DJ Mooney
T Boontheekul
R Chen
K Leach

Actively regulating bioengineered tissue and organ formation

Authors' affiliations:
David J. Mooney, K. Leach, Division of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA
T. Boontheekul, Department of Chemical Engineering, University of Michigan, MI, USA
Ruth Chen, Department of Biomedical Engineering, University of Michigan, MI, USA

Correspondence to:
David J. Mooney
DEAS, Harvard University
29 Oxford St., 325 Pierce Hall
Cambridge, MA 02138, USA
Tel.: +617 384 9624
Fax: +617 495 8543
E-mail: mooneyd@deas.harvard.edu

Dates:
Accepted 10 April 2005

To cite this article:
Orthod Craniofacial Res **8**, 2005; 141–144
Mooney DJ, Boontheekul T, Chen R, Leach K: Actively regulating bioengineered tissue and organ formation

Structured Abstract

Authors – Mooney DJ, Boontheekul T, Chen R, Leach K

Objectives – Describe current and future approaches to tissue engineering, specifically in the area of bone regeneration. These approaches will allow one to actively regulate the cellular populations participating in this process.

Design – Many approaches to actively regulate cellular phenotype are under exploration, and these typically exploit known signal transduction pathways via presentation of specific receptor-binding ligands, and may also deliver mechanical information via the physical bridge formed by the receptor-ligand interactions. Cellular gene expression may also be directly modulated utilizing gene therapy approaches to control tissue regeneration.

Conclusions – Significant progress has been made to date in bone regeneration using inductive molecules and transplanted cells, and FDA approved therapies have resulted. While approaches to date have focused on delivery of single stimuli (e.g. one growth factor), future efforts will likely attempt to more closely mimic developmental processes by the delivery of multiple inputs to the cells in spatially and temporally regulated fashions.

Key words: biomaterials; extracellular matrix; growth factors; peptides

Introduction

Limitations to the current therapies available for the reconstruction and replacement of craniofacial tissues has led to significant interest in tissue engineering approaches to these challenges (1). The tissue engineering approaches include conductive, inductive, and cell transplantation approaches. All three approaches have been successful in various animal models (1), and some have progressed to clinical application (e.g.

InFUSE™). However, for tissue engineering strategies to be useful in the craniofacial complex, which often involve multiple tissue types comprised of many cell types organized in a highly specific manner, it will likely be crucial to actively regulate the cell populations involved in tissue formation.

Materials are often used in tissue engineering to provide certain functions of the native extracellular matrix (ECM) that surrounds the cells in tissues. The physical properties of the ECM (e.g. ability to create space for tissue formation and provide mechanical support) have long been appreciated. The polymeric and ceramic materials used in guided tissue regeneration in dentistry are excellent examples of tissue engineering materials that mimic these functions of the ECM. However, these approaches depend on host cell populations to affect the tissue regeneration, and exert little active control over the cells, which limits the applicability of conductive tissue engineering approaches. Over the past few decades it has become clear that the ECM plays more than a passive spatial and mechanical role in tissues, as both peptides within ECM molecules and cellular receptors for these specific amino acid sequences have been identified (2). In addition, the ability of the ECM to serve as a depot for locally acting growth factors and to convey regulatory mechanical cues to cells residing within the ECM have become clear (3). Exploitation of these ECM functions via design and application of analogs used in tissue engineering may allow one to ultimately regulate, in a precise manner, the function of cells involved in tissue engineering and regenerative processes. This article overviews two aspects of the design of such materials – the presentation of cell binding peptides and delivery of inductive growth factors. These materials are discussed in the context of bone regeneration, using the authors' previous studies as the main examples.

Peptide presenting materials

Many functions of the ECM can be mimicked by small peptide fragments of the entire molecule (2), and these fragments may be produced synthetically and covalently coupled to synthetic polymers. To present the peptides with a high signal to noise ratio, the polymer chosen for peptide presentation should not mediate significant adsorption of contaminant proteins, and a variety of polymers including alginate and poly (ethylene oxide) meet this requirement (4). Peptide-incorporating biomaterials have demonstrated control over the adhesion, proliferation, and differentiation of bone forming cells (5). Recently, the nanoscale organization of the peptides presented from the material has also been demonstrated to regulate the proliferation and differentiation of interacting pre-osteoblasts (6).

An important advance over the past few years is the demonstration that peptide immobilized polymers can specifically enhance bone regeneration (reviewed in 7). RGD-coupled alginate hydrogels can control the formation of cartilaginous and bony tissues, *in vivo* (5,8,9). Strikingly, co-transplantation of both cell types together in this material leads to the formation of growing tissues that structurally and functionally resemble a growth plate (8). It is important to note that the degradation rate of these gels has also been demonstrated to play an important role in bone formation, in concert with peptide presentation (9). This effect is likely due to the formation of new space available for matrix deposition and mineralization with gel degradation. Recently, this system has also been demonstrated to allow bone formation with transplantation of bone marrow derived stromal cell populations (10), although provision of appropriate growth factors was needed for significant bone formation. This latter finding suggests that additional signals must be provided to progenitor cells, as contrasted to differentiated osteoblasts, from the matrix to promote bone formation *in vivo*.

Growth factor delivery systems

There are several important considerations in the design of a delivery system for growth factors used to regenerate or engineer tissues (11). First, the mode of factor delivery must target the desired cell population. Early applications of growth factors involved intravenous injection or injection of solutions containing the factor into the tissue of interest, but these methods did not effectively target the factors to the target tissue. Second, to achieve the desired cellular response, these factors must typically be present for time periods ranging from days to weeks. However, these molecules typically are degraded over time frames of minutes-hours *in vivo*. Polymeric delivery systems have been designed to bypass these limitations and meet the

design criteria for growth factor delivery (11). The factors themselves may be directly incorporated into the polymer, with the subsequent sustained release and local availability being regulated by diffusion of the factor or polymer degradation. Alternatively, plasmid DNA encoding the factor may be immobilized within the polymer, allowing the local production of the factor by cells that take up and express this DNA following implantation of the system at the desired tissue site.

Polymeric protein delivery systems can be successfully utilized to administer small doses of factors (proteins or plasmid DNA) at defined dose rates directly to target cells, and have been successfully used for bone regeneration via delivery of osteoinductive factors (7). Although successful, the controlled delivery of inductive growth factors from matrices to obtain bone regeneration requires supraphysiological protein concentrations, prompting concerns regarding the cost, safety, and efficient delivery of these molecules (7). Simultaneous delivery of two growth factors involved in bone regeneration with osteoprogenitor cells has recently demonstrated significant bone formation when using physiological dosages of these inductive proteins (10). These results suggest there is some level of cooperation between bone-forming cells and inductive factors in bone regeneration, and providing both together may lead to synergistic effects.

The role of vascularization in bone regeneration has been clearly highlighted in recent studies, and vascular endothelial growth factor (VEGF) has been demonstrated to play a particularly important role (12,13). The VEGF is a chemotactic signal for endothelial cells to migrate and form new blood vessels, and osteoblasts have also been shown to both produce VEGF and undergo chemotactic migration in response to this angiogenic molecule (14). The delivery of VEGF can improve angiogenesis and bony bridging of a fracture (12), while VEGF-induced blood vessel ingrowth into a PLG scaffold has recently resulted in enhanced regeneration of mineralized tissue (15). These studies suggest strategies that include both osteogenic and angiogenic stimuli may optimally regenerate bone. Further, the finding that sequential delivery of multiple growth factors that act at varying stages of vessel formation can promote both formation and maturation of vascular networks (16) suggests that appropriately timed delivery of multiple factors may be beneficial in many tissue regenerative processes.

Clinical utility and implications

Osteoconductive, osteoinductive, and cell transplantation strategies all have individually resulted in improved bone regeneration in various animal models or human clinical trials. A variety of guided tissue regeneration materials have been available and are routinely used in clinical practice, although their utility is limited. There are also two currently approved uses of one of the recombinant inductive molecules (BMP-2), and clinical trials examining BMP-7 and cell-based approaches are ongoing. The limited number of inductive and cell transplantation products that are currently FDA approved may be related to several factors, including issues related to cell sourcing, complications in translating results from animal models to humans, and the use of single factors instead of combinations. An improved understanding of the biology of bone healing may allow investigators to address these issues. In addition, improved carriers for the delivery of single factors or cocktails of inductive molecules and cells, as described in this review, may greatly enhance their utility and speed their application to clinical dentistry. We anticipate that the clinical application of these inductive molecules and bone-forming cells will increase in the next few years as these issues are addressed.

A number of challenges must be addressed to commercialize tissue-engineered therapies. Supraphysiologic quantities of recombinant proteins are currently required to elicit the desired response, but providing combinations of molecules with appropriate cell populations may allow physiologic concentrations of inductive molecules to be effective. For cell transplantation strategies, although autologous cells raise the least concerns for immune response, procedures to both first obtain the cells, and then to transplant the cells are required. Bone regeneration with allogeneic cells, on the other hand, would allow for cells and tissues to be produced in large batches, and accelerate the application of these therapies to large numbers of patients. This combination of scientific, engineering, clinical and additional economic issues will likely lead to multiple strategies being used to treat various craniofacial defects in the future.

Acknowledgements: The author's research in this area is supported by the NIH (NIDCR, NHLBI).

References

1. Alsberg E, Hill EE, Mooney DJ. Craniofacial tissue engineering. *Crit Rev Oral Biol Med* 2001;**12**:64–75.
2. Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987;**48**:549–54.
3. Park JE, Keller GA, Ferrara N. The vascular endothelial growth factor (VEGF) isoforms: differential deposition into the subepithelial extracellular matrix and bioactivity of extracellular matrix-bound VEGF. *Mol Biol Cell* 1993;**4**:1317–26.
4. Lee KY, Mooney DJ. Hydrogels for tissue engineering. *Chem Rev* 2001;**101**:1869–80.
5. Alsberg E, Anderson K, Albeiruti A, Franceschi R, Mooney DJ. Cell interactive alginate hydrogels for bone tissue engineering. *J Dent Res* 2001;**80**:2025–29.
6. Lee KY, Alsberg E, Hsiong S, Comisar W, Linderman J, Ziff R et al. Nanoscale adhesion ligand organization regulates osteoblast differentiation in vitro. *NanoLetters* 2004;**4**:1501–6.
7. Leach JK, Mooney DJ. Bone engineering by controlled delivery of osteoinductive molecules and cells. *Expert Opin Biol Ther* 2004;**4**:1–13.
8. Alsberg E, Anderson KW, Albeiruti A, Rowley JA, Mooney DJ. Engineering growing tissues. *PNAS* 2002;**99**:12025–30.
9. Alsberg E, Kong, HJ, Hirano Y, Smith MK, Albeiruti A, Mooney DJ. Regulating bone formation via controlled scaffold degradation. *J Dent Res* 2003;**82**:903–8.
10. Simmons CA, Alsberg E, Hsiong S, Kim WJ, Mooney DJ. Dual growth factor delivery and scaffold degradation enhance in vivo bone formation by transplanted bone marrow stromal cells. *Bone* 2004;**35**:562–9.
11. Chen RR, Mooney DJ. Polymeric growth factor delivery strategies for tissue engineering. *Pharma Res* 2003;**20**:1103–12.
12. Street J, Bao M, de Guzman L, Bunting S, Peale FV Jr, Ferrara N et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc Nat Acad Sci USA* 2002;**99**:9656–61.
13. Gerber HP, Ferrara N. Angiogenesis and bone formation. *Trends Cardiovas Med* 2000;**10**:223–8.
14. Deckers MM, van Bezooijen RL, van der Horst G, Hoogendam J, van der Bent C, Papapoulos SE et al. Bone morphogenetic proteins stimulate angiogenesis through osteoblast derived vascular endoethelial growth factor A. *Endocrinology* 2002;**143**:1545–53.
15. Murphy WL, Simmons CA, Kaigler D, Mooney DJ. Bone regeneration via a mineralized substrate and inducing angiogenesis. *J Dental Res* 2004;**83**:204–10.
16. Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. *Nat Biotechnol* 2001;**19**:1029–34.