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Tissue Engineering: The First Decade and Beyond

Lawrence J. Bonassar* and Charles A. Vacanti

Departments of Anesthesiology, Cell Biology and Biomedical Engineering, University of Massachusetts Medical School, Worcester, Massachusetts 01655

Abstract This article reviews the important developments in the field of tissue engineering over the last 10 years. Research in the area of biomaterials is examined from the perspective of providing the foundation for the development of tissue engineering. Early efforts combining cells with biocompatible materials are described and applications of this technology presented, with particular focus on uses in orthopaedics and maxillofacial surgery. The basic principles of tissue engineering and state-of-the-art technology in cell biology and materials science as used currently in the field are presented. Finally, futures challenges are outlined from the perspective of integrating technologies from medicine, biology, and engineering, in hopes of translating tissue engineering to clinical applications. J. Cell. Biochem. Suppls. 30/31:297–303, 1998. © 1998 Wiley-Liss, Inc.

Key words: tissue engineering; biomaterials; cell culture; polymers; transplants

Interest in the field of tissue engineering has grown tremendously since its inception slightly more than 10 years ago. While the number of researchers in this field has greatly increased during this time and the potential applications have become more widespread, there is a common theme in this work: the concept that the repair and regeneration of biological tissues can be guided through application and control of cells, materials, and chemoactive proteins. As such, tissue engineering is, at its core, an interdisciplinary field, requiring the interactions of physicians, scientists, and engineers.

The motivation for this field has come largely from physicians who are keenly aware of the scarcity of transplant tissue for use in the replacement of tissue lost to cancer or trauma and in the repair of birth defects. This scarcity in combination with potential rejection of allograft tissue makes it preferable to use autologous tissue, which is also at a premium. What is needed is a significant amount of autologous tissue which can be obtained without compromising the function of a donor site. The goal, then, is to harvest a relatively small piece of tissue and remove the cells and then to expand the cell population so that the cells can be reimplanted using a carrier material and generate a substantial amount of tissue. The neogeneration of tissue requires a keen understanding of extracellular matrix as both a structural framework and a regulator of cell behavior. Consequently, this requires considerable input from biological scientists who give insight on the structure of extracellular matrix of the native tissue and the behavior of cells during in vitro culture and after in vivo implantation. This process also requires the active participation of engineers who fabricate and process materials to use as scaffolds for guiding tissue development and develop paradigms for assessing the functional capacity of generated tissue.

HISTORY OF TISSUE ENGINEERING

The roots of tissue engineering can be traced to the field of biomaterials, which is the study of materials used in the body and of the effects such materials have on the host and of the biological environment on the material. In many cases, the objective was to use materials that were as inert as possible, and therefore not degraded or harmful to the host. Tissue engineering, in a sense, began with the use of bioactive materials, that is, materials designed to interact with the body to encourage tissue repair. An important early example is documented in a series of three articles by Yannas and colleagues [Yannas et al., 1980a,b; Dagal-

*Correspondence to: Lawrence J. Bonassar, Tissue Engineering Laboratory, Department of Anesthesiology, University of Massachusetts, 55 Lake Avenue North, Worcester, MA 01655–0300. E-mail: lawrence.bonassar@ummed.edu

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akis et al., 1980], which describe the design and fabrication of an artificial skin analogue from collagen and glycosaminoglycan (GAG). The first use of the term "tissue engineering" was in reference to an observation of an organization of an endothelium-like structure on the surface of a polymethylmathacrylate (PMMA) ophthalmic prosthesis [Wolter and Meyer, 1984]. When used currently, the term tissue engineering has come to imply some combination of cells, scaffold material, and bioactive peptides used to guide the repair or formation of tissue. Two early examples were the growth chondrocytes on a polyglycolic acid (PGA) mesh [Cima et al., 1991] and the culture of hepatocytes in hollow fibers [Jauregui and Gann, 1991]. A report on the early efforts in the field and an explanation of the concepts to a general audience [Langer and Vacanti, 1993] brought the field to prominence in a relatively short time.

During the 7 years since the initial report of these studies, the principles of tissue engineering have been applied to virtually every organ system in the body (Fig. 1). Considerable attention has been focused on orthopaedic and maxillofacial applications, including engineering of bone [Nakahara et al., 1992], cartilage [Cima et al., 1991], tendon [Cao et al., 1995], ligament [Huang et al., 1993], and skin [Bell et al., 1981], as well as significant contributions in the cardiovascular area with engineering of heart valves [Fabiani et al., 1995] and blood vessels [Kempczinski et al., 1985]; endocrinology, with encapsulation of pancreatic islets cells [Lanza et al., 1995]; gastrointenstinal system, with the growth of hepatocytes on synthetic scaffolds [Nyberg et al., 1993]; the nervous system, including guided peripheral nerve regeneration [Guenard et al., 1992] and spinal cord repair [Aebischer et al., 1988]; dental applications such as periodontal tissue repair [Anderegg et al., 1995] and dentin regeneration [Nakashima, 1994]; and studies in ophthalmology to engineer cornea [Chang et al., 1995] and lens tissue

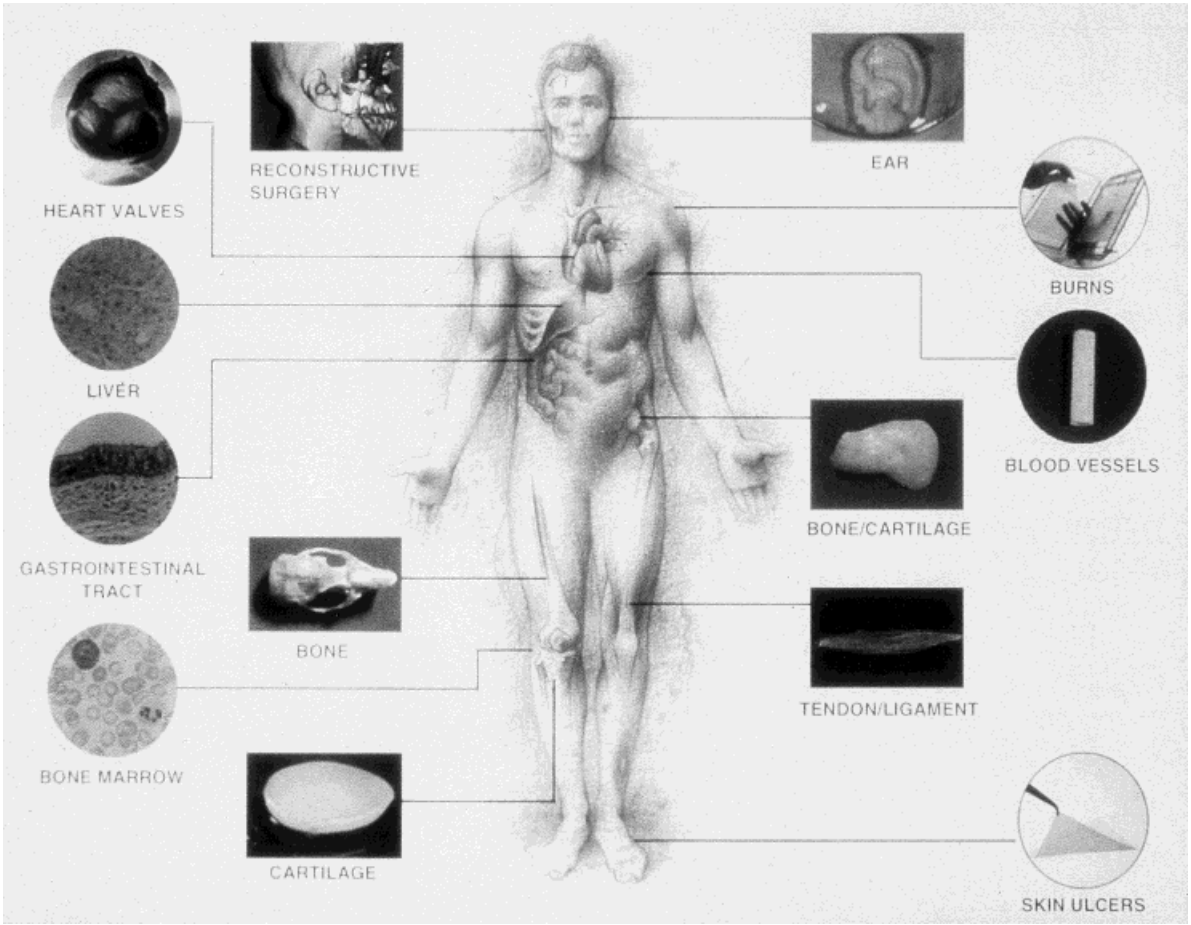

Fig. 1. Schematic diagram illustrating various organ systems for which tissue-engineered constructs have been designed and fabricated. **Color plate on page 336.**

[Nishi et al., 1998]. These efforts have led to a steady increase in the number of publications per year, which specifically refer to tissue engineering (Fig. 2). This indicates that the term tissue engineering is steadily becoming a part of the vocabulary of the scientific community and interest in this work is still growing, even as the field itself it is still in its infancy.

CRITICAL COMPONENTS: CELLS AND MATERIALS

Most tissue-engineered constructs are composed of at least two important components: a group of cells, and a material scaffold on which they can grow. Elements play an important role in the development of new tissue. The cellular component is necessary for the generation of new tissue through production of extracellular matrix and is responsible for the long-term maintenance of this matrix. The scaffold material provides mechanical stability of the construct in the short term and provides a template for the three-dimensional organization for the developing tissue. The interaction of these two components, such as the coordination of polymer degradation rates with cellular biosynthetic rates and the cell-seeding characteristics of polymers, is critical for the success of an engineered tissue construct.

As in native tissue, cells within a construct respond and adapt to the physical and biological stimuli to which they are exposed in vivo.

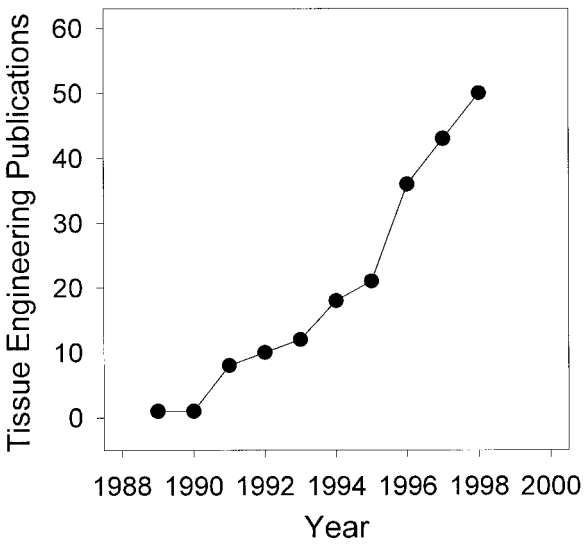

Fig. 2. Number of peer-reviewed journal articles since 1989 that specifically have tissue engineering listed as a keyword.

This is, in fact, one of the great strength of a tissue engineering approach. A noncellular implant or material is subject to degradation by enzymes, hydrolysis, or fatigue, which will ultimately impede its performance. By contrast, a tissue-engineered construct contains cells that have the capacity to repair and remodel its extracellular matrix such that its properties should not degrade with time.

As stated above, the cellular component of a tissue-engineered construct is ultimately responsible for performing the function of the tissue it was designed to replace. This function may be primarily mechanical, as in the case of structural tissues like bone, cartilage, and skin, or biological, as in liver, pancreas, or nerve. The process of assembling a tissue-engineered construct then begins with the identification of the relevant cell type and the process of isolation of these cells from native tissue. Given that the goal is to replace or repair a sizable defect while only harvesting a small tissue sample, the next critical step involves obtaining enough cells from the available tissue.

Expansion of the cell population in vitro then becomes an important step in the process of building a construct. The ease or difficulty of expansion is highly dependent on the cell type—fibroblasts for use in skin or tendon constructs may multiply quickly, in contrast to neurons used in nerve repair. In this process, it is important to ensure that the expanded cell population retains its phenotypic function. This is of great concern for chondrocytes, which will dedifferentiate upon repeated passaging [Benya and Schaffer, 1982], as well as other cell types.

The issue of phenotype expression and dedifferentiation has led to the investigation of pluripotent stem cells as a source for engineered tissues. These have included mensenchymal stem cells, which are capable of differentiating into bone, cartilage, tendon, and muscle [Caplan, 1990]; hematopoietic stem cells, which give rise to bone [Lazarus et al., 1995]; and neural stem cells, which give rise to neurons, astrocytes, and oligodendrocytes [Hulspas et al., 1997]. For these stem cells, the regulation of lineage formation becomes critical to controlling the development of tissue. A common approach to this issue is the use of polypeptide growth factors, which are known to support various terminal phenotype. These include the transforming growth factors-β (TGF-β), which have been used to support the chondrocytic

phenotype [Yaeger et al., 1997] and recruit native cells to the chondrocyte lineage in vivo [Hunziker and Rosenberg, 1996]; the bone morphogenetic proteins (BMP), which have been shown to induce in vivo bone formation [Zegzula et al., 1997]; and fibroblast growth factors (FGF), which are known to support angiogenesis [Sellke et al., 1998].

The formation of tissue produced by implanted cells is influenced greatly by the scaffold onto which they are seeded. As with growth factors, the scaffold itself can regulate cell phenotype, as evidenced by the re-expression of the chondrocyte phenotype in agarose after dedifferentiation during monolayer culture [Benya and Schaffer, 1982]. The primary purposes of the scaffold materials are to provide mechanical stability to the construct in the short term and to provide a framework for three-dimensional organization of the developing tissue. As the tissue develops, the new extracellular matrix takes on both roles. Consequently, it is often preferable to use a biodegradable material scaffold such that, in the long term, all implanted materials are gone and all that remains is the generated tissue.

A variety of biodegradable materials have been used for tissue scaffolds, including ceramics and polymers. The primary use of ceramics has been in tissue engineering of bone, where porous formulations of hydroxyapatite have been used to carry osteoprogenitor cells derived from periosteum or bone marrow. Typically, ceramic materials have long degradation times in vivo, often on the order of years. Polymers have seen widespread use as scaffold materials because of their good processing characteristics. These materials have a range degradation times from very short (days) to long (several months). Typically, polymers scaffolds are in the form of fibrous meshes, porous sponges or foams, or hydrogels. The more common polymers used in fibrous meshes and foams include the linear polyesters, including polyglycolic acid (PGA), polylactic acid (PLA), and polycarpolactone (PCL); polyethylene glycol (PEG); and natural polymers, such as collagen and hyaluronic acid (HA). Polymeric hydrogels have the distinct advantage of being injectable, permitting less invasive delivery of the construct, and thereby reducing surgical risks. Common hydrogel substrates include the coplymers of polyethylene oxide and polypropylene oxide known as Pluronics and natural polymers including alginate and agarose.

Scaffold materials play a critical role in providing mechanical stability to constructs prior to synthesis of new extracellular matrix by the cells. It is then desirable to match the mechanical properties of the material with that of the tissue. Consequently, scaffolds for bone often contain ceramic hydroxyapatite, which has high stiffness like bone; scaffolds for cartilage and tendon tend to be made from more compliant polymers. In addition to mechanical stability, scaffold materials often serve to reduce immune response to allogenic cells. The most notable example of this is encapsulation of pancreatic islet cells for diabetes treatment.

As the demand for new and more sophisticated scaffolds develops, materials are being designed which have a more active role in guiding tissue development. Instead of merely holding cells in place, these bioactive matrices are designed to encourage cell attachment to the polymer through cell surface adhesion proteins. Toward this end, polymers have been synthesized that have an integrin polypeptide sequence (RGD) in the backbone [Shakesheff et al., 1998, Hern and Hubbel, 1998] or branches [Harrison et al., 1997] or constructed entirely of polypeptide sequences [Petka et al., 1998]. This allows the scaffold to effectively mimic the extracelluar matrix and induce attachment of cells directly to the material. This may be particularly important in tissues that bear mechanical loads, as it would allow physical stimuli to be sensed by the cells in the developing tissue in a more physiologic manner.

FUTURE CHALLENGES

While the field of tissue engineering has made significant strides in its first decade of existence, many challenges exist in the biology and engineering that must be addressed to bring these technologies to clinical practice. The processes by which cells seeded onto degradable scaffolds and generate new tissue are still being defined. These processes are known to involve the production of extracellular matrix, but the regulation of matrix production, pattern formation, and morphogenesis in tissue engineering remains largely unexplored. It has been suggested that tissue neogenesis in engineered constructs may involve the same processes present in tissue development during embryogenesis. This is supported by studies in which periosteal

cells on a polyglycolic acid scaffold appeared to first generate hypertrophic cartilage prior to mineralized bone formation [Vacanti et al., 1995], reminiscent of endochondral ossification observed during skeletal morphogenesis. Such an observation raises a variety of biological questions on many levels: What are the levels of BMPs in the construct during tissue neogenesis? What is the time sequence of expression of tissue-specific markers such as osteocalcin, osteopontin, and bone sialoprotein? What are the roles of hox and Indian hedgehog genes in regulating this process? To what extent does this recapitulate the developmental process of bone formation? Understanding these issues will aid not only in describing the observed phenomena, but will lead to the next step in tissue engineering—controlling these events to more effectively guide tissue formation. Clearly, cell and developmental biologists will play a prominent role in this expanding area of tissue engineering.

In addition to understanding the process of tissue generation, it is similarly important to increase the level of sophistication of techniques used to characterize engineered tissues. The most commonly used tool for evaluation of tissue is simple histology. This allows researchers to determine the extent to which the morphology of the generated tissue resembles native tissue. Although clearly a critical step in evaluating the tissue, it should not be the only step or even the last. Immunohistochemistry allows for the detection of tissue-specific proteins, such as type II collagen for cartilage, bone sialoprotein for bone, or neurofilament protein for neurons. In addition, it is desirable to determine the presence of matrix components, but also to quantify their levels in generated tissues. Several common biochemical assays are available for quantification of total collagen (hydroxyproline), proteoglycan (glycosaminoglycan), and elastin, in structural tissues, and these could easily be applied to engineered tissues as well. Similarly, enzyme-linked immunosorbet assay (ELISA) techniques using antibodies to tissue-specific proteins would also permit quantitation of these components in generated tissue. Further, characterization of the quality of matrix composition, such as the size distribution of proteoglycans or the frequency of collagen cross-links, will give further insight as to whether the structure of the native tissue has been reproduced in the generated tissue. Most of the techniques described above are part of a standard repertoire used in the analysis of structural and connective tissues. The collaboration of biochemists and tissue engineers to translate these approaches to understand generated tissues is then of great potential interest to both fields.

An understanding of the structure of engineered constructs needs to be closely associated with an assessment of tissue function. Indeed, the entire motivation for the field of tissue engineering is to restore function of tissue lost to disease, accident, or malformations. Therefore, it is critical to determine the extent to which the functional properties of generated tissues are similar to those of native tissue. Given that the focus of many tissue engineering applications is structural tissues, such as bone, cartilage, tendon, skin, and muscle, this necessitates the analysis of the biomechanical properties of generated tissues. As with the discussion of biochemical composition, an understanding of the biomechanical properties of engineered tissue must start with an understanding of the mechanical properties of the native tissue. And as with the previous discussion, this requires an assessment of the level of sophistication that adequately describes the system. Tissues with nonlinear or time-dependent mechanical responses may not be adequately described by a modulus determined at equilibrium or at a single strain rate. Indeed, soft tissues such as skin, tendon, muscle, and cartilage require viscoelastic or poroelastic parameters in addition to the modulus to describe their mechanical behavior. Consequently, these parameters must also be evaluated to adequately describe the behavior of corresponding engineered tissues.

The concept of assessing tissue function can be extended to nonstructural tissues as well, and similar questions can be asked: Do tissue-engineered nerves have similar conductances to native nerves? Are flow patterns through engineered heart valves similar to those of natural valves? Do encapsulated islet cells sense and respond to blood glucose levels by producing insulin? Do transport kinetics through engineered epithelium match those of native epithelium? To address these issues requires interaction with physiologists and bioengineers, who can assist in developing paradigms for the functional assessment of engineered tissues.

Confronted by these questions, it is clear that many challenges still confront researchers in tissue engineering, despite the considerable accomplishments in the field in its short existence. From the molecular level of gene expression and polymer design to the macroscopic level of organ physiology, and at every level in between, researchers in tissue engineering are challenged to understand the biological and physical processes underlying tissue formation. However, it is precisely these challenges and questions that will push the field to greater accomplishments in the future. Through the interaction of physicians, scientists, and engineers, the field of tissue engineering will continue to expand its limits and applications, which will ultimately lead to new strategies and therapies for the treatment of debility and disease.

REFERENCES

Aebischer P, Winn SR, Galletti PM (1988): Transplantation of neural tissue in polymer capsules. Brain Res 448:364–368.

Anderegg CA, Metzger DG, Nicoll BK (1995): Gingiva thickness in guided tissue regeneration and associated recession at facial furcation defects. J Periodontal 66:397–402.

Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T (1981): Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. Science 211:1052–1054.

Benya PD, Shaffer JD (1982): Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell 30:215–224.

Cao Y, Vacanti JP, Ma X, Paige KT, Upton J, Chowanski Z, Schloo B, Langer R, Vacanti CA (1995): Generation of neo-tendon using synthetic polymers seeded with tenocytes. Transplant Proc 26:3390–3392.

Caplan AI (1990): Stem cell delivery vehicle. Biomaterials 11:44–46.

Chang PCT, Lee SD, Huang JH (1995): Biocompatibility of an artificial corneal membrane— in vivo animal study. Invest Ophthalmol Vis Sci 36:1467–1476.

Cima LG, Vacanti JP, Vacanti C, Ingber D, Mooney D, Langer R (1991): Tissue engineering by cell transplantation using degradable polymer substrates. J Biomech Eng 113:143–151.

Dagalakis N, Flink J, Stasikelis P, Burke JF, Yannas IV (1980): Design of an artificial skin. Part III. Control of pore structure. J Biomed Mater Res 14:511–528.

Fabiani JN, Dreyfus GD, Marchand M, Jourdan J, Aupart M, Latremouille C, Chardigny C, Carpentier AF (1995): The autologous tissue cardiac valve: A new paradigm for heart valve replacement. Ann Thorac Surg 60:189–194.

Guenard V, Kleitman N, Morrissey TK, Bunge RP, Aebischer P (1992) Syngenic Schwann cells derived from adult nerves seeded in semipermeable guidance channels enhance peripheral nerve regeneration. J Neurosci 2:3310–3320.

Harrison D, Johnson R, Tucci M, Puckett A, Tsao A, Hughes J, Benghuzzi H (1997): Interaction of cells with UHMWPE impregnated with the bioactive peptides RGD, RGE, or Poly-L-lysine. Biomed Sci Instrum 34:41–46.

Hern DL, Hubbell JA (1998): Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. J Biomed Mater Res 39:266–276.

Huang D, Chang TR, Aggarwal A, Lee RC, Ehrlich HP (1993): Mechanisms and dynamics of mechanical strengthening in ligament-equivalent fibroblast-populated collagen matrices. Ann Biomed Eng 21:289–305.

Hulspas R, Tiarks C, Reilly J, Hsieh CC, Recht L, Quesenberry PJ (1997): In vitro cell density-dependent clonal growth of EGF-responsive murine neural progenitor cells under serum-free conditions. Exp Neurol 148:147–156.

Hunziker EB, Rosenberg LC (1996): Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane. J Bone Joint Surg Am 78:721–733.

Jauregui HO, Gann KL (1991): Mammalian hepatocytes as a foundation for treatment in human liver failure. J Cell Biochem 45:359–365.

Kempczinski RF, Rosenman JE, Pearce WH, Roedersheimer LR, Berlatzky Y, Ramalanjaona G (1985): Endothelial cell seeding of a new PTFE vascular prosthesis. J Vasc Surg 2:424–429.

Langer R, Vacanti JP (1993): Tissue engineering. Science 260:920–926.

Lanza RP, Ecker DM, Kuhtreiber WM, Marsh JP, Chick WL (1995): A simple and inexpensive method for transplanting xenogeneic cells and tissues into rats using alginate gel spheres. Transplant Proc 27:3322.

Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI (1995): Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): Implications for therapeutic use. Bone Marrow Transplant 16:557–564.

Nakahara H, Goldberg VM, Caplan AI (1992): Culture-expanded periosteal-derived cells exhibit osteochondrogenic potential in poroous calcium phosphate ceramics in vivo. Clin Orthop 276:291–298.

Nakashima M (1994): Induction of dentin formation on canine amputated pulp by recombinant human bone morphogenetic proteins (BMP)-2 and -4. J Dent Res 73:1515–1522.

Nishi O, Nishi K, Mano C, Ichihara M, Honda T (1998): The inhibition of lens epithelial cell migration by a discontinuous capsular bend created by a band-shaped circular loop or a capsule-bending ring. Ophthalmic Surg Lasers 29: 119–125.

Nyberg SL, Shatford RA, Peshvwa MW (1993) Evaluation of a hepatocyte entrapment hollow fiber bioreactor: A potential bioartificial liver. Biotech Bioeng 41:194–203.

Petka WA, Harden, JL, McGrath KP, Wirtz D, Tirrell DA (1998): Reversible hydrogels from self-assembling artificial proteins. Science 281:389–391.

Sellke FW, Laham RJ, Edelman ER, Pearlman JD, Simons M (1998): Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. Ann Thorac Surg 65:1540–1544.

Shakesheff K, Cannizzaro S, Langer R, (1998): Creating biomimetic micro-environments with synthetic polymer-peptide hybrid molecules. J Biomater Sci Polymer Ed 9:507–518.

Vacanti CA, Kim W, Upton J, Mooney D, Vacanti JP (1995): The efficacy of periosteal cell compared to chondrocytes in the tissue-engineered repair of bone defects. Tissue Eng 1:301–308.

Wolter JR, Meyer RF (1984): Sessile macrophages forming clear endothelium-like membrane on inside of successful keratoprosthesis. Trans Am Ophthalmol Soc 82:187–202.

Yaeger PC, Masi TL, de Ortiz JL, Binette F, Tubo R, McPherson JM (1997): Synergistic action of transforming growth factor-beta and insulin-like growth factor-I induces expression of type II collagen and aggrecan genes in adult human articular chondrocytes. Exp Cell Res 237:318–325.

Yannas IV, Burke JF (1980): Design of an artificial skin. I. Basic design principles. J Biomed Mater Res 14:65–81.

Yannas IV, Burke JF, Gordon PL, Huang C, Rubenstein RH (1980): Design of an artificial skin. II. Control of chemical composition. J Biomed Mater Res 14:107–132.

Zegzula HD, Buck DC, Brekke J, Wozney JM, Hollinger JO (1997): Bone formation with use of rhBMP-2 (recombinant human bone morphogenetic protein-2). J Bone Joint Surg Am 79:1778–1790.