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Bioengineered tissues: the science, the technology, and the industry

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Structured Abstract

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Objective – The bioengineering of tissues and organs, sometimes called tissue engineering and at other times regenerative medicine, is emerging as a science, as a technology, and as an industry. The goal is the repair, replacement, and/or the regeneration of tissues and organs. The objective of this paper is to identify and discuss the major issues that have become apparent.

Results – One of the critical issues is that of cell source, i.e. what will be the source of the cells to be employed? Another critical issue is the development of approaches for the fabrication of substitute tissues/organs and/or vehicles for the delivery of biological active molecules for use in the repair/regeneration of tissues. A third critical issue, one very much related to cell source, is that of immune acceptance. In addition, there are technological hurdles; there are additional issues such as the scale-up of manufacturing processes and the preservation of living-cell products for off-the-shelf availability. Although the initial products have been superficially applied skin substitutes, as this fledgling industry continues to evolve, it is beginning to focus on a wider range of more invasive and complicated products. From a public health perspective, the real opportunity may be in addressing chronic diseases, as well as the transplantation crisis (i.e. the tremendous disparity between patient need for vital organs and donor availability) and, equally important is the challenge of neural repair.

Conclusion – These are the grand challenges, and the scientific community, business/private sector, and federal government must mobilize itself together in this emerging area to translate the benchtop science to the patient bedside.

Key words: biomaterials; cell source; regenerative medicine; tissue engineering

Introduction

In discussing bioengineered tissues, what are meant are the replacement, repair, and/or regeneration of tissues/organs. Some call this tissue engineering, a term 'coined' in the late 1980s (1). However, the field predates that considerably with the first mention of the concept of a more biological approach dating back to 1938 (2). It was in the 1970s when there was the first appearance of significant research activities. Those efforts have now expanded to include applications as diverse as skin, bone, blood vessels, kidney, and neural repair. Consistent research efforts in this field led to the National Library of Medicine introducing Tissue Engineering as a Medical Subject Heading (MeSH) in 2002 (3). Recently the term Regenerative Medicine has been used (added as a MeSH heading in 2004) (3). However, the meaning of regenerative medicine varies with the user, where in some cases it is used specifically to describe the use of stem and other progenitor cells in medicine. Others use the terms tissue engineering and regenerative medicine interchangeably, and the authors are members of this group.

Tissue engineering/regenerative medicine is at the interface of the medical implant industry and the biological revolution. This industry is in the process of being revolutionized by the continuing advances in molecular and cell biology. With recent technical advances in molecular and cell biology that result in large volumes of data, combined with bioinformatics, a new field focusing on the collection and manipulation of biological data, great advances in biological knowledge and understanding are underway. These advances will give rise to the next generation of medical implants and related therapies. While in the past most implants were mostly structural and inert, future products will be much more biologic in nature, taking advantage of and mobilizing the inherent biological power of our bodies. In cardiovascular applications, examples of these more biologic implants include the ongoing development of vascular grafts with an antithrombogenic endothelial lining and the future development of viable pediatric aortic valves that can grow and remodel over time. While early efforts were mostly by small pioneering companies, in light of the far-reaching implications of this field, more recently a number of the larger medical implant companies have begun to increase their investments in this emerging area of technology. These companies recognize that regenerative medicine has the potential to become an important part of our future.

With the development of regenerative medicine, there is the opportunity to make some of the current clinical treatments obsolete in the future. Fully realizing this potential, however, is for the most part a ways off. It is thus unfortunate that this field has been so over hyped, as this has led to unrealistic expectations by the public. The media has been very enthusiastic to focus on possible *in vitro* growth of spare body parts (4); however, scientists have also contributed to the hype by being unrealistically optimistic, thus overstating the potential and timeline of accomplishment (5,6). While some predict that tissue engineering will be a $100 billion industry, the reality today consists of only five approved products, annual sales of less than $100 million, and a small patient population being impacted, these in non-life saving applications. The potential, however, still is very much there for we are only at the very beginning. It is in this spirit, one of being only at the very beginning, that this brief review of the science, the technology, and the translation of these to industry are presented.

Critical issues

The industry associated with tissue engineering and regenerative medicine is very much in a fledgling state. Today there are fewer than 100 companies and less than 3000 people working in this industry (Table 1), the latter as compared to more than 3 00 000 employees in the medical implant industry. Last year total sales of products that can be identified with tissue engineering was less than $100 million, where in contrast worldwide sales for the medical implant industry is approaching $200 billion. The initial products have been the more structural tissues, including skin substitutes, and in the future this will broaden to include the vital organs and neural repair. While several of the early pioneering companies have had a variety of problems, from these initial products much has been learned, and this learning will help us to broaden the application of tissue engineering.

Last March the Georgia Tech/Emory Center for the Engineering of Living Tissues, i.e. GTEC, held its annual Workshop at the Sea Pines Plantation on Hilton Head

Table 1. **The status of the tissue engineering industry in 2002, as compared to 2000 (23)**

	2000	2002
Companies	73	89
Annual spending	$610 million	$487 million
FTE's	3079	2611
Industry makeup		
Structural	58%	37%
Cellular	29%	47%
Metabolic	12%	15%
Other	1%	1%
Industry distribution		
United States	80%	54%
Rest of the world	20%	46%

The following subcategories are defined as: structural–skin, cardiovascular disease, and musculoskeletal applications; cellular–stem cell/therapeutic cloning and encapsulated cell therapy; metabolic–bioartificial liver, bioartificial pancreas, and bioartificial kidney.
FTEs, full time employees.

Island, South Carolina. As part of this workshop, a panel on critical issues was held, and out of this discussion there were four key issues that were identified. These are as follows:

- Off-the-shelf availability.
- Cell source.
- Matrix.
- Immune acceptance.

Of the above, the issue of off-the-shelf availability was identified as the key to the success of a product. Off-the-shelf availability is instrumental for both commercial and clinical needs. Commercially, a business model is more viable if it is based on the manufacturing of large batches of product, as opposed to need-based production. Such an approach results in less consumption of product for quality testing, a steady manufacturing schedule, and a reliable supply of product, together potentially leading to greater profits. Off-the-shelf availability also better services clinical needs. There clearly are surgeries that must be carried out on short notice, in some cases extremely short notice, and for these off-the-shelf availability is essential. But what about those cases where the time of surgery is elective? Why for cases where the time of surgery is elective does one need off-the-shelf availability? Why can one not adopt the approach of extracting cells from the patient, expanding them, and seeding the substitute that then is implanted? The answer is that, even for a case where the time of surgery is elective, unless there is off-the-shelf availability this approach will not be used at the large variety of hospitals required to impact the wider patient population that is out there.

Thus, off-the-shelf availability seems crucial for long-term success of tissue-engineered products. Within this context, the next sections will address the other key issues: cell source, matrix, and immune acceptance.

Cell source

If one is to truly harness the power of biology in developing new strategies for therapy and treatment, then one must be able to mobilize the involvement of cells. For obvious reasons, early clinical trials in many cases are using autologous cells, bypassing immunogenicity issues. However, unless one can recruit these autologous cells directly to the tissue engineered implant, one cannot have a product that is off-the-shelf available. It is important to note that the skin substitutes, ones that incorporate cells and have made it to market, employ allogeneic cells. These have been carefully selected to be immune acceptable, e.g. dermal fibroblasts, and have provided the off-the-shelf availability that was desired. However, there will be cases where the cell to be employed is immunogenic. One example is the use of the vascular endothelial cell where one would need to, in some manner, employ a strategy for the engineering of immune acceptance. This will be discussed in a later section.

There are, however, other cell source-related issues. Not only are there differences associated with species, there are differences associated with location in the body. For example, vascular endothelial cells from different locations in the vasculature can have phenotypic and functional characteristics that are very different (7). There also can be differences with age (8,9), and little attention has been paid to differences in allogeneic cells associated with the age of the donor. Even with autologous cells there can be age-related issues, including cellular functionality and availability (10). This includes differences in bone marrow-derived cells that are dependent on age. To this one must add differences due to gender (11). Taken together and using cartilage repair as an example, it is clear that a chondrocyte is not a chondrocyte.

Is there an autologous cell source that might allow for off-the-shelf availability? Certainly one attractive source is adult stem cells (12), such as the bone marrow-derived stem/progenitor cells. The bone marrow is a rich repository of cells of a variety of types, and in the studies reported to date, at least in many cases; the cell population harvested and then used was poorly characterized (13). Still, there is the possibility that in the future one might be able to recruit bone marrow-derived cells to a non-cellularized implant *in vivo* so as to create either a living cell substitute or some other type of cell-based therapy.

Finally, there is the potential of human embryonic stem cells. This is an exciting area, and one where advances are reported regularly. Still, there is so much that we do not know. This is certainly true when it comes to our knowledge of the signals that drive the differentiation of a stem or progenitor cell into a specific type of differentiated cell, one with a very specific phenotype. Furthermore, there is the concern that the cues *in vivo* may then induce tumorgenesis in highly proliferative undifferentiated cells (14).

Once one understands the basic biology and moves beyond that, in moving forward to larger scale production, there are issues with both an autologous and an allogeneic product concept. For an autologous implant, a protocol that consistently and sufficiently recruits only the appropriate cells becomes necessary. For an allogeneic implant, the scale up and the expansion of cells, while maintaining the appropriate phenotype is critical. How will this be done? What type of environment will be needed in a bioreactor to optimize this process? How will one be able to ensure quality control in any scale-up process? These all are important issues that need to be addressed.

The matrix

To mimic native tissue requires a three-dimensional structure, and one way to achieve this is by seeding cells into/onto a scaffold. This scaffold could be biological, made of a synthetic material, e.g. a polymer, or be some type of hybrid, and there have been several excellent reviews on scaffold technology (15–18). If the cells are the key to harnessing the biology, then they will need the right cues. In some ways tissue engineering/regenerative medicine can be viewed, as a remodeling problem and the state of the matrix will dictate this remodeling. Matrix properties, such as composition, architecture and biocompatibility, control the remodeling, both by influencing the donor and recipient cells/tissues. In addition, as the field of biomaterials has advanced, other sophisticated scaffolds, some affixed with growth factors (19) or specific peptide sequences (20) and others designed to support a multi-cellular system (21), provide even greater possibilities for influencing the microenvironment of the cells, and thus the type and timing of the remodeling. It is for this reason that at the 2004 GTEC Hilton Head Workshop there was a focus on the matrix, including the related issues of tissue growth and the integration of the substitute into the surrounding, connected tissue. Due to the long-term implications of implantation, many there expressed that there is a need to move away from synthetic scaffolds to more biologic scaffolds. Alternatively, if one needs to use a synthetic scaffold, it should be as short lived as possible; although it must maintain its viability long enough for the cells to make their own matrix.

Once one has a scaffold and has selected the cells to be employed, then the issue is what will be the environment that will foster the growth and/or conditioning of the tissue substitute. Bioreactors can provide both chemical and mechanical signals that allow for optimizing the development of the substitute. Recognizing that a tissue substitute can be remodeled *in vitro* using a bioreactor, one must also realize that once implanted there will be additional remodeling that takes place *in vivo*. Thus, one's goal is not to generate an implant *in vitro* that mimics the final tissue, but one that meets the criteria needed upon implantation for long-term success. Unfortunately, in many tissue-engineering cases, these criteria are not known. Taking tissue-engineered cartilage as an example, a tissue substitute where the mechanical properties are all important, should the cartilage grown in a bioreactor be 'matured' to the point where its mechanical properties are virtually identical to those of adult native cartilage? Alternatively, if integration with the host and *in vivo* remodeling are more important, then perhaps at the time of implantation the tissue-engineered cartilage may need to be more like that of developing young cartilage. Thus, clearly there is an open question as to when in the growth process the engineered tissue

should be transferred from the *in vitro* bioreactor to the *in vivo* 'bioreactor,' i.e. the body.

Immune acceptance

If allogeneic cells are to be used in order to achieve off-the-shelf availability, then the issue of immunogenicity needs to be considered. This will of course depend on cell type. Even with stem cells one must assume that when such a cell becomes fully differentiated into a specific cell type, then it should exhibit all the characteristics of that cell type, including the expression of those molecules that lead to immune rejection.

This does, however, raise the following question. Are the cells associated with a particular tissue engineering strategy there only for a transitional period, one that is very transient, or is the intent that they be there long term? If only there are for a transient period, then any immunogenicity of the cells may not be particularly important.

On the other hand, if there long term, then there may need to be a strategy for the engineering of immune acceptance, this of course depending on the cell type employed. One could of course employ the kind of immunosuppressive drugs used in organ transplantation; however, this approach has never been desirable. It is for this reason that the transplant immunology community has been actively doing research focused on developing other strategies. The tissue engineering/regenerative medicine community is fortunate to be able to take advantage of the advances being made in transplant immunology for the two cases: both can be viewed as allogeneic cell transplantation. In the one case it is the transplantation of a tissue or organ containing allogeneic cells, while in the other the allogeneic cells are extracted, combined into a substitute, and then implanted. In both of these cases, however, what is being done is allogeneic cell transplantation.

One progressive strategy under development is a chimeric approach using multi-potent or pluri-potent stem cells. In such a chimeric approach, from a single donor one normally harvests bone marrow and also takes the organ to be transplanted. One then implants into the patient both the bone marrow as well as the organ (22). This creates the chimerism. The 'donor' however, could be a stem cell source that is used to produce hematopoietic cells to be implanted into the bone marrow and also used to create the differentiated, tissue specific cells needed for the tissue/organ substitute. Whether an approach like the chimeric one represents what we will be doing in the future remains to be seen. What is clear, however, is that research in immunology and the strategies addressing the immunogenicity of tissue engineered substitutes need to be further developed.

From benchtop to bedside

As important as the advances are that are being made in the research laboratory, this represents only a start in moving from the benchtop to the bedside. A successful tissue engineered implant will also need to address manufacturing, economic, and regulatory issues.

It is one thing to make one of a kind of something in a research laboratory; it is quite different to make a 1000 per week with the reproducible quality that would be required to obtain FDA approval. In most inert implants the final attributes are the most salient in defining the product. However, with a living cell product precise characterization is difficult, so the process of formation needs to be critically controlled. This becomes particularly difficult to translate when scaling up production from a few to many, as required for off-the-shelf availability. For example, certain problematic areas arise with the testing of the initial cells and the growth/handling of large volumes of cells, such as maintaining sterility and phenotype. Here the field of bioreactor design needs to take both a basic science and applied engineering approach to meet the needs of cell expansion and tissue production, while preserving the vital biological attributes of the product. Whereas generating large cell numbers can be problematic, generating large volumes of another raw material, the matrix, may also be difficult. As matrices for engineered tissues become more specialized, high throughput production will require the development of new manufacturing techniques.

Once the product is being manufactured, additional hurdles include quality control of the engineered tissue. Advances in cellular and molecular techniques now allow us to assess biological parameters with great sensitivity. However, our ability to detect biological changes is much greater than our understanding of the significance of those changes. To date, setting the

specifications for quality control of engineered tissues has focused mostly on maintaining manufacturing consistency. However, for the long term success of the field of tissue engineering, a better understanding of the mechanism of action is needed, these then to be used to set product specifications.

Ultimate success of a tissue-engineered product is also dependent on its product market. This is influenced by its novelty, accessibility, cost, and the market competition. In the field of tissue engineering, many products will emerge for which there is no suitable alternative. Clearly, these products have the potential to have a great impact. However, certain factors can mitigate that impact. Accessibility of the product is very much dependent on the clinical skills required to apply the product. For example, if extensive training are required for application, this would slow down, or even block, the assimilation of the product. With the various areas of expertise within medicine, it is important that the clinician have the appropriate skills for the product application. In addition, it is important that the value of the product justify its cost. This product value is considered in terms of its efficacy, the nature of its application, and the market competition. For example, no one would pay $1000 for a tissue-engineered bandage that would heal a paper cut perfectly when they could buy a sufficiently suitable synthetic bandage for less than $1. Overall, it is important when designing a tissue-engineered product that one considers the clinical and economic factors that will influence its use.

The nature of the tissue engineering industry has changed over the last few years. Between 2000 and 2002, the number of companies increased while the number of employees and the annual spending in tissue engineering substantially decreased (Table 1). As there is yet to be a single, truly profitable engineered tissue product, many of the early pioneering small companies have disbanded, refocused or drastically cut back. More recently, larger more established companies in the medical implant industry are starting to invest in this field. Since the private sector provides much of the funding for tissue engineering, this changing of the guard has resulted in a significant change in the landscape of the field. One analysis of the field categorizes companies as having structural, cellular, or metabolic product approaches (Table 1). Over the last few years, there has been a refocusing of efforts away from structural and towards cellular approaches. The advent of many stems cell-based companies, as well as the restructuring of two prominent companies with structural product approaches, accounts for most of that change.

Much of the shift in focus in tissue engineering over the last few years is due to the hurdles and problems faced by the early pioneering companies. From their efforts has emerged the need to take a more basic science approach to understanding engineered tissues. However, many practical issues have arisen as well. Many of the early companies did not accurately assess the size of their market, account for regulatory delays from the FDA, or take into account the importance of reimbursement approval from CMMS. The time to market of an engineered tissue concept most likely is more than a decade, outlasting a typical economic cycle. Thus, for a company to have long term success in tissue engineering, they need to develop a sound strategic and business model that will provide for short, mid- and long-term success.

In regard to obtaining FDA approval, the cost of clinical trials has risen significantly. The types of products that will come out of tissue engineering and regenerative medicine are ones that will be of a combination nature. By that is meant a product or strategy that may involve surgical implantation like a device and yet also have biologic activity or perhaps be a drug. Within the structure of FDA, an organization that categorizes products as a device, a biologic, or a drug, how is such a combination product to be regulated? To which part of FDA is it to be assigned? What is the regulatory pathway or process for such a combination product?

This is something with which FDA has been struggling, and there now is an Office for Combination Products. This office, however, does not have regulatory authority; it has mainly a coordinating, oversight role. There needs to be some changes made, and even though there are groups working to foster such changes, we still have a long way to go. In the end, we need a separate, timely and predictable regulatory process for combination products.

Concluding discussion

The field of tissue engineering/regenerative medicine has enormous potential, and in the area of science and

technology, exciting advances are being made. Even so, if this emerging area is to impact patients, sooner rather than later, we need to accelerate our efforts. This can be achieved through a national initiative. Such an initiative should include as a minimum the following four components:

- Increased funding for research.
- Support for early stage product development.
- A redesigned regulatory process that accelerates bringing new technology to patients.
- A more patient 'friendly' reimbursement process.

Each of these four components is necessary if the potential of tissue engineering/regenerative medicine is to be realized. It is not enough to be out in front in the area of research, the science needs to be translated into enabling technology and this in turn into products. These products or strategies then will need to go through clinical trials, receive FDA approval, and then be approved for reimbursement. This is a time-consuming process, too time consuming. For example, it can take an additional 18 months after regulatory approval from FDA before reimbursement is approved.

It should also be noted that there are other parts of the world that are aggressively pursuing tissue engineering. This includes parts of Europe and such countries as China, Japan, Korea, and Singapore. While in the US much of tissue engineering is funded by the private sector, in Europe and Asia funding comes primarily from the federal governments. Together with less stringent regulatory processes, and in some cases more permissive legislation (in particular with regards to stem cell research), the tissue engineering industry is increasingly found outside of the US (Table 1) If the US is to retain its leadership position, it must implement a national initiative with the four components proposed earlier.

In summary, the advances in the science and the technology have been and continue to be exciting; however, the industry is still very much a fledgling one. To date, tissue engineering has been over promised, but undelivered. Still, there is tremendous potential, a potential that, if realized, could dramatically alter the practice of medicine, as we know it today.

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