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Controlled delivery of inductive proteins, plasmid DNA and cells from tissue engineering matrices

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It has been estimated that half the annual health care budget in the United States is spent on patients suffering from tissue loss and late stage organ failure. Critical limitations inherent in traditional therapies call for novel tissue and organ replacement strategies. This paper discusses development of biomaterials for conductive, inductive and cell-based tissue replacement strategies. Biodegradable polymer scaffolds can be used as space-filling matrices for tissue development and barriers to migration of epithelial cells in tissue conductive approaches. Inductive approaches involve sustained delivery of bioactive factors, such as protein growth factors and DNA, to alter cell function in localized regions. Factors can be released from highly porous polymer scaffolds to allow factor delivery and tissue development to occur in concert. Cell-based approaches involve seeding of cells onto polymeric scaffolds *in vitro* and subsequent transplantation of the scaffold. New scaffold materials are being developed that address specific tissue engineering design requirements, and in some cases attempt to mimic natural extracellular matrices. These strategies together offer the possibility of predictably forming specific tissue structures, and may provide solutions to problems such as periodontal ligament detachment, alveolar bone resorption and furcation defects.

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Two of the most substantial health problems in the United States, from both a financial and humanitarian standpoint, are tissue loss and late-stage organ failure. It has been estimated that half the annual health care budget is spent on patients suffering from these conditions, an allocation in excess of $400 billion per year (1). At the present time the standard therapies are transplantation from either an autologous or allogenic donor site. These therapies are severely limited by the amount of tissues available for transplantation, and demand for tissues significantly outweighs supply (Fig. 1).

The critical limitations inherent in traditional therapies have prompted the development of a new strategy for tissue and organ replacement (2), termed tissue engineering. The emerging field of tissue engineering is concerned with the development of natural biological surrogates that restore, maintain, or improve upon tissue structure and function (3). Tissue engineering strategies have been applied to virtually all tissue types, and particularly significant progress has been made in the areas of skin (4), cartilage (5) and bone (6) regeneration. Specifically in the field of dentistry, new strategies for replacing diseased or damaged tissue offer widespread potential. Oral diseases (e.g. oral cancer, caries, periodontitis) are extremely prevalent and effect a large percentage of the population.

Three general strategies have emerged for engineering of tissues. The first is a conductive approach, in which synthetic scaffold materials amenable to infiltration of specific cell types are implanted into a site of disease or damage. The materials provoke conduction of desired cell types

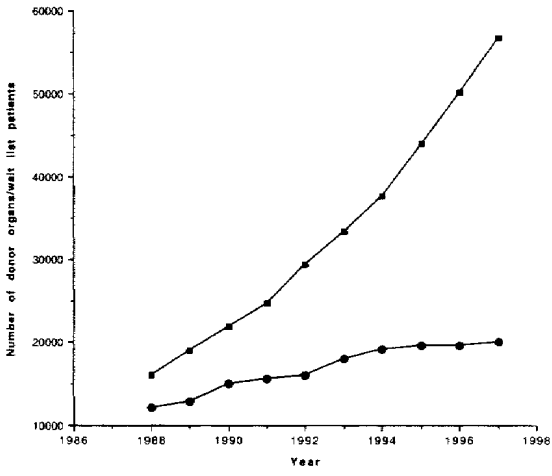

Fig. 1. Graph showing the divergence in the amount of organs recovered from cadaveric donors (●) vs. patients on the waiting list (■) over time: data taken from the United Network for Organ Sharing (UNOS), 1998 annual report.

while blocking conduction of unwanted cell types (e.g. epithelial cells). The second approach involves the inclusion of bioactive factors (e.g. growth factors) into the aforementioned synthetic scaffolds. The factors are chosen to spur the infiltration of the appropriate cell types, and induce the formation of a specific type of tissue. The third approach is based on seeding scaffolds with cells *in vitro*, followed by implantation of the cell construct. Our laboratory is interested in designing novel biomaterial scaffolds for use in each of the 3 general tissue engineering strategies.

Basic requirements in designing scaffolds for tissue engineering applications include degradability, biocompatibility, high surface area/volume ratio, mechanical integrity and vascular and neural infiltration (2). Materials used as scaffolds for tissue development must be degradable over a predictable and controllable time scale so that one can synchronize material degradation and natural tissue formation. This requirement is even more important in the context of recent inductive tissue engineering techniques in which material degradation is the major parameter determining factor delivery. The most widely used tissue engineering materials are the poly(α-hydroxy acids), specifically poly(L-lactic acid) (PLLA), poly (glycolic acid) (PGA) and their copolymers (7). They have a simple, predictable and controllable degradation mechanism and their products of degradation are natural metabolites, and thus pose minimal threat of inflammatory response in the host tissue (8). Simply by varying the relative amounts of lactic and glycolic acid present in a matrix formulation, one can vary degradation time from days to several months, while also controlling the scaffold's mechanical properties (9). Another key material requirement is a large surface area/volume ratio to support cell adhesion, and facilitate nutrient transport. A highly porous material promotes cell activity by extending the substrate area for growth and proliferation while also allowing for optimal diffusion of nutrients between cells in the scaffold and the surrounding tissue. Macropores are also intended to promote the ingrowth of granulation tissue from the host to allow for development of a vascular supply. Vascularization expedites mass transport, which is essential in the region of a developing tissue. Methods abound for producing PLLA, PGA and poly(lactide-co-glycolide) (PLG) materials with interconnected pores (open-pore structure) (10–13). Mechanical integrity of the scaffold material is necessary for resistance of contractile cellular forces, which can cause collapse of a scaffold's 3-dimensional structure during tissue growth. Some materials, such as PLG and alginate hydrogels, can be processed so that their mechanical strength is controlled. This can imply control over the shape of the final tissue product, resulting in a scaffold system with "shape memory" (14).

Conductive approach

Scaffolds can be designed to simultaneously conduct desired cell types and discourage conduction of undesired cell types, a strategy termed "guided tissue regeneration" (GTR). In most cases, a bioabsorbable synthetic construct is chosen so as to degrade and disappear in concert with new tissue development. The basic requirements of the system are degradability of the scaffold material over a controllable time scale and selectivity of cell conduction. Currently, the standard therapy for treatment of advanced periodontal disease involves the use of synthetic membranes (both degradable and non-degradable) as conduits for progenitor cells and barriers to infiltration of epithelial cells (15–21). Highly porous scaffolds for GTR could augment the barrier function of currently used membranes by providing a space-filling matrix for cell migration and tissue formation. This approach could provide a barrier to migration of epithelial cells by controlling the porosity and pore size distribution of the scaffold (22), while also defining a bioabsorbable matrix for tissue development. Recent work has shown growth of bone tissue in porous degradable PLG scaffolds (12), and porosity of the scaffolds can be readily controlled (23). The biocompatibility and degradability of these PLG scaffolds can also be combined with the osteoconductivity and mechanical integrity of a bone-like

mineral (BLM). Coating the inner pore surfaces of PLG matrices with a BLM layer significantly enhances their compressive modulus (24), and a BLM layer has been shown to enhance osteoconductivity of various orthopedic and dental implant materials (25).

Inductive approach

Recent tissue engineering approaches have been concerned with more accurately mimicking the processes of embryonic development and wound healing. Inductive approaches to engineering tissues are aimed at manipulating the process of tissue formation by controlled delivery of various bioactive factors involved in developmental processes. These factors can provide the means for manipulating cell proliferation, chemotaxis, differentiation and matrix synthesis, and thus exhibit potential for regenerative medicine. Factors examined as possible directors of tissue development include polypeptide growth factors and deoxyribonucleic acids (DNA) that encode for bioactive factors.

Factor delivery

Delivery of bioactive factors locally can induce cellular regenerative activity, potentially extending the natural wound healing process to full regeneration of the appropriate tissue, even in the absence of a conduit for regeneration. Delivery systems often take advantage of the controlled degradation of synthetic polymer scaffolds to release significant quantities of drug over an extended time scale (26). It is possible to tune material properties to manipulate the release profile over time, and thus precisely direct the regenerative process. For example, vascular endothelial growth factor (VEGF) can be released in a highly active form for periods from days to weeks from hydrogels composed of the polysaccharide alginate (Fig. 2) (27).

In periodontal tissue engineering, many of the bioactive factors relevant to periodontal ligament fibroblast (PLF), gingival fibroblast and osteoblast proliferation and differentiation have been identified (28). PDGF is the most potent factor in periodontal ligament regeneration, and has received the most attention from researchers (29–31). Investigators have developed various delivery systems (32–34), specifically for the delivery of PDGF to periodontal tissues, and in each case cellular activity was increased when factors were delivered in a sustained and controlled manner compared to the direct addition of factors *in vivo* or *in vitro*. Transforming growth factor beta (TGF-β), and basic fibroblast growth factor (bFGF) have

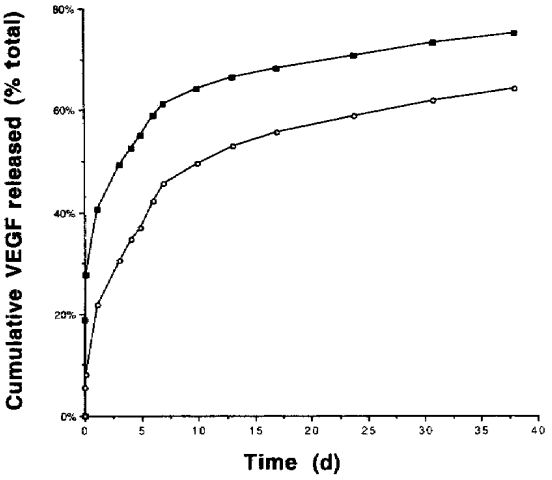

Fig. 2. Cumulative release of VEGF from alginate hydrogel beads gelled in either 1.0 M $CaCl_2$ (○), or 0.1 M $CaCl_2$ (■) as a function of time (from Peters *et al.* (27)).

also shown strong chemotactic and mitogenic effects on PLFs (34–36), and have been delivered from collagen (37), alginate (38), PLG (39) and methylcellulose gels (40). Bone morphogenetic proteins (BMPs) are potent factors related to alveolar bone regeneration and have been delivered to the periodontium via a collagen matrix carrier to induce cementogenesis and development of a functional periodontal ligament (41).

Factor release from scaffolds

Ideally, one would like to deliver bioactive factors directly from degradable, synthetic, 3-dimensional scaffolds capable of guiding the size, shape, and structure of regenerating tissues. This approach would allow for simultaneous induction of tissue formation and conduction of progenitor cells onto the matrix. Also, the inclusion of angiogenic factors into a scaffold could induce vascular infiltration, a critical factor in tissue development. Most processing methods for polymer scaffolds are dependent on a phase transition, and require the use of organic solvents or high temperatures which can denature proteins and DNA. We have developed a processing technique in which high-pressure gas foaming of poly(lactide-co-glycolide) (PLG) scaffolds is combined with particulate leaching to produce an open-pore structure (Fig. 3) without cytotoxic residuals. The unique feature of the process is that it maintains factor bioactivity by eliminating the use of organic solvents. The scaffolds are over 95% porous, and are thus amenable to cell infiltration and tissue growth. The controlled release of VEGF has been accomplished (42) by incorporating the factor into the gas-foaming process (12).

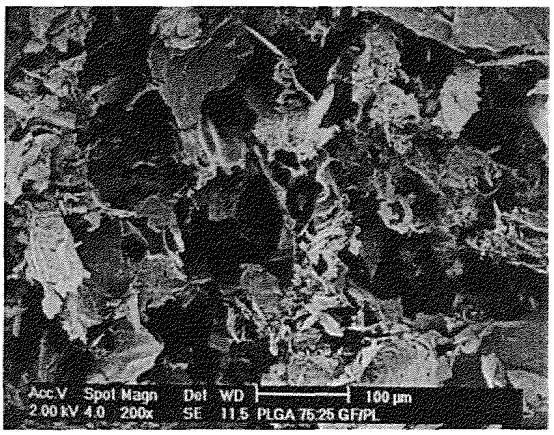

Fig. 3. SEM micrograph of the cross-section of a gas-foamed/particulate-leached 75 : 25 PLG scaffold (from Shea *et al.*).

The release from 75 : 25 PLGA was sustained over 60+ d *in vitro*, and the VEGF retained over 90% of its bioactivity.

Delivery of plasmid DNA from a polymer scaffold may lead to transfection of large numbers of cells at localized sites, inducing cells to produce therapeutic proteins that may ultimately enhance tissue development. Sustained release of DNA encoding for platelet derived growth factor (PDGF) from gas-foamed PLG scaffolds has been achieved (29). Release was sustained for periods of up to a month *in vitro*, and the delivery led to the transfection of a large number of cells *in vivo*. Delivery of DNA enhanced extracellular matrix deposition and blood vessel formation *in vivo*, exhibiting the functional potential of the technique (Fig. 4). Inducing certain cells to act as microbioreactors may improve the efficiency and specificity of drug delivery, and exert a new level of control over cell activity during tissue development.

Induction of new tissues is an exciting approach with clinical relevance in specific situations, but there are limitations. Even if inductive factors are able to control cell migration and activity, the factors have not been identified for all cell types, and there may be cell types for which factors do not exist. Also, these approaches rely on the postulate that progenitor cells reside in the tissue adjacent to a defect, and these cells will conduct themselves onto a material construct *in vivo* when provided with an appropriate migratory environment. This process may not occur consistently, and may be hindered in regions of significant tissue trauma or disease.

Cell-based approaches

An intrinsic limitation of tissue engineering using delivery of inductive factors is the inability to

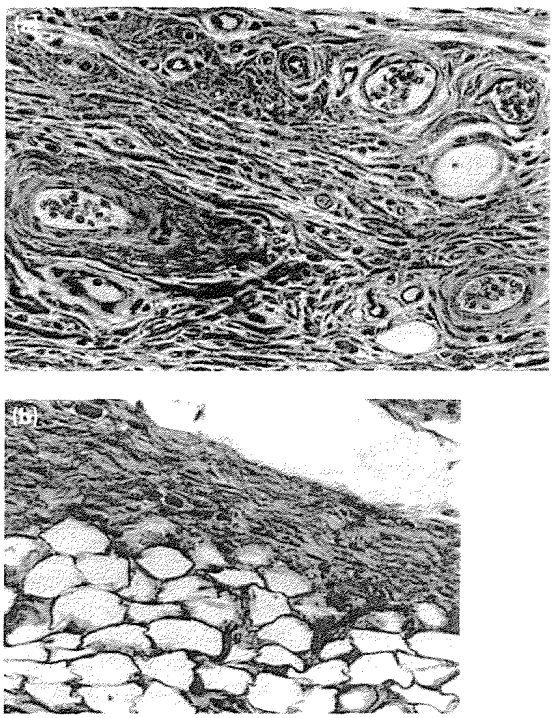

Fig. 4. Photomicrographs of tissue cross-section after 4 wk of implantation for (a) a matrix-releasing plasmid encoding PDGF and (b) control plasmid delivered by direct injection at magnification of 400 ×. Photomicrographs have labels for polymer (P), adipose tissue (A) and granulation tissue (G).

precisely predict and control which cell types conduct onto a construct and which biological factors are involved in the process of tissue development. Each of these parameters may be more accurately controlled using cell transplantation. Cell-based tissue engineering schemes involve seeding of cells onto a synthetic construct *in vitro* and subsequent transplantation of the construct (43). The most exciting advantage of this method is the possibility of treating a multitude of patients from a small tissue supply, which circumvents the problem of tissue and organ donor shortages. One can obtain the necessary cells for *in vitro* tissue development or cell transplantation via allogenic, autologous or xenogeneic tissues. Allogenic and xenogeneic cell-harvesting techniques are desirable because they involve minimal patient trauma; however, they also run the risk of tissue rejection and may necessitate the use of immunosuppressant drugs. Autologous cell-sourcing eliminates concerns about cell or tissue rejection, but requires 2 surgical procedures (cell isolation and cell transplantation), with a time lapse between the procedures to allow for cell expansion in culture.

Synthetic PLLA, PGA and PLG scaffolds have been used to engineer 3-dimensional tissues *in vitro*

and *in vivo*. Smooth muscle cells have been cultured in non-woven meshes of PGA bonded at their fiber cross-points with PLLA molecules. The fiber bonding mechanically stabilizes the matrices and allows them to resist contractile cellular forces during smooth muscle tissue development, resulting in maintenance of scaffold shape, and thus control over the shape of the tissue formed (14). A 3-dimensional culture system for hepatocytes has been developed using a porous PLLA scaffold. Hepatocytes seeded onto these matrices adhered and remained viable for 14 d *in vitro*, and immobilizing cells within the PLLA scaffolds with a collagen gel led to enhanced cell survival and function (44). These results suggest that a PLLA matrix could be used as a long-term culture medium for hepatocyte function and formation of liver tissue. In addition, transplantation of hepatocytes with these systems has led to the formation of new liver-like tissues (45). Similarly, a 3-dimensional culture system for osteogenic cells has also been developed using PLG scaffolds (23). Osteoblast progenitor cells follow their classic differentiation pathway within a 3-dimensional engineered tissue, and are able to form a 3-dimensional tissue with architecture similar to that of native bone after 12 wk in culture. The ability of osteoblast precursors to form new bone tissue *in vivo* following transplantation on porous biodegradable scaffolds has also been demonstrated (46). Each of these results exhibits the potential of cell-based methods to engineer tissues *in vitro* or *in vivo*.

Novel materials are currently being developed that exert a higher level of control over cell substrate interactions, and thus may mimic more closely natural extracellular matrices than the biodegradable polyesters (e.g. PLLA, PLG, PLGA). Synthetic extracellular matrices can be used to organize cells into a 3-dimensional architecture, provide mechanical integrity during tissue development, and provide a space for nutrient diffusion to and from the cell (47, 48). For example, cell anchorage and interaction with the extracellular environment can be controlled via covalent modification of alginate hydrogels with specific cell adhesion ligands. Skeletal myoblasts have been shown to adhere specifically to alginate hydrogels covalently modified with RGD-containing cell adhesion sequences with minimal adhesion to unmodified alginate (47). Variation of adhesion ligand density changes the local mechanical stress on cells and implies control over cell function, since mechanical signaling via the adhesion substrate is critical in the development of many different tissue types (49). Control over cell adhesion and interaction with the extracellular environment could prove critical in predictably engineering tissues using cell-based methods. Biodegradable versions of alginate in which the degradation time can be varied from days to months have recently been developed (50), and these may find widespread use in tissue engineering applications.

Future applications of tissue engineering to periodontal disease

All 3 approaches under development to engineer new tissues may find applications in treating periodontal disease. Since the periodontium includes 3 separate tissue types, control over tissue formation is at a premium. A conductive, porous, space-filling scaffold may be adequate for cell infiltration and new tissue development in the case of a small-scale periodontal defect and a single tissue type (e.g. alveolar bone). Regeneration of tissue for larger defects requires a vascular supply for the developing tissue, and thus inclusion of angiogenic factors into a scaffold may be necessary. Also, the inclusion of inductive factors can potentially be used to spur the infiltration, proliferation and differentiation of PLFs, cementoblasts and osteogenic cells while blocking infiltration of epithelial cells. This could result in more efficient tissue development with minimal interference. Defects involving multiple tissue types (e.g. periodontal ligament and alveolar bone) may require the application of cell-based tissue engineering methods. Implantation of scaffolds containing the necessary cells for tissue development could facilitate regeneration of specific tissues, and separation of different cell types within a single scaffold could result in development of hybrid tissues. The combination of conductive, inductive and cell-based methods could potentially lead to engineering of replacements for large-scale defects that require multiple tissue types. This general strategy holds the promise of controllably and predictably guiding the development of specific tissues and offers a new level of precision in tissue engineering.

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