides stereolithography, are attempting to deliver. Research groups have built stand alone machines that utilize materials such as thermoplastic hydrogels and agarose.[31] Therics Inc. uses a particle binding system with six printheads offering increased production, while maintaining high resolution required for internal morphology (Therics Inc., Princeton, NJ). Sciperio Inc. has created a system that is able to print on a complex surface with a variety of materials including fibrin glue, cells and polymers, and eventually live cells. The machine uses two lasers to guide the printheads to deposit material on an object, even one moving at 10 Hz.[62] Mironov *et al*. discuss

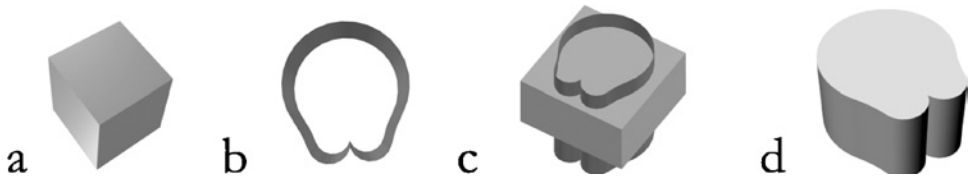

FIGURE 5. Explanation of one type of a Boolean difference function. Simple shape (a) is overlaid with a complex border (b) shown in panel C. Adapted from a process described previously.[55] After the Boolean difference, the simple shape now has the complex border with the excess material deleted (d).

the development of a rapid prototyping system capable of delivering cells, gels, and cell aggregates into defined locations and whole cells into predetermined locations.[39] However, if the resolution cannot generate the structures needed for a functional implant, then even a versatile rapid prototyping machine is limited, as is currently the case with these systems.

Fused deposition modeling is currently the most versatile rapid prototyping process and works like an inkjet printer with a movable *z*-stage. The printhead deposits a thermoplastic material onto the stage in a two-dimensional pattern resulting in a slice of the final geometry. A second material is printed surrounding the build material, enabling oblique angles. The PatternMaster (SolidScape, Manchester, NH) wields two thermoplastic waxes for build and support, and several different solvents to remove the reinforcement material.[56] The ability to remove support materials makes FDM a good candidate for investment casting. By printing the inverse of an object and removing the support material, a mold of the object is created. This mold will be a template for injection of the casting material, following removal of the build material.[34] This process can be used with any material (even metal) as long as the melting temperature and the chemical solvents are conducive with the casting material. Interpore Cross currently uses this technology to generate titanium VB replacements (Interpore Cross, Irvine, CA). More complex molds can be generated that allow the implementation of multiple biomaterials.[47,64] Incorporating separate rapid prototyped parts into a single mold allows the use of a wide range of materials, while maintaining the resolution required for a bone-like architecture.

Functional Integration

Functional integration of the implant into the anatomic site is essential to address load transfer demands. After crafting the implant based on perceived specifications for loading and mechanical stability, functional integration is necessary to prevent implant failure and loosening. Loosening persists for any metal implant resulting in its dislodge from the bone in 10–15 years, and generally causes severe pain.[42] To prevent stress shielding and subsequent implant failure, it is the current belief that the biomechanical properties of the implant must approximate the original bone properties and that the implant must be functionally integrated into the surrounding tissue.

The successful implantation of the vertebral replacement requires that the muscles, tendons, ligaments, and other tissue be rejoined to the VB in the same location as they were before they were removed. While this is currently not possible, the intended future attachment must be in a manner that promotes their functional integration during tissue healing. For example, specific fillets could be designed into the VB shell that would allow for connective tissue to be sutured or tied to the outside of the implant. Resorbable fixation devices could be useful for the fixation of the constituents of the spine as well as the external parts; previous studies have already demonstrated the efficacy of joining tendons and ligaments with screws and pins.[29] Other options include the use of bone cements that contain stiffness and strength much closer to bone than other polymeric materials.[5] Additionally, optimal performance of the VB *in vivo* requires that posterior elements are matched with the spinal cord to protect and enact load transfer. Tissue glues such as fibrin glue, a popular natural adhesive, do provide connections between two tissue counterparts and are fully resorbable, yet questions remain regarding its adhesive threshold for this application. The hollow channel present in the posterior elements is a convenient anatomical attachment point for connecting the two lateral elements.[41,50] Figure 6 depicts the splitting of the vertebrae that would be required for implantation to work surrounding the spinal cord. The invertebral disc above and below the VB could also be joined to the surface of the implant with fibrin glue, combined with therapeutic drugs, sutured, or fixed closed with bioresorbable materials.

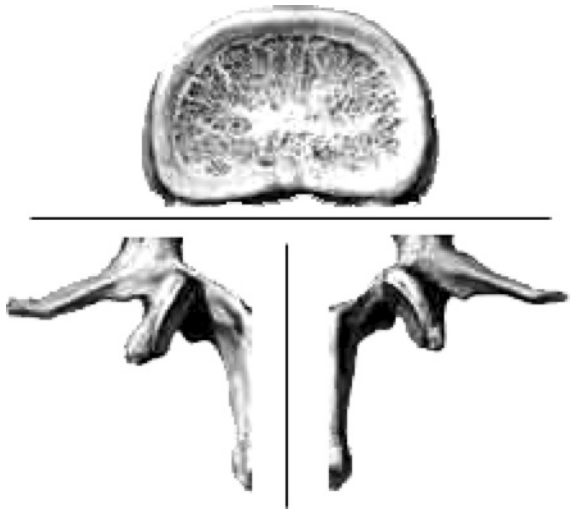

FIGURE 6. Diagram of lumbar vertebral body. Functional integration of the vertebral body into the location in the body would required the splitting of the body into three segments. These three segments will facilitate the assembly of the vertebrae around the spinal cord without damaging it. Tissue glue can be used to rejoin the three parts together.

Material Selection

The scaffold material determines both the mechanical robustness and the biological coupling of the scaffold with its environment. The material should be selected with prior knowledge of the anatomical implant site as well as the production method used in its manufacture. For example, various pretreated titanium products have shown detrimental effects caused by unexpected surface oxide reactions

in vivo that differ depending on base material.[33] Likewise, if a specific microstructure is desired, or resolution is an issue, the use of stereolithography or FDM would be advisable.[20] There is a limit on the types of materials that can be produced with rapid prototyping technologies and also the types of materials suitable to be cast into molds.[64] Materials such as hydroxyapatite and calcium phosphates, which are formed from sintered powder particulates, are certainly less conducive to fabrication though technologies utilizing subtractive molds do exist.[57] Furthermore, the preparation of biodegradable polymers commonly used as templates for tissue engineering involves toxic solvents or cross-linking agents, some of which will not be completely denatured from the construct.[13] The personal selection of any material will have to gain the final approval of the FDA.

As discussed in the previous sections, through spatial redistribution and organization of a material, the structural properties can be significantly increased.[18] Materials are often chosen based on their mechanical and biological compatibility which is a factor of anatomic location. A large problem noted early on in the development of hip replacements was stress shielding: a phenomenon whereby the brunt of the load carried by the stiffer material, usually titanium, which resulted in reduced deformation of the surrounding bone to below equilibrium states, stimulating osteoclastic bone resorption and eventual implant loosening.[43] Other shortcomings in mechanical properties can be attributed to ultimate strength, fatigue life, and elasticity. Unfortunately, biocompatible materials—materials that minimize an immune response—do not in most cases have the mechanical strength or stiffness to mimic bone. These materials should not be discarded, however, as they may be favored biologically through hydrolytic breakdown or their ability to cause minimal pH changes in the environment.

Composites have been explored as a natural compromise, since no one material possesses all of the ideal properties for a given problem. Composites can be as simple as the application of peptide sequences onto a carbon–carbon backbone, or as complex as developing negative stiffness or "smart" materials. Many biocomposites seek to offer surface modifications or specify binding for a particular application. Other types of composites make use of a biocompatible base material enforced with stronger nodules such as carbon nanotubes.[19] These materials offer a good compromise in gross properties, but increase the complexity of chemical interactions occurring within the architecture, making them more difficult to study and safely apply.

RESULTS

As a demonstration of both the possibility and limitations of this technique, a simple model of a VB was generated. Using the governing concepts for CATE, a lumbar vertebrae was scanned using a QCT and was reconstructed using Analyze (Analyze Direct 5.0, Lenexa, KS). The elastic moduli were calculated from mapping pixel values onto FEA grids and then were matched with predefined correlations between Hounsfield units and bone mineral density. The mean trabecular density was calculated as 0.13 g/(cm^3 (range 0.01–0.25 g/(cm^3) with an average cortical density of 0.6 g/(cm^3. Modulus values for trabecular bone averaged 390 MPa (range 0.01–770 MPa) using accepted correlations.[28] Modulus values per region were grouped into four regions corresponding to four different building blocks evaluated in a previous study[35] shown at the right of the figure. Building blocks were modified to global dimensions of 3.0 mm $\times$ 3.0 mm $\times$ 3.0 mm, which would result in 1008 total blocks for the complete approximation of the VB. The porosities ranged from 80 vol.% porosity up to 92% porosity, values encompassing bone porosities. On the basis of the generated modulus map, the building blocks were arranged into their respective locations. CAD processes reached the upper limits of memory usage long before the assembly of the entire 1008 building blocks. The memory limit of Windows XP was also a limiting factor. As a result, only half of the approximated VB was built using the PatternMaster (Fig. 7). While the overall dimensions were replicated, fine features were often error prone. In conclusion, we have demonstrated that this process can be completed on a rudimentary scale but that fine tuning is required for each step of the process. For this process to be successful in the future, both computing processes and the resolution of rapid prototyping will have to increase.

CONCLUSION

The presentation for a patient-specific approach for constructing a complete VB via building blocks has been conducted. A summary of the procedure starting with image capture through surgical implantation is depicted in Fig. 8. Though some of the methods described cannot be realized with current technology, the necessary advances are not far off. Computing resources do not currently allow the generation and manipulation of models larger than we have proposed. Computing power and CAD programs need to improve slightly to allow the rapid generation of complex models that would ease the fabrication of an appropriate number of building blocks. In materials research, there is much effort directed at varying the modulus and surface chemistry of materials, especially important for load-bearing implants. Already, previous studies have evidenced rapid prototyping models which use a repeated structure in large size models.[12,52] With the improvement of rapid prototyping speed and resolution, machines will be able to print with a wider range of materials. A current goal of several companies is the ability to incorporate live cells into their printed scaffolds.[48]

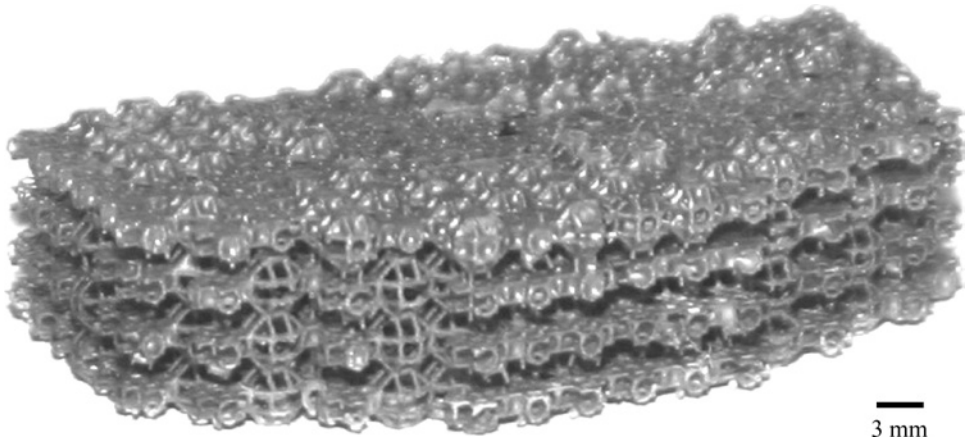

FIGURE 7. Approximation of a human lumbar vertebral body generated using computer-aided tissue engineering principles and the PatternMaster.

The main bottleneck of the process described in this study is the general lack of knowledge of human mechanobiology and the role of cellular interactions on artificial substrates. Assuming these biological parameters can be identified, a scaffold may be designed with a proper pore size and interconnectivity, microstructure, degradation rate, and surface chemistry. The advantage of the outlined process lies in adjustment of the vertebral compliance first to ensure adequate load transfer, an important property for vertebral replacement. Subsequently, net biological properties can be fine tuned by simply scaling the final construct. Further alterations can be accomplished by choosing a new microstructure from the available library of shapes with a different biological property but similar unit stiffness.

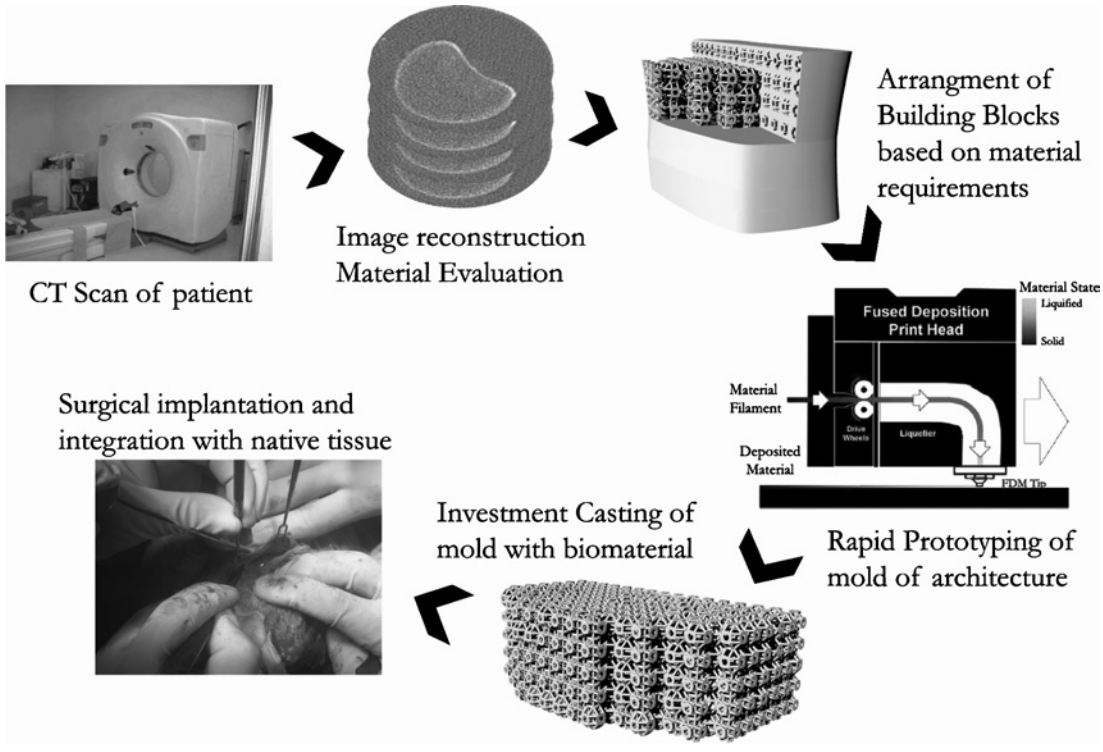

FIGURE 8. Conceptual diagram of the process of computer-aided tissue engineering. Three-dimensional model of tissue is obtained using an imaging modality. Reconstruction of the image occurs with a sectioning program. Following this, the CAD model of the part is generated using building blocks. This architecture is then built using rapid prototyping and cast with the biomaterial of choice to yield an implant, which is then surgically inserted into the body.

Mixing and matching of geometries may be utilized to design asymmetric scaffolds or scaffolds that exhibit a discontinuous microstructural stiffness with the goal of accentuating fluid flow. Finally, while these techniques lend themselves to the formulation of bone constructs, they can be used for other parts of the body as well that do not require load-bearing support.

REFERENCES

[1]Adachi, T., K. Tsubota, Y. Tomita, and S. J. Hollister. Trabecular surface remodeling simulation for cancellous bone using microstructural voxel finite element models. *J. Biomech. Eng.* **123**(5):403–409, 2001.

[2]Asano, S., K. Kaneda, S. Umehara, and S. Tadano. The mechanical properties of the human L4-5 functional spinal unit during cyclic loading. The structural effects of the posterior elements. *Spine* **17**(11):1343–1352, 1992.

[3]Borah, B., G. J. Gross, T. E. Dufresne, T. S. Smith, M. D. Cockman, P. A. Chmielewski, M. W. Lundy, J. R. Hartke, and E. W. Sod. Three-dimensional microimaging (MRmicroI and microCT), finite element modeling, and rapid prototyping provide unique insights into bone architecture in osteoporosis. *Anat. Rec.* 265(2):101–110, 2001.

[4]Brody, A. S. CT scanner design and patient radiation exposure. *Pediatr. Radiol.* 32(4):268–271, 2002.

[5]Chu, K. T., Y. Oshida, E. B. Hancock, M. J. Kowolik, T. Barco, and S. L. Zunt. Hydroxyapatite(PMMA composites as bone cements. *Biomed. Mater. Eng.* 14(1):87–105, 2004.

[6]Cooke, M. N., J. P. Fisher, D. Dean, C. Rimnac, and A. G. Mikos. Use of stereolithography to manufacture critical-sized 3D biodegradable scaffolds for bone ingrowth. *J. Biomed. Mater. Res.* 64B(2):65–69, 2003.

[7]Danesi, L., R. Cherubini, L. Ciceri, G. Graziadei, M. D. Cappellini, F. Cavagnini, and S. Ortolani. Evaluation of spine and hip bone density by DXA and QCT in thalassemic patients. *J. Pediatr. Endocrinol. Metab.* 11(Suppl. 3):961–962, 1998.

[8]Davidson, E. T., J. G. Evans, and Y. D. Coble, Jr. Bone mineral density testing by DEXA. *J. Fla. Med. Assoc.* 83(8):567–568, 1996.

[9]Davis, J., Till death do us part. *Wired* 110–120, 2003.

[10]Dean, D., K. J. Min, and A. Bond, Computer aided design of large-format prefabricated cranial plates. *J. Craniofac. Surg.* 14(6):819–832, 2003.

[11]3DSystems. 3D Systems—Rapid Prototyping, Advanced Digital Manufacturing, 3D Printing, 3D CAD, 2004.

[12]Feinberg, S. E., S. J. Hollister, J. W. Halloran, T. M. Chu, and P. H. Krebsbach. Image-based biomimetic approach to reconstruction of the temporomandibular joint. *Cells Tissues Organs* 169(3):309–321, 2001.

[13]Fisher, J. P., T. A. Holland, D. Dean, P. S. Engel, and A. G. Mikos. Synthesis and properties of photocross-linked poly(propylene fumarate) scaffolds. *J. Biomater. Sci. Polym. Ed.* 12(6):673–687, 2001.

[14]Gibson, L. J., and M. F. Ashby. Cellular Solids: Structure and Properties. Elmsford, NY: Pergamon Press, 1988.

[15]Gordon, R., R. Bender, and G. T. Herman. Algebraic reconstruction techniques (ART) for three-dimensional electron microscopy and x-ray photography. *J. Theor. Biol.* 29(3):471–481, 1970.

[16]Guan, H., M. W. Gaber, F. A. DiBianca, and Y. Zhu. CT reconstruction by using the MLS-ART technique and the KCD imaging system–I: Low-energy X-ray studies. *IEEE Trans. Med. Imaging* 18(4):355–358, 1999.

[17]Hansson, T., B. Roos, and A. Nachemson. The bone mineral content and ultimate compressive strength of lumbar vertebrae. *Spine* 5(1):46–55, 1980.

[18]Hollister, S. J., R. D. Maddox, and J. M. Taboas. Optimal design and fabrication of scaffolds to mimic tissue properties and satisfy biological constraints. *Biomaterials* 23(20):4095–4103, 2002.

[19]Horch, R. A., N. Shahid, A. S. Mistry, M. D. Timmer, A. G. Mikos, A. R. Barron. Nanoreinforcement of poly(propylene fumarate)-based networks with surface modified alumoxane nanoparticles for bone tissue engineering. *Biomacromolecules.* 5(5):1990–1998, 2004.

[20]Hutmacher, D. W. Scaffold design and fabrication technologies for engineering tissues—state of the art and future perspectives. *J. Biomater. Sci. Polym. Ed.* 12(1):107–124, 2001.

[21]Ito, M., *et al*. Bone mineral and other bone components in vertebrae evaluated by QCT and MRI. *Skeletal Radiol.* 22(2):109–113, 1993.

[22]Jacobs, C. R., B. R. Davis, C. J. Rieger, J. J. Francis, M. Saad, and D. P. Fyhrie. NACOB presentation to ASB Young Scientist Award: Postdoctoral. The impact of boundary conditions and mesh size on the accuracy of cancellous bone tissue modulus determination using large-scale finite-element modeling. North American Congress on Biomechanics. *J. Biomech.* 32(11):1159–1164, 1999.

[23]Jakobs, T. F., C. R. Becker, B. Ohnesorge, T. Flohr, C. Suess, U. J. Schoepf, and M. F. Reiser. Multislice helical CT of the heart with retrospective ECG gating: Reduction of radiation exposure by ECG-controlled tube current modulation. *Eur. Radiol.* 12(5):1081–1086, 2002.

[24]Jee, W. S. S. Integrated bone tissue physiology: Anatomy and physiology. In: Bone Mechanics Handbook, edited by S. C. Cowin. New York: CRC Press, 2001, pp. 1–1 to 1–68.

[25]Jones, L. M., A. Goulding, and D. F. Gerrard. DEXA: A practical and accurate tool to demonstrate total and regional bone loss, lean tissue loss and fat mass gain in paraplegia. *Spinal Cord* 36(9):637–640, 1998.

[26]Kai, C. C. Three-dimensional rapid prototyping technologies and key development areas. *Comp. Control Eng. J.* 5(4):200–206, 1994.

[27]Keller, T. S., T. H. Hansson, A. C. Abram, D. M. Spengler, and M. M. Panjabi. Regional variations in the compressive properties of lumbar vertebral trabeculae. Effects of disc degeneration. *Spine* 14(9):1012–1019, 1989.

[28]Kopperdahl, D. L., E. F. Morgan, and T. M. Keaveny. Quantitative computed tomography estimates of the mechanical properties of human vertebral trabecular bone. *J. Orthop. Res.* 20(4):801–805, 2002.

[29]Kotani, Y., B. W. Cunningham, A. Cappuccino, K. Kaneda, and P. C. McAfee. The effects of spinal fixation and destabilization on the biomechanical and histologic properties of spinal ligaments. An *in vivo* study. *Spine* 23(6):672–682; discussion 682–683, 1998.

[30]Kusnoto, B., and C. A. Evans. Reliability of a 3D surface laser scanner for orthodontic applications. *Am. J. Orthod. Dentofacial. Orthop.* 122(4):342–348, 2002.

[31]Kusnoto, B., and C. A. Evans. Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering. *Biomaterials* 23(23):4437–4447, 2002.

[32]Landers, R., U. Hubner, R. Schmelzeisen, and R. Mulhaupt. Correlation of mechanical properties of vertebral trabecular

bone with equivalent mineral density as measured by computed tomography. *J. Bone Joint Surg. Am.* 70(10):1531–1538, 1988.
[33]Larsson, C., P. Thomsen, B. O. Aronsson, M. Rodahl, J. Lausmaa, B. Kasemo, and L. E. Ericson. Bone response to surface-modified titanium implants: Studies on the early tissue response to machined and electropolished implants with different oxide thicknesses. *Biomaterials* 17(6):605–616, 1996.
[34]Liebschner, M. A. K., K. Sun, and M. A. Wettergreen. Conceptual analysis of a novel bone anchor system. *J. Biomech.*, submitted.
[35]Liebschner, M. A., and M. A. Wettergreen. Scaffold optimization for load bearing applications. In: Southern Biomedical Engineering Conference. Bethesda, MD: Medical and Engineering Publishers, 2002.
[36]Markel, M. D., M. A. Wikenheiser, R. L. Morin, D. G. Lewallen, and E. Y. Chao. Quantification of bone healing. Comparison of QCT, SPA, MRI, and DEXA in dog osteotomies. *Acta Orthop. Scand.* 61(6):487–498, 1990.
[37]Mayo, J. R., J. Aldrich, and N. L. Muller. Radiation exposure at chest CT: A statement of the Fleischner Society. *Radiology* 228(1):15–21, 2003.
[38]McCubbrey, D. A., D. D. Cody, E. L. Peterson, J. L. Kuhn, M. J. Flynn, and S. A. Static and fatigue failure properties of thoracic and lumbar vertebral bodies and their relation to regional density. *J. Biomech.* 28(8):891–899, 1995.
[39]Mironov, V., T. Boland, T. Trusk, G. Forgacs, and R. R. Markwald. Organ printing: Computer-aided jet-based 3D tissue engineering. *Trends Biotechnol.* 21(4):157–161, 2003.
[40]Nishitani, H., M. Yasutomo, M. Tominaga, H. Fukui, and H. Yagi. Radiation exposure in CT. *Nippon Igaku Hoshasen Gakkai Zasshi* 62(7):347–351, 2002.
[41]Ono, K., J. Shikata, K. Shimizu, and T. Yamamuro. Bone-fibrin mixture in spinal surgery. *Clin. Orthop.* (275):133–139, 1992.
[42]Prendergast, P. J. Bone prostheses and implants. In: Bone Biomechanics Handbook, edited by S. C. Cowin. New York: CRC Press, 2001, pp. 35-1 to 35-29.
[43]Prins, S. H., H. L. Jorgensen, L. V. Jorgensen, and C. Hassager. The role of quantitative ultrasound in the assessment of bone: A review. *Clin. Physiol.* 18(1):3–17, 1998.
[44]Rangayyan, R. M., and R. Gordon. Computed tomography from ordinary radiographs for teleradiology. *Med. Phys.* 10(5):687–690, 1983.
[45]Riggs, B. L., and L. J. Melton, 3rd. The worldwide problem of osteoporosis: Insights afforded by epidemiology. *Bone* 17(Suppl 5):505S–511S, 1995.
[46]Ruimerman, R., B. Van Rietbergen, P. Hilbers, and R. Huiskes. A 3-dimensional computer model to simulate trabecular bone metabolism. *Biorheology* 40(1–3):315–320, 2003.
[47]Sachlos, E., N. Reis, C. Ainsley, B. Derby, and J. T. Czernuszka. Novel collagen scaffolds with predefined internal morphology made by solid freeform fabrication. *Biomaterials* 24(8):1487–1497, 2003.
[48]Sciperio Inc. A Science Revelation. 2003.
[49]Sciperio Inc. A Science Revelation. 2004.
[50]Soffer, E., J. P. Ouhayoun, and F. Anagnostou. Fibrin sealants and platelet preparations in bone and periodontal healing. *Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod.* 95(5):521–528, 2003.
[51]SolidScape. SolidScape, 2004.
[52]Starly, B., W. Lau, Z. Fang, and W. Sun. "Biomimetic" Model For Heterogeneous Bone Scaffold. In: Southern Biomedical Engineering Conference. Washington, DC: Medical and Engineering Publishers, 2002.
[53]Sun, W., B. Starly, A. Darling, and C. Gomez. Computer aided tissue engineering part I: Overview, scope and challenges. *J. Biotechnol. Appl. Biochem.* 2003.
[54]Sun, W., B. Starly, A. Darling, C. Gomez. Computer aided tissue engineering part II: Application to biomimetic modeling and design of tissues. *J. Biotechnol. Appl. Biochem.* 2003.
[55]Sun, W., and P. Lal. Recent development on computer aided tissue engineering—a review. *Comput. Methods Programs Biomed.* 67(2):85–103, 2002.
[56]Taboas, J. M., R. D. Maddox, P. H. Krebsbach, and S. J. Hollister. Indirect solid free form fabrication of local and global porous, biomimetic and composite 3D polymer-ceramic scaffolds. *Biomaterials* 24(1):181–194, 2003.
[57]Tan, K. H., C. K. Chua, K. F. Leong, C. M. Cheah, P. Cheang, M. S. Abu Bakar, and S. W. Cha. Scaffold development using selective laser sintering of polyetheretherketone-hydroxyapatite biocomposite blends. *Biomaterials* 24(18):3115–3123, 2003.
[58]Templeton, A. K., C. D., and M. A. K. Liebschner. Updating a 3-D vertebral body finite element model using 2-D images. *Med. Eng. Phys.*, submitted.
[59]Therics, Inc. Therics, Inc.—Tissue Engineering Specialists, 2003. Available at http://www.therics.com
[60]Toffoli, T. Cellular automata. In: The Handbook of Brain Theory and Neural Networks, edited by A. M. Cambridge, MA: MIT Press, 1995, pp. 166–169.
[61]Ulrich, D., B. van Rietbergen, A. Laib, and P. Ruegsegger. The ability of three-dimensional structural indices to reflect mechanical aspects of trabecular bone. *Bone* 25(1):55–60, 1999.
[62]Warren, W. L. Enabling tools for computer aided tissue engineering. In: Advances in Tissue Engineering. Houston, TX, 2003.
[63]Webb, P. A. A review of rapid prototyping (RP) techniques in the medical and biomedical sector. *J. Med. Eng. Technol.* 24(4):149–153, 2000.
[64]Wettergreen, M. A., M. D. Timmer, J. J. Lemoine, A. G. Mikos, M. A. K. Liebschner. Design of a three-dimensional composite scaffold with varied engineered micro-architecture. Groupe de Recherche Interdisciplinaire sur les Biomateriaux Osteoarticulaires Injectables. Baltimore, MD, 2003.
[65]Wettergreen, M. A., and M. A. K. Liebschner. Scaffold optimization for load bearing applications. In: Southern Biomedical Engineering Conference. Washington, DC: Medical and Engineering Publishers, 2002.
[66]Wiest, P. W., J. A. Locken, P. H. Heintz, and F. A. Mettler, Jr. CT scanning: A major source of radiation exposure. *Semin. Ultrasound CT MR* 23(5):402–410, 2002.
[67]Winder, J., R. S. Cooke, J. Gray, T. Fannin, and T. Fegan. Medical rapid prototyping and 3D CT in the manufacture of custom made cranial titanium plates. *J. Med. Eng. Technol.* 23(1):26–28, 1999.
[68]Winslow, R. L., and M. S. Boguski. Genome informatics: Current status and future prospects. *Circ. Res.* 92(9):953–961, 2003.
[69]Yang, K. H., and A. I. King. Mechanism of facet load transmission as a hypothesis for low-back pain. *Spine* 9(6):557–565, 1984.
[70]Yang, S., K. F. Leong, Z. Du, and C. K. Chua. The design of scaffolds for use in tissue engineering. Part II. Rapid prototyping techniques. *Tissue Eng.* 8(1):1–11, 2002.