Peer reviewed article

Biomaterials-based tissue engineering and regenerative medicine solutions to musculoskeletal problems

Myron Spector

Tissue Engineering, VA Boston Healthcare System, and Orthopaedic Research Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Summary

Tissue engineering and regenerative medicine offer solutions to a number of compelling clinical problems that have not been adequately addressed through the use of permanent replacement devices. The challenge will be to select the optimal combination of a biomaterial scaffold, cells, and soluble regulators for a particular clinical problem. For many connective tissues of the musculoskeletal system, with microstructures that reflect the mechanical environment, it may be more advantageous to regenerate the tissue *in vivo* than to fully engineer the tissue *in vitro* for subsequent implantation. The porous material to be used as the scaffold to facilitate this regeneration needs to have certain pore characteristics, chemical composition and mechanical properties. One approach has been to employ substances that serve as analogues of the extracellular matrix of the tissue to be regenerated. For selected indications in which the supply of endogenous precursor cells is limited it may be more efficacious to employ a scaffold as a delivery vehicle for the cells rather than to inject the cells into the defect. Investigations of cell-scaffold interactions *in vitro* not only offer the opportunity for modification of scaffold composition and structure to improve the outcome *in vivo*, but also offer the opportunity to discover cell biological behaviour when cells grow in the three-dimensional tissue-like environment. Selected clinical applications may also require the implantation of regulatory proteins such as growth factors. That the action of such polypeptides released from biomaterials is short-lived has led to recent work wedding tissue engineering and gene therapy. Genes can be bound to certain biomaterial scaffolds to be released *in vivo* over extended periods (eg weeks) in order to genetically modify cells in the defect to produce the desired growth factors. Thus a new role for biomaterials is as a delivery vehicle for genes, as well as for cells and growth factors. These endeavours are notable particularly because there is a growing consensus that the challenge of developing biomaterials for tissue engineering, regenerative medicine, and gene therapy exceeds the challenge that was faced in the cell biological work that led to the proliferation of cells *in vitro* (in such a way that they retain their phenotypic characteristics) and in the genetic engineering that has led to the production of growth factors and cloning of their genes.

Key words: tissue engineering; regenerative medicine; biomaterials; scaffolds; cells; collagen

Introduction

The term "tissue engineering" has now come to encompass a wide range of strategies employing cells, synthetic and processed natural materials, tissues, cytokines and genes for the regeneration of tissue *in vivo* or the production of tissue *in vitro*. Cell therapies and tissue transplant procedures are thus now often considered under the rubric of tissue engineering. In this respect tissue engineering is not so much a revolution in reconstructive surgery but part of the evolutionary process that this discipline has continuously undergone since its inception over 100 years ago.

While tissue engineering investigations have generated promising results, it is important to point out that no "tissue engineering" procedure, or any other treatment, has yet been successful in fully regenerating a tissue that does not have the capability to spontaneously regenerate (eg bone). One such tissue that does not spontaneously regenerate is articular cartilage. It has been known for many years that the long-term function of articular cartilage is related to its composition and architecture, and to the associated mechanical properties. Even relatively small defects in the

This work was supported by the U.S. Department of Veterans Affairs and Geistlich Biomaterials, Wolhusen, Switzerland, which also provided the type II collagen material.

articular surface (figure 1), which can commonly occur, will not heal with a tissue resembling articular cartilage, and in some cases no reparative tissue will form in the defect. Left untreated, such defects can extend themselves, eventually (over decades) resulting in degeneration of the entire joint. A promising observation, however, has been that under certain circumstances – sometimes those occurring in an untreated cartilage defect – regeneration of articular cartilage can take place, albeit only in a region of the lesion. This indicates that articular cartilage regeneration is a possibility. The challenge, then, is to identify the elements of the regeneration process that need to be supplied to a particular defect: cells, matrix, cytokines, or a combination.

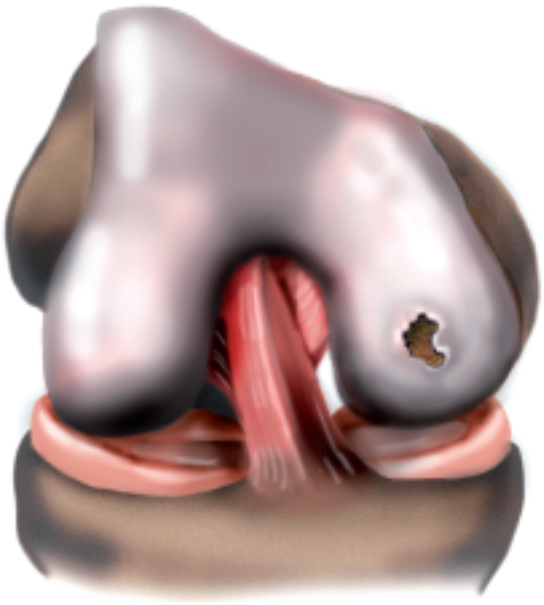

Figure 1

Sketch of a human knee joint showing the type of defect in the articular surface that can commonly occur. Healing of the defect will not result in the formation of articular cartilage, and in many cases no reparative tissue will form in the defect. Such defects in avascular tissue with limited potential of the host cells to participate in a reparative response represent clinical challenges that are addressed by tissue engineering.

It is also important to recognise that despite the absence of full regeneration of a tissue such as articular cartilage, many patients report a dramatic relief of pain as a result of certain treatments. This has raised fundamental questions about the criteria for success that should be adopted in the evaluation of new procedures. For example, a clinically meaningful outcome might be one that provides pain relief for 5 years, and this may be achieved through the formation of a tissue that falls short of replicating the composition and structure of articular cartilage. Thus, is it more appropriate to use histological or clinical criteria for success of a new tissue engineering procedure? The problem with using the clinical endpoint is that patients may report a relief of symptoms for a few years only to experience a precipitous decline in their condition. There is no reason to expect that there would be a gradual decline in function that signals potential problems and thus allows adjustments in the procedure or for it to be abandoned before large numbers of patients are operated on. Fundamental questions thus remain as to how to gage the success of a new tissue engineering procedure.

The scientific basis of tissue engineering

As with any engineering discipline the working goal of "tissue engineering" is the implementation of existing knowledge for the creation of a product – tissue [30]. At the same time, the engineering process often provides opportunities for the discovery of new knowledge, ie the process of science. It is becoming apparent that the unique circumstances related to the growth of cells in three-dimensional scaffolds *in vitro* in the course of tissue engineering are revealing aspects of the phenotypes of a wide variety of cells and insights into cell behaviours that would have otherwise escaped view [30]. In this regard, tissue engineering is likely to contribute important new knowledge to cell and molecular biology, drawn from the advancement of health care through the production of tissue *in vitro* or the facilitation of tissue regeneration *in vivo*.

One unique aspect of tissue engineering science is the investigation of the interactions of cells with absorbable matrices and environmental factors (eg mechanical loading) that relate to the formation of tissue. The cell responses to these interactions include cell proliferation and biosynthesis of matrix molecules. More recently it has been observed that cell contraction is another important aspect of the cell response to scaffolds employed for tissue engineering [30]. As we acquire more knowledge about the interaction of cells with matrices we will better be able to prepare new scaffolds to more specifically elicit the responses from cells that best suit a tissue engineering application.

The challenging engineering aspect of tissue engineering, as with any engineering endeavour, is the judicious use of existing knowledge for the production of a useful product, in this case, tissue. There are so many physical and biological issues related to the production of tissue *in vivo* or *in vitro*, and so few hard facts to guide the engineering process that tissue engineering is much more of a demanding field than other engineering disciplines. Moreover, that the risks of failure include death, greatly increase the stakes of tissue engineering pursuits.

Tissue engineering can now be pursued because of recent advances in enabling technologies related to the tissue engineering triad of cells, matrices, and regulators. Only recently technologies have been developed that focus on the proliferation of cells *in vitro* under conditions that allow maintenance or recovery of the cell phenotype. A critical aspect of most tissue engineering strategies is the expansion of cell number in culture in order to generate the requisite number of cells for the production of tissue *in vitro* or the implantation of cells alone or seeded in matrices for the re-

generation of tissue *in vivo*. Many cell types loose critical phenotypic traits with increasing time in culture. Advances in cell biology allowed the discovery of culture conditions that favour the proliferation of cells while a) preserving their phenotype, or b) recovering lost phenotypic gene expression post-expansion. There have been many advances in the control of culture conditions employed for the preparation of tissue engineering constructs. Some of these new developments have come from the work of Swiss investigators [36], including work employing stem cells [4].

One of the most important technological advances enabling tissue engineering regards the production of the porous, absorbable scaffolds that are required to contain the cells for the production of tissue *in vitro* and or *in vivo*. Synthetic and natural polymers and calcium phosphates have been developed as scaffolds for the engineering of soft and hard tissues. Control of the pore characteristics including pore volume fraction, pore diameter and pore orientation, as well as the chemical composition of the matrix, has played a critical role in the advance of tissue engineering. Another important enabling technology that has had an impact on tissue engineering is the genetic engineering of selected cytokines, such as the bone morphogenetic proteins. These growth and differentiation factors and agents that stimulate biosynthetic activity are playing important roles in efforts to form a tissue *in vitro* and to facilitate regeneration *in vivo*.

Tissue engineering: historical perspective

There are too many investigations that have served as the antecedents of tissue engineering to include in a review. The following provides a brief summary of just a few [3].

Perhaps the earliest successful application of tissue engineering is the implementation of porous collagen-glycosaminoglycan matrices for the *in vivo* regeneration of dermis [39]. This work that led to the term "artificial skin" served as the basis for the subsequent use of these matrices in tubular form for the regeneration of peripheral nerve [38]. The underlying concept was to develop analogues of the extracellular matrix of the tissue to be regenerated. In addition to demonstrating that selected analogues could facilitate the regeneration of tissues that did not have the capability to spontaneously regenerate, these studies showed that tissue-specific pore characteristics (ie pore diameter and orientation) were necessary for the optimal performance. The use of these regeneration templates also revealed the importance of having the degradation rate of the matrix, controlled by cross-linking, match the regeneration rate in a process referred to as isomorphous replacement. Later work in this line of investigation [10] showed for the first time, in a rat model, that an off-the-shelf scaffold could serve better than an autograft in the case of treating gaps in peripheral nerve.

Other early studies [37] that used the term "tissue engineering" investigated the endothelium-like cell layer that formed on the polymethyl methacrylate implants in the eye. Later important work demonstrated the ability of cell-seeded matrices made from a synthetic polymer to form and maintain a viable cartilaginous tissue of a selected shape when implanted in an animal model [11]. These and other studies formed the basis of a review article [18] that established tissue engineering as a distinct discipline.

Tissue engineering versus regenerative medicine

The term "tissue engineering" was initially introduced to describe the technology for producing tissue *in vitro* [18]. More recently the term "regenerative medicine" has been used to describe the development of technology and surgical procedures for the regeneration of tissue *in vivo*. There are advantages and disadvantages to both strategies. One advantage of the synthesis of tissue *in vitro* is the ready ability to examine the tissue as it forms, and to make certain non-destructive measurements to establish its functions prior to implantation. However, a disadvantage, particularly in the production of musculoskeletal tissue that must play a load-bearing role, is the absence of a physiological mechanical environment during the formation of the tissue *in vitro*. It is now well established that mechanical force serves as a critical regulator of cell function, and can profoundly influence the architecture of tissue as it is forming. Because the mechanical environment during the formation of most musculoskeletal tissue *in vivo* is not well understood, it is not yet possible to recreate such an environment *in vitro* during the engineering of most tissues. Another disadvantage of the formation of musculoskeletal tissue outside of the body is the necessary incorporation of the tissues after implantation. This incorporation requires that the engineered tissue be mechanically coupled to the surrounding structures. Union of the implanted tissue with the host organ requires remodelling – degradation and new tissue formation – at the interfaces of the implant with the host tissues. That remodelling of the implanted tissue is essential for its functional incorporation.

Thus, for certain tissues (eg musculoskeletal), an effective strategy may be to facilitate tissue formation *in vivo*, under the influence of the physiological mechanical environment. However, one disadvantage of this approach is that the regenerating tissue may be dislodged or degraded by the mechanical forces normally acting at the site before it is fully formed and incorporated.

In most cases a distinction is not made between tissue engineering and regenerative medicine, with both being referred to as tissue engineering. Just as they are the three components of tissue, matrix, cells and soluble regulators are the elements of strategies to engineer tissue *in vivo*, or *in vitro* for subsequent implantation. Decisions as to which elements might be required for regeneration of tissue *in vivo* can be guided by an understanding of the deficits of the natural (ie spontaneous) healing processes that prevent regeneration.

Scaffolds for tissue engineering and regenerative medicine

For most of the decades of the 20th century, biomaterials have played a critical role in enabling the fabrication of a large number and wide variety of medical implants. Except for a few examples, however, these were permanent devices meant to fix or replace the function of tissues and organs. Stainless steel devices were developed for the fixation of fractures and to fix allografts to host bone. Implants fashioned from metallic, ceramic and polymeric materials facilitated life-saving procedures in many patients (eg vascular prostheses and artificial heart valves) and profoundly improved the quality of the lives of other individuals (eg joint replacement prostheses). Despite these remarkable successes, the new roles for biomaterials in medicine will likely exceed these achievements. The new roles include the use of *porous*, absorbable biomaterial *(sponge-like)* scaffolds in tissue engineering, regenerative medicine, and gene therapy.

Scaffolds for engineering bone and the soft tissues have been synthesised from an array of synthetic and natural calcium phosphates and myriad synthetic (eg polylactic acid and polyglycolic acid) and natural (eg collagen and fibrin) polymers. Scaffolds for engineering tissue *in vitro*, or to be used as implants to facilitate regeneration *in vivo*, need to have a microstructure and chemical composition able to accommodate cells and their functions. In this regard a porous structure is generally necessary. The required porosity and pore diameter, pore distribution, and pore orientation, might be expected to vary with tissue type. The chemical composition of the matrix is important with respect to its influence on cell adhesion and the phenotypic expression of the infiltrating cells. Moreover, because the objective is the regeneration of the original tissue, the scaffold needs to be absorbable. The degradation rate of the material generally may be determined based on the rate of new tissue formation and the normal period for remodelling of the tissue at the site of implantation. Of course, it is important to consider the effects of moieties released during degradation of the matrix on the host and regenerating tissue. Finally, the mechanical properties of the biomaterial employed as a scaffold for tissue engineering are important for providing temporary support of applied loading *in vivo* during the regeneration process and for resisting the contractile forces that may be exerted by the seeded cells prior to implantation and by cells infiltrating the scaffold *in vivo*.

There have been numerous reviews on the characteristics of the biomaterial scaffolds generally employed for tissue engineering [1, 17]. Using a specific biomaterial system (a poly(ethylene glycol)-terephthalate-poly(butylene)-terephthalate block copolymer), Swiss investigators and their collaborators recently provided an example of how the composition and architecture of the scaffold can affect the behaviour of the cells grown in the scaffold [22]. A comprehensive review of biomaterial scaffolds is outside the scope of this article. Rather, the author will draw from his personal experience employing collagen-based biomaterials (figure 2) to address certain issues related to the use of scaffolds for specific tissue engineering applications.

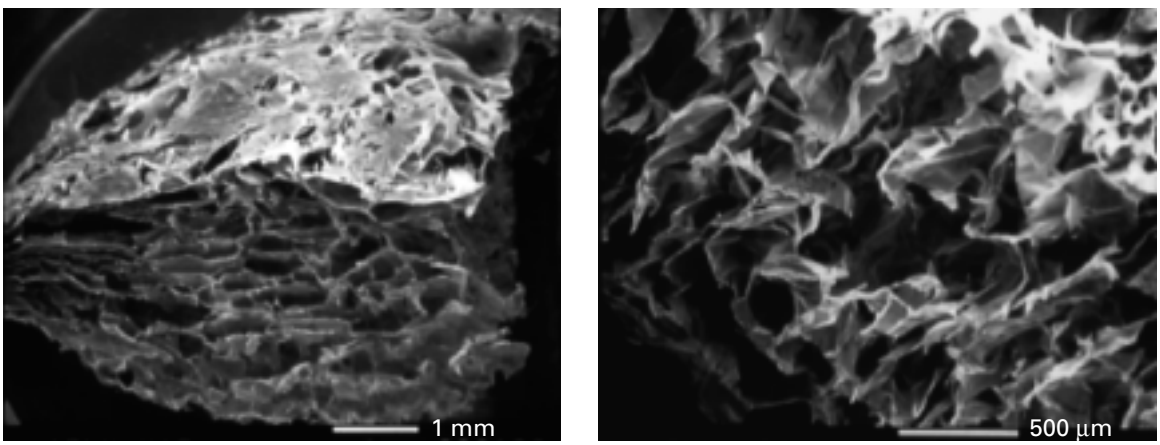

Figure 2

Scanning electron micrograph of a porous collagen-glycosaminoglycan scaffold employed for tissue engineering

Roles of a scaffold in tissue engineering and regenerative medicine

There are many roles that a scaffold can play in the tissue regeneration process:

- The scaffold can serve as a framework to support cell migration into the defect from surrounding tissues; especially important when a fibrin clot is absent.
- Before it is absorbed, a scaffold can serve as a matrix for endogenous or exogenous cell adhesion, and can facilitate/regulate certain cell processes including mitosis, synthesis, and migration. This may be mediated by ligands for cell receptors (integrins), on the biomaterial and/or the biomaterial may selectively adsorb cell adhesion proteins.
- The scaffold may serve as a delivery vehicle for exogenous cells, growth factors, and genes. This activity is enabled by a large surface area for attachment and the possible control of the density of the agents (ie agents/unit volume).
- The scaffold may structurally reinforce the defect to maintain the shape of the defect and prevent distortion of surrounding tissue.
- The scaffold can serve as a barrier to prevent the infiltration of surrounding tissue that may impede the process of regeneration.

The potential role of the scaffold as a delivery vehicle for exogenous cells has become increasingly important in a wide variety of tissues and organs in the light of recent advances in the investigation of cell therapy for local repair. Injection of exogenous cells, expanded in number in monolayer culture, is being studied for the treatment of defects and degenerative conditions in many tissues:

- chondrocytes for the repair of defects in articular cartilage on the surface of joints [8],
- intervertebral disc cells for herniated disc [15],
- stem cells into spinal cord lesions [13],
- myoblasts and stem cells for myocardial infarction [31], and
- cells into the retina [33].

An alternative to injection of cells is implantation a cell-seeded scaffold. As noted above, the large surface area of porous scaffolds allows the delivery of an exceedingly large number of attached cells, and facilitates the retention of the cells at the implant site.

Another potential role of the scaffold is the delivery of genes for selected growth factors [25]. The regeneration of tissue may in some circumstances require the administration of certain therapeutic factors (eg growth factors). For example, selected growth factors, given as a single bolus dose at the beginning of the cartilage repair process, have been shown to accelerate the production of a hyaline-like reparative cartilage matrix [23]. However, none of these growth factors have been able to maintain their effectiveness during the remodelling phase that ensues a few weeks to months after the initial repair procedure. The limited efficacy of the bolus dosing of growth factors may be due to its inherent inability to maintain therapeutic levels of the cytokine for prolonged periods. The transitory effects of bolus dosing of polypeptide growth factors are a consequence of their relatively short *in vivo* half-lives (minutes to hours), the temporal nature of growth factor signalling on cellular differentiation and metabolic function, and the fact that many exogenous cytokines do not stimulate endogenous production.

Transfer of the gene for a selected cytokine to the cells involved in the reparative process using a scaffold as the delivery vehicle is one means of maintaining therapeutic levels of the protein through the later phases of the cartilage repair process [27]. Non-viral vector systems offer the advantages of low immunogenicity, simplicity of vector design, and relative ease of large-scale production [12, 25]. The major disadvantage of this approach is related to the lower efficiency of transfection. However, for some reparative processes (eg articular cartilage) even relatively small amounts of the cytokine produced by a few transfected cells may be of significant value. This approach has provided promising results in recent studies directed toward enhancing bone regeneration using a collagen matrix as a carrier for selected genes [2, 14].

Prolonged release (over several weeks or months) of DNA from an implant is necessary in cases where there is a benefit in transfecting selected cells that only appear at the implant site days or weeks post-operatively, and in which there is a rapid loss of expression in transfected cells or in which transfected cells migrate from the defect site.

In one recent study, porous gene-supplemented collagen-GAG (GSCG) matrices were loaded with plasmid DNA coding for the luciferase reporter gene, and the effects of cross-linking and pH (during gene loading) on release kinetics and DNA integrity were determined [25]. The optimal conditions showed luciferase expression in chondrocyte-seeded GSCG constructs up to 28 days demonstrating continuous transfection of articular chondrocytes throughout the culture period. In a prior study investigating release of plasmid DNA from copolymers of D,L-lactide and glycolide, less than 10% of the DNA remained in the synthetic polymer construct after 28 days in leaching studies performed using Tris-EDTA buffer [26]. Other matrix materials may lend themselves to modification for gene-supplementation for more prolonged release of genes.

Methods for the production of scaffolds and design rationale

Many methods have been used for the production of porous materials to be used as scaffolds for tissue engineering and regenerative medicine. These include a) the manipulation of fibers into non-woven and woven structures [18], b) incorporation of sacrificial pore-forming agents including ice (through freeze-drying [39]; figure 2) and soluble particles (eg NaCl and sucrose), c) self-assemblying molecules (eg certain peptides [40] and collagen-hydroxyapatite composites [21]), and d) solid free-form fabrication.

The underlying concepts guiding the development of scaffolds can be predicated on the selected biomaterial or on the method of production of the scaffold. Examples of biomaterials-based approaches include 1) use of biomaterials that have been frequently used for other implant applications (eg PLA-PGA) [18], 2) treated natural extracellular matrix materials (eg anorganic bone [28]), 3) biomimetics and analogues of extracellular matrix (eg collagen-glycosaminoglycan [39] (figure 2) and collagen-hydroxyapatite scaffolds [21]), 4) biopolymers for nanoscale matrix (eg self-assemblying peptides) [40], and 5) new types of biomaterials designed specifically for tissue engineering scaffolds. Alternatively the driving force for the design of scaffolds may be the precision (computer) multi-scale control of material, architecture, and cells: solid free-form fabrication technologies. This has become possible with the introduction of a wide array of solid free-form fabrication techniques and apparatus [32].

As noted above, one design approach has been to employ materials that can serve as analogues of the extracellular matrix of the tissue to be engineered [39]. This concept recognises that the molecular composition and architecture of the extracellular matrix displays chemical and mechanical properties required by the parenchymal cells and the physiological demands of the tissue. For scaffolds for regeneration of bone, this approach has led to the use of natural bone mineral produced by removing the organic matter of bovine bone [28]. For soft tissue applications collagen-based biomaterials have been employed [39]. There are special issues that need to be considered in the selection of scaffold materials for the engineering of specific tissues.

Investigations of cell-scaffold interactions *in vitro*: contraction of connective tissue cells

Investigations of cell-scaffold interactions *in vitro* can inform the rationale formulation of scaffold composition and structure for improved performance in tissue engineering and regenerative medicine applications. These investigations of cells in three-dimensional scaffolds that may mimic certain aspects of the natural extracellular matrix *in vivo* can also provide insights into, and discoveries of, cell biology. In this respect, studies of the behaviour of cells in collagen-GAG analogues of extracellular matrix (figure 2) have been particularly informative. An advantage of this material system is the ability to alter selected properties through cross-linking: mechanical behaviour, degradation rate, and alteration of ligands for the integrins of cells. Prior work has demonstrated the effects of cross-link density on the mitosis and synthesis of matrix molecules by chondrocytes in type I collagen-GAG scaffolds with increasing cross-link density [20].

Several years ago, in the course of investigations on the behaviour of articular chondrocytes in collagen-GAG scaffolds, the observation was made that the disc-shaped scaffolds were decreasing in size. The reduction in volume did not appear to be due to dissolution of the scaffold. Subsequent histological studies demonstrated a reduction in the pore diameter of the matrices and suggested a cell-mediated process. Because fibroblasts were known to adopt a contractile phenotype as the result of expression of the muscle actin isoform, α-smooth muscle actin (SMA), chondrocytes were examined for their expression of this cytoskeletal protein. This led to a series of findings that adult canine and human articular chondrocytes and many other connective tissue cells and their mesenchymal stem cell progenitor express SMA and can contract [29]. These findings have suggested roles for the contractile behaviour of connective tissue cells in the control of the architecture of the extracellular matrix and in the response of the tissue to injury. The contribution of muscle actin-expressing and contracting connective tissue cells to the process of dermal wound closure has been recognised for three decades. Muscle actin-enabled cell contraction may also be playing important roles in many other connective tissues including those comprising the musculoskeletal system: tendon, ligament, meniscus, intervertebral disc, articular cartilage, and bone.

In the context of tissue engineering and regenerative medicine, the mechanical stiffness of the scaffold is important in resisting SMA-enabled cell-mediated contraction that can alter the shape of the implant and compress the pores. Recent work, however, has demonstrated that the cell-mediated contraction of a scaffold can be employed to favour chondrogenesis *in vitro* [35]. Chondrocyte seeded-scaffolds of varying cross-link densities were cultured for 2 weeks to evaluate the effect of

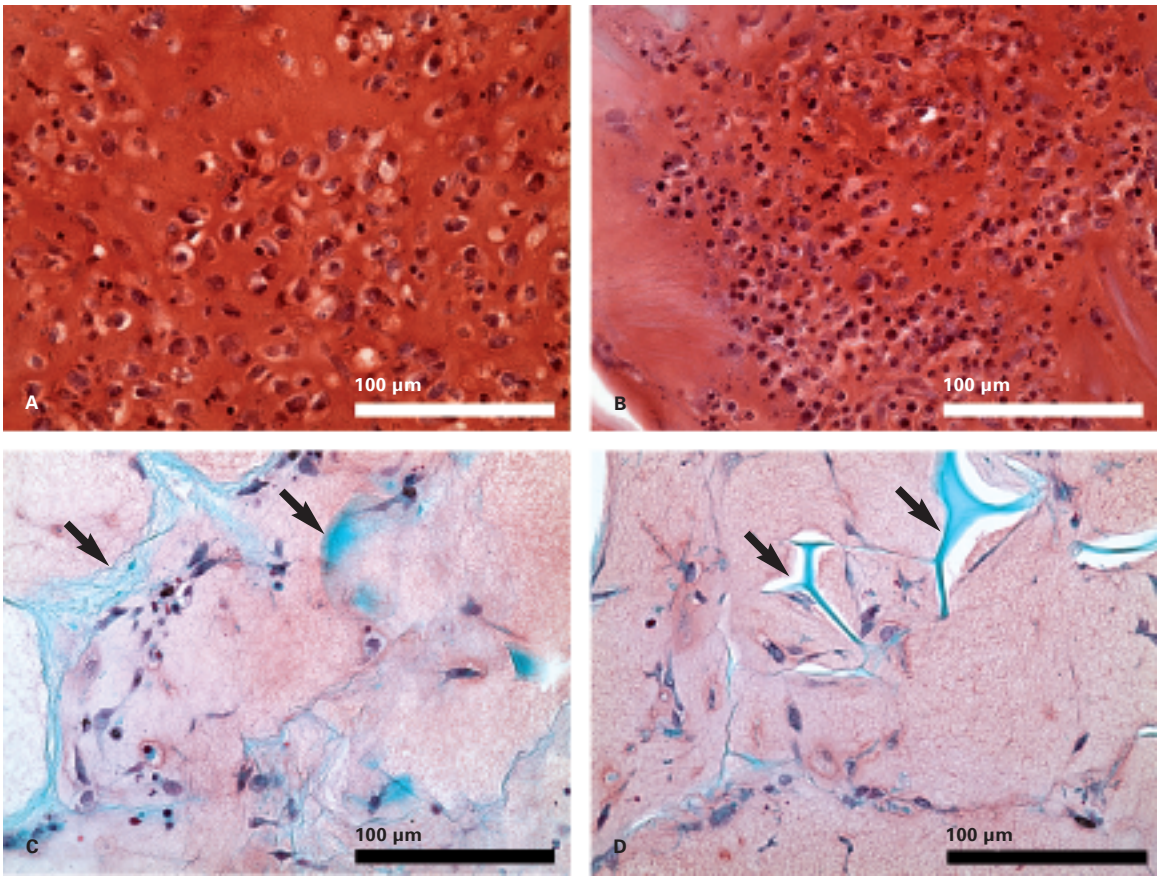

Figure 3

Light micrographs of chondrocyte-seeded collagen-glycosaminoglycan scaffolds with cross-link density increasing from (A) to (D), following a 2-week culture period. The microtomed 7-µm thick sections paraffin-embedded specimens were stained with a Safranin-O/fast green stain that labels proteoglycans, a major constituent of cartilage, red. The arrows in panels (C) and (D) show the residual collagen-glycosaminoglycan scaffold (stained green). It appeared that a higher rate of resorption of the scaffold was associated with a higher rate of chondrogenesis.

cross-link density on scaffold contraction and chondrogenesis. Scaffolds with low cross-link densities experienced cell-mediated contraction, increased cell number densities, and a greater degree of chondrogenesis and an apparent increase in the rate of degradation of the scaffold compared to more highly cross-linked scaffolds that resisted cellular contraction (figure 3). The results of this study suggest the promise of "dynamic pore reduction" of scaffolds for articular cartilage tissue engineering. In this approach scaffolds would have an initial pore diameter large enough to facilitate cell seeding and a mechanical stiffness low enough to allow cell-mediated contraction to yield a reduced pore volume favouring chondrogenesis. This approach may provide a useful alternative to traditional means of increasing cell number density and retention of synthesised molecules that promote cartilage formation in tissue engineered constructs.

Chondrocyte-seeded collagen-gag scaffolds for cartilage repair

Studies demonstrating the potential benefit of injection of culture-expanded chondrocytes for cartilage repair date back to rabbit studies first performed in the mid nineteen eighties [9, 16]. Subsequent experiments in a canine model [6, 7] found significantly more hyaline cartilage in the autologous chondrocyte implantation (ACI)-treated group after 3 and 6 months compared to the untreated control. At 6 months there was a promising amount of defect filling with articular cartilage-like tissue. However, by 1 year there were no significant differences among the treated and control (periosteum alone and non-treated defects) groups. By 18 months neither complete filling, nor the restoration of the architecture was found [7]. Moreover, cartilage surrounding the defect showed degenerative changes, some of which were related to suturing of the periosteal flap. Despite the absence of compelling animal findings using ACI, the procedure has been introduced into widespread clinic use [8] with promising symptomatic relief in many patients [8, 24].

Current efforts in many laboratories around the world are being directed to determine whether the results of ACI can be improved when the cells are implanted as a cell-seeded scaffold rather than delivered by injection. One recent series of studies compared the reparative tissue in chondral defects in adult dogs implanted with cultured autologous chondrocytes (CACs) alone, ie ACI [6], and CAC-seeded type II collagen-GAG scaffolds cultured for 24 hours [5] and 4 weeks [19] prior to implantation. The cell-seeded scaffolds yielded a greater amount of reparative tissue than the sites implanted with the CACs alone. The cell-seeded scaffolds cultured for 24 hours induced more reparative tissue formation than the injection of cells alone. However, this tissue consisted of fibro-

cartilage and fibrous tissue with virtually no hyaline cartilage. The question remains as to the relative importance of the amount versus composition of the reparative tissue with respect to providing symptomatic relief for individuals with focal cartilage defects. Related to this point is the fact that the hyaline cartilage found at sites treated by CACs alone and in the collagen scaffolds did not display the architecture of articular cartilage. Of note was that the greatest amount of reparative tissue was induced by the CAC-seeded scaffold cultured for 4 weeks prior to implantation, and that this group also demonstrated the same amount of hyaline and articular cartilage as found in defects implanted with the cells alone [19]. Although these studies on implementing tissue engineering scaffolds for cartilage repair are promising, there are potential problems and significant expenses associated with culturing a cell-seeded scaffold for 4 weeks prior to implantation. This draws attention to the implementation of growth factors to accelerate cell proliferation and matrix synthesis in the scaffolds prior to implantation [34].

The future of tissue engineering and regenerative medicine

Just as several technologies enabled the development of tissue engineering as a viable discipline, *new* technologies will provide continuation of its growth and maturation. One of these emerging technologies is the isolation and expansion of stem cells and the identification of the signals required for their differentiation into specific cell types. Another related technology is the genetic modification of cells *in vitro* or *in vivo*. These technologies will address the difficulties that are often encountered in obtaining a sufficient amount of tissue for the isolation of autologous cells.

New matrix materials will likely be developed with selected chemical compositions that allow them to better serve as insoluble regulators of cell function. Finally, methods will likely be introduced to control the mechanical environment of the cells *in vitro* to better regulate their biosynthetic behaviour. Collectively these approaches will enable the synthesis of tissue *in vitro* that better replicates the native material and will be of value in preparing implants employed for strategies to facilitate tissue regeneration *in vivo*.

The proliferation of cells in monolayer culture and their subsequent growth in three-dimensional scaffolds for tissue engineering continues to provide unique opportunities to observe selected cell behaviour. Tissue engineering science will thus provide critical new knowledge that will deepen our understanding of the phenotype of many cell types and this knowledge will likely enable meaningful advances in tissue engineering and regenerative medicine.

Correspondence:
Myron Spector, Ph.D.
VA Boston Healthcare System
Boston Campus, Room D1-152
Mail Stop: 151 Research
150 S. Huntington Ave.
Boston, MA 02130, USA
E-Mail: mspector@rics.bwh.harvard.edu

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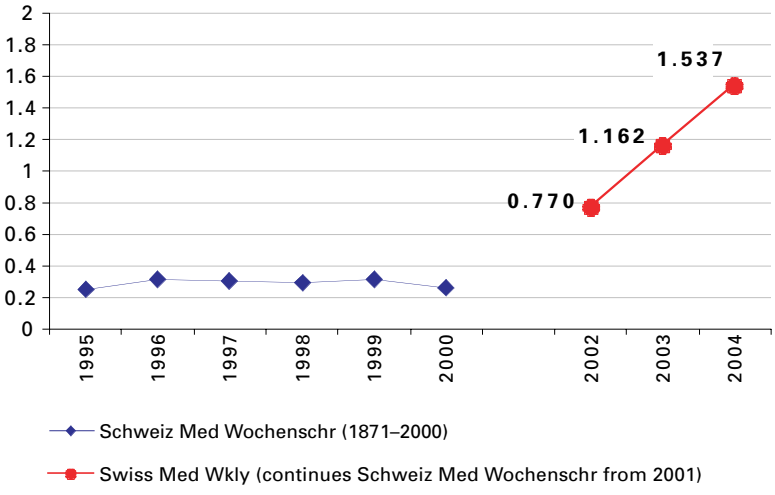

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