Tissue Engineering

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INTRODUCTION

There is little doubt that tissue engineering is a revolutionary addition to the therapeutic armamentarium of medicine. The dilemma of adequately repairing either failing or traumatized organs has been looming larger as patients either become older or are in dire need of grafts. Compounding some of the intrinsic problems of transplantation is the chronic shortage of tissues and organs. Tissue engineering allows the hope of a regular creation of spare parts for the human body. This is a most significant approach to reconstruct, replace, or repair organs in a way that could not be foreseen 25 years ago.

Reconstructive medicine is, in a way, not a very recent concept. If one stays away from punctilious definitions, one of its forms, reconstructive surgery, has been practiced for quite some time, with a surge of development after the Second World War. In 1970s, the development of microsurgery allowed distant tissue transfer and reimplantation.[1–5] Since then, the introduction of various biomaterials has allowed vast and diversified types of reconstruction of the human body. Vascular grafts and prosthetic articulation are two prominent examples.[6]

However, tissue engineering does open a radically new chapter in reconstructive medicine, for it is now deemed possible to reconstruct in the laboratory human living tissues and organs for either in-vivo, ex-vivo, and even in-vitro applications.[7–13] This new domain of biotechnology is remarkably multidisciplinary, bringing together cell and molecular biologists, biochemists, engineers, pharmacologists, physicians, and others.

When the aim of tissue engineers is to obtain grafts for in-vivo applications, then the biological and mechanical functions are of utmost importance. In some subdivisions of the field, one can essentially choose between a biological function, as in cell therapy, and a principally mechanical function, as in the use of tissue templates[14] (Fig. 1).

Tissue-engineered substitutes are three-dimensional reconstructions that can be implanted into the human body, leading to rapid host integration and acceptance. These substitutes must have at least minimal biological and mechanical functions for such a reparative role.

HISTORICAL PERSPECTIVE

Even though the field of tissue engineering is relatively young, it has already enjoyed a fascinating evolution over the last quarter of a century. Tissue engineering has also been considered one of the most influential new technologies for the future of biomedicine.[15–17]

The development of tissue engineering can be seen as having two phases. The phase of exponential development and potential application is still continuing to evolve. But it seems reasonable to also identify a second phase, brought about by a flurry of discoveries about stem cells. Even though these cells had been known of for many years and had certainly been involved in many tissue-engineered efforts, some very important aspects of embryonic stem cells were revealed. Indeed, the isolation and stable culturing of either the totipotent or germinal stem cell was a pivotal breakthrough.[18–20] In parallel, adult stem cells were found to be much more ubiquitous and to have more lineage plasticity than previously thought.[21]

Since 1998, these discoveries have brought a tremendous amount of energy and expectation to the field of tissue engineering and reconstruction. Indeed, their therapeutic potential since then has been estimated to be greatly enhanced.

APPROACHES TO TISSUE-ENGINEERED SUBSTITUTES

The different approaches to tissue-engineered substitutes involve deceptively few elements: cells and various natural or artificial matrices combined in such manner to obtain a tissue substitute. These tissue substitutes can then in turn be sequentially integrated to create more complex organs (Fig. 2).

Three main approaches are being utilized for tissue engineering. The first is the seeding of cells into various gels. The seminal work by Dr. Eugene Bell and collaborators has set the stage for various applications of such an approach with collagen gels.[11] Previous work had already indicated that cells could be cultivated on different collagen gels at times with incorporation into the depth

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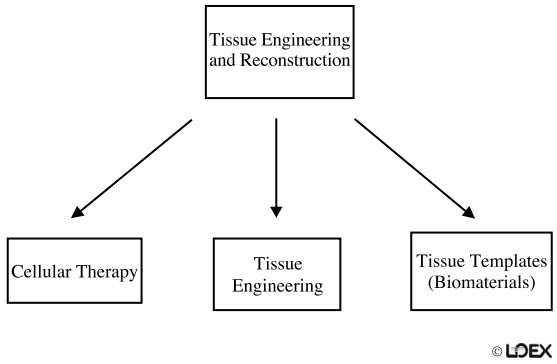

Fig. 1 Overview of the different approaches leading to tissue engineering and reconstruction.

of this matrix.[22,23] However, the true integration of cells into gels allowed them to reorganize the surrounding matrix.[12,24–30] A few investigators are pursuing this technology with either collagen gels or other matrix components such as fibrin.

Even though this approach allows the creation of many substitutes and can recreate excellent in-vitro models, it has a few drawbacks. The main one is the weak mechanical resistance of the obtained substitutes. The structural integrity may be sufficient for such tissues as skin, but not so for substitutes in the vascular or orthopedic system. This problem has been addressed with some original solutions, such as glycation and magnetic alignment of the collagen fibers.[31–33] It remains to be seen if these modifications are truly applicable to a clinical setting.

A second approach entails the seeding of cells into scaffolds. These scaffolds can be of a natural or biosynthetic origin. The cells then thrive in the porous material and secrete various amounts of extracellular matrix depending on their nature. Much of the original concept was developed by Dr. Robert Langer's group at MIT.[34] It also builds on the prior work of Dr. Burke[35,36] and Dr. Odile Damour,[37,38] with the creation of sponge-like structures from mainly collagenous material. Most of the MIT work has centered around synthetic scaffolds such as PGA [Poly(glycolic acid)].[34] There have been countless modifications and additions to the different types of synthetic materials used over the last decade in this approach of tissue engineering. The obvious advantage of this approach is the immediate creation of a three-dimensional structure that already has significant structural properties. However, the intrinsic nature of most of these polymers, which are suture materials, entails slow degradation with an ensuing lowering of the pH of surrounding tissues. This leads to a slow but rather protracted low-level inflammatory process. Many groups are thus attempting to alter the chemical nature of these biomaterials for not only better tissue acceptance, but also better control over the resorption time frame and acidic conditions. These efforts would inhibit the inflammatory process and ensure that the disappearance of the material is synchronous with the integration and constructive phase of the graft. How successful these attempts are will be apparent within the next few years.

More recently, the LOEX group in Canada has developed a different approach to tissue engineering. It has some roots in the very initial phases of in-vitro tissue reconstruction as exemplified by Dr. Howard Green with cultured epithelia for burn patient therapy,[39] combined with many observations about the reorganization of the extracellular matrix in various substitutes. In this approach, various types of cells, mostly of mesenchymal origin, are grown in such a fashion within a culture flask that they literally embed themselves in their very own extracellular matrix. Among many factors, the addition of sodium ascorbate allows the significant appearance of the various components of the extracellular matrix. These sheets are then either stacked or rolled to obtain various tissue substitutes.[17] The most convincing demonstration was the re-creation of a totally biological vascular substitute. The final substitute was not only three-dimensional and three-cellular, but also had very valuable mechanical and biological properties. The main advantage in this approach is the absence of extraneous collagens and any synthetic material.[40] This method is truly close to some of the in-vivo processes found during organogenesis in utero.

This team has also championed for many years the use of self-generated mechanical forces with mechanical stresses also applied to these substitutes. This combination not only leads to a significant in-vitro cellular and extracellular matrix reorganization, but also achieves a physiological final result. It is felt that such substitutes could

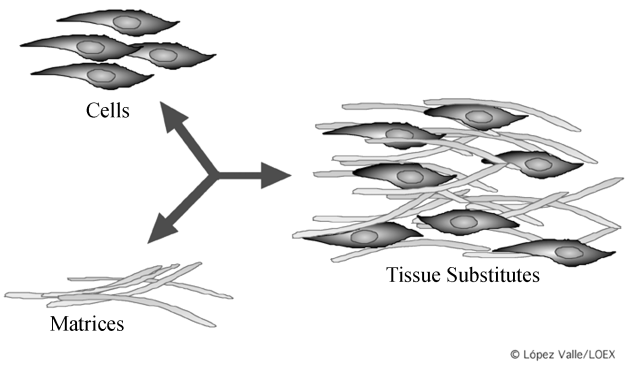

Fig. 2 Simplified illustration of the components necessary for tissue engineering. (*View this art in color at www.dekker.com.*)

be grafted with minimal inflammatory response, but also with a rather unique state of readiness for in-vivo integration and remodeling.

However, one must also note that this approach is time-consuming. Thus, it will demand some form of automation and acceleration of maturation time for the culture of these substitutes. On the other hand, they can be considered, at the present time, as the purest form of in-vitro models for various physiological, pathophysiological, pharmaceutical, and toxicological studies.

CRITICAL INTEGRATIVE ASPECTS OF TISSUE-ENGINEERED SUBSTITUTES

As tissue engineering enters the clinical area in a more and more significant fashion each passing year, some very critical aspects of tissue integration must be addressed.

Two of these critical aspects, namely vascularization and innervation, have not received all the attention they deserve. This may be due to the fact that most of the efforts leading to clinically applicable substitutes centered at first on skin grafts. Then, because of their very nature, the animal experiments did not reveal the need to take into account these two important aspects of tissue integration.

In a way, this may be seen as quite surprising since reconstructive surgery devotes a lot of attention to the revascularization and reinnervation of autologous or allogeneic grafts. This is evidently less true if a graft has a nearly exclusively mechanical function such as an aortic synthetic graft or hip prosthesis. But even then some surgeons and scientists have pointed out that some biological responses to the graft should be enhanced for a better integration. As an example, the team of Dr. Zilla has extensively studied and optimized the seeding of endothelial cells on the internal side of vascular grafts.[41] The field of tissue engineering is now much more attuned to the necessity of responding to such a challenge.

In regard to vascularization, even with a paucity of results at first, the approach of stimulating the ingrowth of blood vessels into solid organs has not been successful. Such organs rapidly, within hours or even minutes, demand blood irrigation for survival and proper function. This necessity was the basis for a new endothelialized skin substitute developed at LOEX.[42] Drawing on clinical lessons showing that cadaver skin could rapidly take and demonstrate a capillary blood flow, the team of Dr. Auger has strived to recreate a microvascular system in the dermal component of skin substitutes. Such a design was even more plausible with understanding of the phenomenon that allows the rapid take of cadaver skin in burn patients. Inosculation, the physiological anastamosis between blood vessels of the graft and the wound bed, was only recently clearly described in an animal model.[43] Thus, at first using a scaffold technique, this group has combined in the dermal layer fibroblasts and endothelial cells in such a fashion that a capillary-like system was reconstituted. A series of analyses, including histology, immunohistology and electron microscopy, has shown the microvascular nature of this endothelialized skin equivalent. Furthermore, preliminary grafting experiments in animals have shown that blood flow within the graft is reestablished at a faster rate than in substitutes without a capillary-like system.[44]

There is now a flurry of interest in such microvascularization in various soft tissues.[45] But the next level of complexity will undoubtedly obtain for solid organs the full spectrum, from small arteries to capillaries and finally outflow small-diameter veins. This certainly is a lofty goal and will necessitate a tremendous multidisciplinary effort.

Of a less immediate nature is the matter of tissue substitute reinnervation. Too little attention has been focused on this element if the final goal is full tissue integration. Most tissues have many types of neurological receptors that play an important role in not only the biological function but also ultimately their very own homeostasis. Thus, these receptors allow the body to receive the appropriate messages, from nociception to positional information, which then translate into local and frequently systemic reactions. This loop is an integral part of the true physiological function we are hoping for in order for tissue-engineered organs to obtain their full potential. Not only will the appropriate neurological signals be sent by the grafted tissues, but many physiological responses will ensue or be set in motion, such as vasoreactivity, hormonal secretion, and enzymatic delivery.

Until now efforts have been mostly centered on evaluating the reappearance of the neural network in various tissues. Once again, the most studied tissue has been the skin. Early experiments have shown the level and time schedule of pain perception reappearance in the first generation of epidermal tissue grafts for burn patients.[46,47]

Our LOEX group has now focused its observation on some reconstructed tissues such as full-thickness reconstructed skin substitutes for reinnervation after animal implantation. The results are quite encouraging.[48] But once again the challenges will be much more complex in large solid organs with various cell types. This is even more true when there is a specialized nervous tissue within the organ with a precise physiological function, such as the cardiac pacing system.

Another layer of complexity is added when one considers secretory or filtrating organs such as the pancreas, liver, or spleen. The successful three-dimensional arrangement of such organs is at the present time a

daunting task, but the future may hold answers that are not yet foreseeable. Some investigators have targeted embryonic stem cells as an answer to such complex issues. It remains to be seen if the science of tissue engineering will ever find the right condition for such a demanding differentiation program to occur ex vivo.

ENABLING TECHNOLOGIES

Some technologies can be seen as being of incontrovertible value on the path to better tissue engineering. We shall review a few of them.

Tissue Morphometrics

Some scientists in the field of tissue engineering having noted the lack of quantitative and qualitative tissue values and analysis and have proposed measures to remedy this perceived shortcoming.

With an appropriate database for various morphometric tissue parameters, the science of reconstructive medicine should be more accurate and lead to better results. This ongoing effort is certainly quite interesting, and its impact should be felt in the near future according to its proponents.

New Biomaterials

The search for innovative biomaterials seems to be developing along two pathways that will probably intertwine. The first avenue is a worldwide effort, already alluded to, in searching for biomaterials that have better integrative properties. This entails a minimal inflammatory reaction to such materials combined with optimal resorption time depending on the targeted tissues or organs. One must note that inflammation will always be present since any surgical operation entails such a reaction. However, it is quite probable that the least reaction, the better in this context.

The second avenue is to attach signaling molecules to the utilized biomaterial. This can be as direct as a slow, sequential release of various cytokines or growth factors. However, the more technically sophisticated approach of adding DNA sequencing for appropriate messaging may be of high value for all those seeking to induce favorable integration of these biomaterials.[49]

Mechanical Stimulation

In the last decade it has become quite clear that nearly all body tissues can attain a higher level of complexity by being submitted to mechanical stimulation.[12] Although such a concept was rather evident in the orthopedic field, where load bearing is known to be of great importance in tissue differentiation, it also turned out to be valuable for other tissues, such as blood vessels, heart valves, and skin.[26,32,34]

Bioreactors

If one wants to combine the previous technology with any significant scaling-up process, then bioreactors are a pivotal step in tissue engineering. A great part of the answer lies not only in obtaining large amount of cells, but also exposing them to the appropriate mechanical conditions.

Furthermore, this is a critical pathway for automating at least some of the processes involved in recreating tissue substitutes.

Gene Therapy

If the combining of DNA into biomaterial has already been established, one must look upon gene therapy as an additional and more permanent technology for obtaining better tissue-engineered organs.

For example, in re-creating the pancreas, at the present time a high number of Langerhans cells are necessary for an appropriate level of insulin secretion. If these cells were amenable to higher levels of secretion, this would be of great advantage in the treatment of diabetic patients.

Another example is reconstructed blood vessels with anti-atherosclerotic molecules secreted by either smooth muscle cells or endothelial cells. Here, the patient most prone to restenosis could benefit from a superior type of graft. However, this technology brings these substitutes into an entirely different regulatory environment. It may be a step that will be valuable, but the time frame of such a step is difficult to predict today.

This does not deter many groups working on various types of ''universal'' donor cells. Such cells, devoid of specific immunological markers, usually targeted in rejection, would allow the creation of well-tolerated substitutes. But once again, this task may entail more complexity than meets the eye.[50]

MULTIDISCIPLINARY TEAMWORK IN TISSUE ENGINEERING

It seems to us that the best way to meet all these challenges is to combine various types expertise that have not always been tied together in the past. Many regional and national programs in tissue engineering are addressing this issue.

The LOEX group has always brought together a triad of specialties into our tissue engineering efforts:

- Cell and molecular biologists
- Bio-engineers
- Biomedical clinicians

When we set about the project of re-creating ligaments, the orthopedic surgeons declared the anterior cruciate ligament to be of the utmost clinical importance. They also gave the team a sense of alternative therapies and what additional advantages a tissue-engineered substitute should bring. This enabled the team to reach a set of biological and mechanical criteria for each substitute. This combination has proven to be quite fruitful in forging ahead with tissue engineering projects that are clinically focused. Thus, as a first step the clinician investigators help the whole team set the stage in pinning down the most significant goals to be achieved for a particular substitute. This interaction helps in determining the most acute need in tissue reconstruction for any body system. Then the cell biologists strive to reconstruct the organ or tissue with what is deemed to be the best tissue engineering approach. The pluses and minuses of each approach have already been presented and will, in a sense, dictate the best fit. One of the most crucial roles of the cell biologists at this point is to obtain the various cells necessary for the reconstruction. Their challenge is not only to find appropriate sources of cells, but also to establish efficacious extraction methods. The purity of the cells is of paramount importance if the tissue engineering effort is to be successful.

Afterward, the expansion of cells must be efficacious enough to obtain the amount of cells and the appropriate quality necessary for the reconstruction. The cell and molecular biologists will have also settled on some functional biological requirements for the tissue-engineered organs. This may be a cellular function such as the antithrombotic properties of endothelial cells or an exocrine secretory function such as insulin for pancreatic Langerhans cells.

The role of bio-engineers is also of great value. They frequently can create or refine bioreactors to facilitate and accelerate the expansion of the necessary cells. They maintain a close relationship with the biologists so that the processes are not deleterious to the cellular phenotypes and function. But usually at the inception of a project, the biomechanical engineers are in close interaction to create different types of apparatus that will induce the appropriate mechanical strains in the reconstructed tissues. These strains may appear passively or be induced by machines that, for example, bring pulsatile flow to a reconstructed blood vessel. There is now clear evidence that such mechanical preparation is advantageous not only for the structural integrity, but also for some functions of tissue-engineered substitutes. Many cells have been shown to acquire their in-vivo phenotype when exposed in vitro to strains similar to those encountered in the human body.[12,30,34,51]

All this multidisciplinary work forms a working loop of interaction between teams. This is quite understandable, as advances in each sector have some bearing on the other members of the team.

There is also a two-step paradigm to our efforts in a given tissue engineering project. The first step, while taking into account what has been previously described, aims at recreating a three-dimensional structure that will have a satisfactory histological aspect. However, the biological and mechanical properties are not the main purpose. The team strives to demonstrate the possibility of assembling the various cells and tissues to form an organ that will have acceptable tissular organization and enough stability to maintain it in culture. The second step is optimization of the desired mechanical and biological functions. This leads to an intensive collaboration between the biologists and the engineers.

This two-step paradigm can be well observed in our own effort at recreating a tissue-engineered blood vessel (TEBV). Our first publication, using the self-assembly approach to tissue engineering, had allowed us to re-create all three layers of a blood vessel.[40] The histological analysis revealed well-defined adventitia, media, and intima with no crossover contamination from the different cell types between layers. Furthermore, a few very important functions were shown to be present, such as incorporation of acetylated LDL by endothelial cells and antithrombogenic activity.[51] But no vasoreactivity was noted, and the mechanical resistance was very weak. This TEBV was thus not regarded as acceptable for grafting. The second generation, utilizing the self-assembly approach, answered those pitfalls in an interesting manner. The vasoreactivity of these vessels was clearly established as their supraphysiological burst pressure settled around 2500 mm Hg.[40] This is a fitting example to a stepwise road to the completion of a tissue-engineered organ in vitro. Thereafter the role of the clinician is of paramount importance in creating protocols for grafting these substitutes first in animals and then into the human host.

CONCLUSION

Tissue engineering has opened fascinating perspectives in the biomedical armamentarium. The astounding clinical demand for such biotechnological solutions ensures that this research domain will continue to forge ahead.

On the other hand, the road to success has also a few meanders that should be carefully addressed if this field is

to keep its pace. There is a tremendous level of trepidation related to the expanding knowledge of both embryonic and adult stem cells. The question surrounding the ethical acceptability of using embryonic stem cells is already partly out of the scientists' hands. Various nations will have to define the boundaries of acceptable utilization of embryonic stem cells.

Next we will have to solve the important aspect of host acceptance of the tissue-engineered graft. There is no problem for an autologous construct; however, allogeneic grafting brings about the eventual problem of rejection. If tissue engineers want to stay clear of the immunosuppressive drug used in classical organ transplantation, new solutions will be necessary. The two main efforts have, up to now, aimed at either rendering the graft less of a target or inducing tolerance in the receiver. Here, tissue engineers are accompanied by all the transplantation community, thus heightening the possibilities of successes.

Finally, the regulatory aspect will eventually become clearer. The examining agencies in many countries are responding to these new therapies. However, the channel of communication must be well established between regulators and tissue engineers to ensure a safe but not too constraining introduction of this form of therapy into the clinical arena.

Whatever lays ahead for tissue engineering, it is already clear that this field will alleviate much suffering and improve life for many patients around the world.

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