# To engineer is to create: the link between engineering and regeneration

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**Tissue engineering is a radically different approach to reconstruction of the body following degenerative diseases, trauma or chronic debilitating conditions. Although there have been some successes, tissue engineering is not yet delivering significant progress in terms of clinical outcomes and commercialization. Part of the problem is that we have failed to understand what tissue engineering really means and to appreciate that engineering is synonymous with creation. These processes involve many different phases and there has been minimal integration of these phases within tissue-engineering paradigms. The conventional concept, based upon a temporal sequence from sourcing cells through to the incorporation of generated tissue into a host, has to be transformed by a systems engineering approach in which all biological and technological phases, and the inter-relationships between them, are fully integrated. It might be that real success will not be achieved until systems biology is superimposed onto this systems engineering paradigm.**

### Introduction – the engineering in tissue engineering

The reason why there is the word 'engineering' in the term 'tissue engineering' is not intuitively obvious. Leaving aside the more trivial suggestions that engineering is the part of an organization that deals with machines and engineers are train drivers, the majority of definitions of engineering invoke the use of scientific knowledge to solve practical problems and/or the systematic analysis of physical data to yield tangible end-products. Although not entirely unconnected, neither of these concepts is readily translated into the paradigms that are now represented by tissue engineering. Tissue engineering does have practical end-products, but the underlying science is far more related to cell, molecular and developmental biology, and to pharmacology, than to the physical sciences that normally underpin classical engineering. There is, however, another meaning of engineering, appreciated best when we consider that the origin of the term is the Latin '*ingenium*', from which we can see that it is ingenuity, or creativeness, that is really at the heart of the subject. This is not a matter of semantics, but of immense importance in both the philosophy and practical development of tissue engineering and the broader area of regenerative medicine. We should bear in mind that tissue engineering, after some 15 years, has yet to really make its mark, either clinically or commercially, and it could be argued that this is related to a misunderstanding of what it actually is.

### Tissue repair, replacement and regeneration

A wide-range of diseases and injuries affect tissues and organs in ways that might result in a loss of some degree of function. Primarily, these conditions are associated with acute injury or chronic degenerative changes. Without medical intervention, the response of the body is relatively limited and is mainly restricted to repair processes. Repair might lead to the restoration of continuity in the affected part by the synthesis of scar tissue, which is essentially collagenous and not reminiscent of the indigenous damaged tissue. This might be an effective front-line response to injury, but does not lead to the restoration of normal structure and function and might, if uncontrolled, lead to detrimental effects in the patient.

During the past 50 years, the accepted mode of treatment for many of these conditions is to excise the affected part, either a tissue or an organ, and replace it with some form of structure that could replicate part, or all, of the function that has been lost. This replacement or augmentation of tissues could be achieved through the use of synthetic implanted devices, which clearly do not lead to restoration of structure given that they are non-viable, or through the use of grafts or transplants, which should restore both structure and function. We need not rehearse the difficulties with both the logistics of supply, and the immunological responses with respect to transplantation, here. With replacement devices, referred to as implants or prostheses, there have been excellent clinical performances with, for example, hip and knee replacements [1], intraocular lens for the treatment of cataracts [2], cardiac pacemakers for arrhythmias [3] and prosthetic heart valves [4]. However, in addition to failing to restore structure, the nature of the function they can replace is largely limited to the simple mechanical and physical, whereas problems of biocompatibility lead to restricted longevity in most cases [5].

The logical conclusion to the discussions that emphasize that repair is not an effective outcome, and that replacement has serious limitations with respect to logistics and lack of biological functionality, is to consider tissue regeneration as the only possible alternative. Tissue regeneration is aimed at restoring normal structure and function through the production of new tissue that replicates exactly that which has been lost. The only problem is that, as explained by Yannas in his monograph on tissue and organ regeneration [6], adult mammals do not spontaneously regenerate many of their organs that are damaged, and have only limited ability to regenerate certain tissues. If we wish to persuade the human adult to

regenerate whole organs or tissues that do not spontaneously regenerate, then we have to give them some cues or signals and superimpose on them a mechanism that is not the natural response to those conditions. Induced regeneration is the essence of tissue engineering, which is, of course, very different to either repair or replacement of tissues. In particular, repair processes in most tissues do not lead to fully functional tissues, and clinical outcomes would be far inferior to those achieved through functional regeneration. Tissue engineering is, therefore, a matter of the creation of new tissue and to engineer here is, simply, to create. The creative process has to be achieved by cells, and they are stimulated into this unnatural regenerative mode by a variety of factors, which can be collectively described as either biomolecules or supporting structures, the former providing molecular signals and the latter mechanical signals. Although there are several very general definitions of tissue engineering, my preference, in line with this concept, has been that 'tissue engineering is the persuasion of the body to heal itself through the delivery, to the appropriate site, of cells, biomolecules and/or supporting structures' [7].

### The central tissue engineering paradigm

Clearly, persuading cells to produce new tissue under circumstances in which they do not normally do so is not a trivial process. Moreover, it is of the utmost importance that during this process, exactly the right type of tissue is generated; that the signals given to the cells can be switched off when the process is complete; and that the resulting tissue is fully functional. The process of tissue engineering starts with the sourcing of the relevant cells, and ends with the full incorporation of the functional regenerated tissue into the host. The pathway between these two points can take many forms but is essentially represented by the central tissue engineering paradigm (Box 1). The types of cells include those derived from autologous, allogeneic or, possibly, xenogeneic sources and they can be fully differentiated cells or stem and/or progenitor cells. The degree of cell manipulation will depend on the origin of the cells and the complexity of the tissue [8] and might be dependent on gene transfer to optimize processes of, for example, cell expansion or to control phenotype under these abnormal circumstances. Normally, the cells will require some supporting structure, either a scaffold, a matrix, or a membrane [9], within, or on, which they will express the new tissue. They will be persuaded to do this by the molecular signals provided by relevant cytokines, growth factors or other molecules [10], and by mechanical signals transmitted through the support and the fluid medium. The environment in which this takes place is usually described as a bioreactor [11]. The tissue that forms is often referred to as a construct and will, if generated *ex vivo*, have to be placed within the host where it has to be fully and functionally incorporated [12]. This process must take into account the responses that should be avoided, such as excessive inflammation, an immune response and carcinogenicity or teratogenicity, in addition to the responses that might be required, such as vascularisation and innervation, and, indeed, the further development and maturation of the tissue itself [13]. It should be borne in mind that this paradigm does not have to be rigidly followed and many tissue engineering processes are evolving with, for example, much of the regeneration actually occurring *in vivo* rather than *ex vivo*. [14].

### Crucial barriers to progress

Having set out the framework of the generic tissue engineering approach, we have to identify the scientific and infrastructure factors that have so far held back progress. There are several prime candidates but probably the most important is the difficulty of integrating all of these components into a coherent system. Such a system needs to accommodate the requirements and specifications for each phase of this paradigm into an efficient and cost-effective process within a quality-validated, clinically-oriented environment; furthermore, it must take into account the impositions of regulatory, ethical and reimbursement schemes. A systems engineering approach to regenerative medicine appears to be an essential element of future developments with respect to this integration, and it is possible that this will also require some elements of systems biology with respect to the underlying science. I shall come back to these points at the end. It should be borne in mind that, a systems engineering approach has already made a difference to other areas of biotechnology, including the production of engineered antibodies; microarray technologies used in cancer drug development; and the use of metabolic models for optimizing fermentation processes.

When considering the individual components of this new paradigm, there are several crucial issues, a few of which are discussed below, regarding the context of complexity and the challenges of engineering new tissue.

#### *The expansion and differentiation of stem cells*

With so many options for cell sourcing, it is unlikely that tissue engineering processes will be confined to one or even a small group of cell-types and origins. Early products and processes have focused on either allogeneic cell lines, such as foreskin-derived fibroblasts and keratinocytes for skin tissue engineering [15], or autologous differentiated cells, such as chondrocytes, for autologous chondrocyte transplantation in cartilage lesions [16]: with limited clinical success in both cases. Embryonic stem cells are still associated with logistics issues based on the ethical dilemma and safety concerns related to the possibility of teratogenicity. Many now believe that adult stem cells provide the most relevant source of cells for tissue engineering [17]. The most significant questions facing this use, however, concern whether the standard of current knowledge of stem cell science is sufficient to direct the tissue engineers into the optimal processes to precisely control the expansion and differentiation of these cells, from wherever sourced, such that they provide the right phenotype, with the right activity, to generate the right tissue. The key here will be the transition from purely stochastic control of stem cell behaviour to that of environmental or extrinsic regulation, [18], and the application of those factors known to influence this regulation, including growth factors,

cytokines, morphogens and adhesion factors, in robust biomanufacturing processes. It is possible that random fluctuations in the signalling reactions and the presence of feed-back transcription loops will both be very significant. Therefore, inherent stochastic events might interfere with the imposed process of extrinsic control, perhaps in a bioreactor, or in an *ex vivo* tissue engineering process. In this context, it is, as yet, uncertain how significant will be, in practical tissue engineering, the effects of telomerase down-regulation and telomere erosion; the associated senescence of the cells during mesenchymal stem cell expansion (or indeed in culture-expanded cells in general); or the sustained and proper functioning of the re-implanted tissue [19].

### *Gene transfer and tissue engineering*

One of the reasons why tissue engineering has not reached the clinical targets initially thought possible with autologous or allogeneic differentiated cells has been the difficulty of generating significant volumes of tissue of the appropriate structure and architecture under bioreactor conditions, given the state-of-the-art knowledge for optimization of molecular and mechanical signals. During the past few years, there have been many attempts to improve this efficiency through the use of *ex vivo* gene transfer, in association with tissue-engineered constructs [20]. This has been applied in bone-tissue engineering with extensive use of bone morphogenetic proteins, particularly recombinant BMP-2 [21], and also transcription factors, such as Runx2/Cbfa 1 [22]. It is known that several BMP-transduced cell types, such as osteoblasts and fibroblasts, can produce bone in ectopic sites. In other situations, bone marrow stromal cells have been genetically engineered to constitutively express the osteoblast specific Runx2/Cbfa 1, with concomitant expression of multiple osteoblast specific genes, such as osteocalcin and osteopontin [23]. We have also seen attempts to deliver plasmid DNA to cells in the vicinity of an implanted construct through release from a biodegradable polymeric scaffold [24,25]. It is clear that gene transfer can be employed within the tissue engineering paradigm in several circumstances to enhance cell performance within *in vitro* systems and small animal models [26]. It is far from clear, however, whether existing gene-transfer materials and techniques can be applied efficiently and safely in human patients. Furthermore, the perception of tissue engineering as a vehicle for radical changes in therapy could be profoundly altered if the application of this 'unnatural selection process of genetic engineering' [27] ended in errors.

### *Growth factor delivery*

One of the simplest examples in tissue engineering involves the sourcing and expansion of cells and their subsequent signalling by growth factors in a bioreactor. Besides the cost, some of the drawbacks of this include the instability of many of these molecules and the fact that their optimal effects are usually observed *in vivo* rather than *ex vivo*. However, the *in vivo* use of growth factors in tissue engineering is problematic, largely in view of their short biological half lives – often measured in minutes – and the potential for systemic toxicity. This has led to the search for methods to immobilize, or protect, growth factors on or within the materials used for the supporting matrices and then arrange for their sustained and controlled release. Biodegradable synthetic polymers [28]; biopolymers, including proteins such as collagen [29], and polysaccharides, such as alginate and hyaluronan [30]; and bioceramics, such as the various calcium phosphates [31], have all been used for this purpose. The sustained, local release of transforming growth factors(TGFβ1) and osteogenic protein (OP1) to influence osteogenesis; basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) for nerve regeneration [32]; platelet derived growth factor (PDGF) for enhancement of wound healing; and vascular endothelial growth factor (VEGF) for angiogenesis [33] have been achieved. Of considerable importance are the attempts to use matrix-immobilized growth-factors to mimic the release of growth factors from natural extracellular matrix (ECM) *in vivo* through cell-controlled proteolysis [34]. However, in general, these developments have been largely of an unsystematic manner, and little information is available about procedures for orchestrating release-profiles, particularly the sequential release of multiple factors to optimize their effectiveness. In this context, most therapies suggested, so far, rely on the supply of a single factor, whereas the natural process is multifactorial, such that there is a need for a better orchestration of growth factor profiles [35].

### *Scaffold materials and design*

One of the main tenets of the now classical tissue engineering process is the need for a supporting structure for the control of cell behaviour and scaffold biomaterials have had a major role in developments so far. The majority of scaffolds are porous polymers, usually synthetic biodegradable polyesters, prepared by solvent casting, with porosity achieved by porogen or leaching technology; by fibre spinning processes; or by solid free-form fabrication methods. There is a certain logic to this because the cells need a substrate on which to adhere and function, but these materials and architecture hardly mimic the ECM that a cell normally expects; therefore, neither the physico-chemical-support role nor the provision of active mechanical signalling can be considered at all optimal, even if the cells are provided with adhesive peptides, immobilized on the material surface to encourage adhesion [36]. Many would argue that a far more realistic strategy would be to mimic the gel morphology of the ECM, and a variety of protein, polysaccharide and synthetic hydrogel structures have been developed for this purpose. One advantage, here, is that the cells within this three-dimensional matrix will experience the same type of stimulation received in their normal ECM, rather than the random nature of the interaction between a cell suspended in culture medium and the porous polymeric network into which it is seeded. There is also the added advantage that growth factors can be incorporated into these gels in addition to adhesion peptides. Two issues are of considerable importance. The first is the paramount importance of distancing the design of tissue engineering matrices and scaffolds from the

classical development of biomaterials for implantable devices, and it is probably the hitherto slavish approach to concentrate on FDA medical-device-approved materials for tissue engineering that has been the biggest obstacle to progress. Second, if we are to make significant progress in biomimickry of the viscous, biologically active ECM, there is a great deal more we need to know about the functioning of this matrix, including events of cell adhesion and migration, growth factor distribution and interactions, and molecular recognition events.

### *Mechanotransduction and bioreactor design*

The final point for consideration is that of mechanotransduction, which effectively follows on from the discussion of the ECM. The fibrillar structures within the ECM distribute and focus mechanical stresses, such that the integrin receptors on the cell membranes transmit forces productively to the cytoskeleton, which then modulates cell behaviour, including motility and mitosis [37]. The science of mechanotransduction is rapidly developing, but, apart from recognizing its potential importance in the regulation of cell behaviour in the pre-conditioning of cells before implantation in patients, little has been done to actually target the molecules that could affect mechanotransduction within a tissue engineering construct, including cell–cell adhesion molecules, cytoskeletal filaments and signal transduction molecules. All knowledge, so generated, has then to be translated into the design of the bioreactors, within which, through either or both structural mechanical and fluid shear-stresses, mechanotransduction is put into practice in the art of generating new tissue.

## Reassessing the future of tissue engineering

The above examples, and there are many more should further rationalization be necessary, point clearly to the generic difficulties facing the engineering of new tissues. There are many scientific and technological components of the paradigm and although relevant data and knowledge are emerging fast, there has been no way of incorporating these individual components into a complete system. These scientific barriers to progress are compounded by the significant cost-implications of current tissue engineering processes and the considerable global uncertainty regarding the regulatory procedures for tissue engineering products and processes.

It should be of no surprise that both systems engineering and systems biology are being discussed in terms of solutions to this situation. Starting with systems biology, it has become clear that it is the dynamic interactions of molecules and cells that give rise to biological function and that knowledge about individual biological components, from genes through to proteins, subcellular components, cells, tissues, organs and whole organisms does not in itself lead to an understanding of cell and organ function [38]. It is rather the understanding of the inter- and intra-cellular processes that will do this, leading, for example, to a far greater appreciation of disease causation and drug design. So it is within tissue engineering and regenerative medicine. The paradigm discussed in this paper is not hierarchical, but temporal. It is based on the practical transition from cell derivation to tissue construct integration. This, as concluded below, is appropriate for a systems engineering approach, but not one of systems biology. Here, we should follow a bottom-up approach for the generation of *de novo* tissue, in an un-natural situation, that is based on interaction pathways, such as those used in the broader modelling of biological systems. If we do not understand how the physical and genetic components within a cell that we have sourced for a tissue engineering process operate together within their system, how can we hope to provide the optimal conditions for them to create the correct tissue?

The problem with a systems biology approach is that there are far too many gaps in our understanding of these pathways for us to use it in a practical way at the moment; meanwhile, there are patients to treat and a biotechnology industry waiting eagerly for some return on their investment. Furthermore, it has to be said that, patients are already being treated with some 'first generation' tissue engineering processes, ranging from the treatment of chronic foot ulcers in diabetics to artery generation in congenitally deformed children and nerve regeneration after trauma. In the interim, therefore, and to make some tangible progress based upon these beginnings, we must turn to systems engineering. Here, we can stay with our temporal paradigm but from a practical rather than a theoretical point of view. It is essential that these disparate components are integrated so that the interactions between them are factored into the process. One of the essential dogmas of systems biology is that in, for example, the classic bottom-up approach – from gene to organism – there are multiple feed-back loops, and these are often studiously ignored in the technology of tissue engineering. A major consortium, funded by the European Commission, is just starting to engage with systems engineering solutions to regenerative medicine based on these considerations (European Commission Sixth Framework Integrated Project Consortium).

## Concluding remarks

There is, therefore, a profound link between engineering systems and regenerative medicine. One of the consequences of this evaluation of the current status of tissue engineering is the realization that not only might the paradigms be wrong, but also some of the concepts and even the definition. Having worked with, and refined, the conceptual definition given earlier in this paper for more than a decade, I now believe that we should move on to one that is more precise and I suggest, 'Tissue engineering is the creation of new tissue for the therapeutic reconstruction of the human body, by the deliberate and controlled stimulation of selected target cells, through a systematic combination of molecular and mechanical signals'.

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**References**

1 National Institute of Clinical Excellence (UK) Guidance on Artificial Hip Joints Issued 31st March 2000, 2000/0003a, (http://www.nice.org.uk)
2 Lloyd, A.W. *et al.* (2001) Ocular biomaterials and implants. *Biomaterials* 22, 769–785
3 Bourke, M.F. and Healey, J.S. (2002) Pacemakers, recent directions and developments. *Current Opinions in Anesthesiology* 15, 681–686
4 Moffatt-Bruce, S.D. and Jamieson, W.R.E. (2004) Long-term performance of prostheses in mitral valve replacement. *J. Cardiovasc. Surg. (Torino)* 45, 427–447
5 Williams, D.F. (2003) The inert bioactivity conundrum. In *The Implant Tissue Interface* (Ellingsen, J.E. ed.), pp. 407–430, CRC Press
6 Yannas, I.V. (2001) *Tissue and organ regeneration in adults*, Springer
7 Williams, D.F. (1999) *The Williams Dictionary of Biomaterials*, Liverpool University Press
8 Qian, L. and Saltzman, W.M. (2004) Improving the expansion and neuronal differentiation of mesenchymal stem cells through culture surface modification. *Biomaterials* 25, 1331–1337
9 Karageorgiou, V. and Kaplan, D. (2005) Porosity of 3D scaffolds and osteogenesis. *Biomaterials* 26, 5474–5491
10 Boontheekul, T. and Mooney, D.J. (2003) Protein-based signalling systems in tissue engineering. *Curr. Opin. Biotechnol.* 14, 559–565
11 Lichtenberg, A. *et al.* (2005) A multifunctional bioreactor for three dimensional cell co-culture. *Biomaterials* 26, 555–562
12 Babensee, J.E. *et al.* (1998) Host response to tissue engineered devices. *Adv. Drug Deliv. Rev.* 33, 111–139
13 Zisch, A. (2004) Tissue engineering of angiogenesis with autologous endothelial progenitor cells. *Curr. Opin. Biotechnol.* 15, 424–429
14 Cassell, O.C.S. *et al.* (2002) Vascularisation of tissue-engineered grafts; the regulation of angiogenesis in reconstructive surgery and in disease states. *Br. J. Plast. Surg.* 55, 603–610
15 Omar, A.A. *et al.* (2004) Treatment of venous leg ulcers with Dermagraft. *Eur. J. Vasc. Endovasc. Surg.* 27, 666–672
16 Tibesku, C.O. *et al.* (2004) Hyaline cartilage degenerates after autologous osteochondral transplantation. *J. Orthop. Res.* 22, 1210–1214
17 Zanstra, P.W. and Nagy, A. (2001) Stem cell bioengineering. *Annu. Rev. Biomed. Eng.* 3, 275–305
18 O'Neill, A. and Schaffer, D.V. (2004) The biology and engineering of stem cell control. *Biotechnol. Appl. Biochem.* 40, 5–16
19 Parsch, D. *et al.* (2004) Telomere length and telomerase activity during expansion and differentiation of human mesenchymal stem cells and chondrocytes. *J. Mol. Med.* 82, 49–55
20 Oakes, D.A. and Lieberman, J.R. (2000) Osteoinductive applications of regional gene therapy: *ex vivo* gene transfer. *Clin. Orthop. Relat. Res.* (379 Suppl), S101–S112
21 Laurencin, C.T. *et al.* (2001) Poly (lactide-co-glycolide)/ hydroxyapatite delivery of BMP-2 producing cells: a regional gene therapy approach to bone regeneration. *Biomaterials* 22, 1271–1277
22 Yang, S. *et al.* (2003) *In vitro* and *in vivo* synergistic interactions between the Runx2/Cbfa 1 transcription factor and bone morphogenetic protein-2 in stimulating osteoblasts differentiation. *J. Bone Miner. Res.* 18, 705–715
23 Byers, B.A. and Garcia, A.J. (2004) Exogenous Runx2 expression enhances *in vitro* osteoblastic differentiation and mineralization in primary bone marrow stromal cells. *Tissue Eng.* 10, 1623–1632
24 Bonadio, J. *et al.* (1999) Localized direct plasmid gene delivery *in vivo*: prolonged therapy results in reproducible tissue regeneration. *Nat. Med.* 5, 753–759
25 Madsen, S. and Mooney, D.J. (2000) Delivering DNA with polymer matrices: applications in tissue engineering and gene therapy. *Pharm. Sci. Technol. Today* 3, 381–384
26 Hutmacher, D.W. and Garcia, A.J. (2005) Scaffold–based bone engineering by genetically modified cells. *Gene* 347, 1–10
27 Snow, A. (2003) Genetic engineering: unnatural selection. *Nature* 424, 619
28 Xu, X. *et al.* (2002) Polyphosphoester microspheres for sustained release of biologically active nerve growth factor. *Biomaterials* 23, 3765–3772
29 Luginbuehl, V. *et al.* (2004) Localised delivery of growth factors for bone repair. *Eur. J. Pharm. Biopharm.* 58, 197–208
30 Eckardt, H. *et al.* (2005) Recombinant human bone morphogenetic protein 2 enhances bone healing in an experimental model of fractures at risk of non-union. *Injury* 36, 489–494
31 Kroese-Deutman, H.C. *et al.* (2005) Bone-inductive properties of rhBMP-2 loaded porous calcium phosphate implants inserted in ectopic site in rabbits. *Biomaterials* 26, 1131–1138
32 Begley, D.J. (2004) Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. *Pharmacol. Ther.* 104, 29–45
33 Gu, F. *et al.* (2004) Sustained delivery of vascular endothelial growth factor with alginate beads. *J. Control. Release* 96, 463–472
34 Zisch, A. *et al.* (2003) Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularised tissue growth. *FASEB* 17, 2260–2262
35 Richardson, T.P. *et al.* (2001) Polymeric systems for dual growth factor delivery. *Nat. Biotechnol.* 19, 1029–1034
36 Jeschke, B. *et al.* (2002) RGD-peptides for tissue engineering of articular cartilage. *Biomaterials* 23, 3455–3463
37 Ingber, D.E. (2003) Mechanobiology and diseases of mechanotransduction. *Ann. Med.* 35, 564–577
38 Wolkenhauer, O. *et al.* (2005) The dynamic systems approach to control and regulation of intracellular networks. *FEBS Lett.* 579, 1846–1853

## Box 1. The central tissue engineering paradigm

Cell sourcing

Cell expansion and manipulation

Cell seeding and extracellular matrix expression

Mechanical and molecular signalling

Implantation of construct

Full incorporation into host