Introduction

This issue is a chronicle of original research concerning Repair and Regeneration of Oral and Craniofacial Tissues. The articles elaborate the contributions of participants at four integrated sessions at the 32nd Annual Meeting and Exhibition of the American Association for Dental Research that was held in San Antonio, Tex., from March 12 to 15, 2003. The Co-Organizers and Co-Chairs were Arthur English, PhD and Julie Glowacki, PhD.

The overall goal of the symposium and of this dedicated issue of *Cells Tissues Organs* was to highlight important advances in basic and applied research that hold promise for repair and regeneration of oral and craniofacial tissues. An array of tissues was included in order to uncover the similarities in scientific approaches as well as the unique problems and solutions applicable to specific tissues and organs. These include dental enamel, bones, cartilage, muscle, skin, oral mucosa, and nerves.

The first group of four papers concerns discovery technologies and, in particular, some of the ways to discover new genes and new roles for genes, with emphasis on leading edge methodology and use of microarrays and macroarrays, novel models, and complex tissues. The report by Paine et al. describes an experimental test of the possible role of a proteolytic fragment of amelogenin, known as tyrosine-rich amelogenin peptide (TRAP), in mature enamel structure and morphology. Inasmuch as amelogenin protein is degraded during enamel mineralization, identification of measurable quantities of the TRAP fragment suggested that it may play a specific role in mineralization. Overexpression of TRAP in mice was confirmed and produced some variations in mineral voids by scanning electron microscopy, but with little detectable effect

on enamel structure or function. The study provides a systematic guide on use of transgenic technology for hypothesis testing.

The contribution by Perrino and Yelick is an elegant immunohistochemical analysis in zebrafish (*Danio rerio*) of a BMP/TGF- β receptor, Alk8, that had been known to be involved in early embryonic dorsoventral patterning, neural crest development, and patterning of some of the pharyngeal arch cartilages. Because of its short gestational time, large number of clutches per mating, and optical clarity of the embryo, the zebrafish has become very useful for genetic analysis of vertebrate development. Although the zebrafish jaws are not tooth-bearing, its pharyngeal arches support the continuous eruption of replacement teeth appropriate for the species' feeding behavior. Understanding tooth regeneration in zebrafish may provide insights for human applications. This paper documents Alk8 in various structures in zebrafish from 2 days postfertilization to 1 year of age. The authors conclude that similar molecular cascades may regulate development of primary and replacement teeth.

Rutherford and colleagues use microarrays to discover genes that participate in skeletal cell differentiation. The analysis of genes altered by BMP-7 revealed novel potential regulators of Runx2/Cbfa1 in osteoblast and chondroblast differentiation. Finally, Glowacki and colleagues review the principles of gene expression analysis by high-throughput macroarrays and representational difference analysis, functional data reduction, and discovery of novel chondrocyte genes in a model of chondroinduction of human skin fibroblasts by culture with demineralized bone.

The second group of three papers concerns new findings in molecular and epigenetic mechanisms of tissue growth and repair. Ogle et al. describe the roles of dura mater and sutures in orchestrating cranial morphogenesis and growth. Further, this work begins to dissect the signaling pathways by which mechanical forces act on calvariae and how the FGF system mediates tensional forces that delay fusion of sutures in a rat *in vitro* model. Horseley and Pavlath compile knowledge about the calcium-regulated transcription factor NFATC2 in myoblast fusion. Muscle regeneration fails if there is disorder in the process that begins with activation of muscle precursor (satellite) cells, formation of multinucleated myofibers, and maturation of the myofibers. Factors such as interleukins 4 and 13 that act on macrophages also act on myoblasts. Myofiber development is altered by pharmacological agents that block NFAT-mediated transcription and may be manipulated by proteins controlled by NFAT. They use mouse masseter muscles to test whether genetic engineering can be used to provide specific agents that promote repair. Gomes et al. review knowledge about the large heparan sulfate proteoglycan, perlecan, that is found in embryonic and adult cartilage. The current model is that perlecan provides an organizational matrix for chondrogenesis and suggests that it may provide a therapeutic approach for replacing damaged cartilage.

The third group of three papers concerns potential enhancement of innate repair mechanisms with factors or genes. Le et al. report that VEGF is overexpressed and overproduced in human keloids and that these tissues are relatively hypoxic compared to normal skin. These findings are paralleled by reported decreases in VEGF found in diabetic skin. Possible pathophysiological mechanisms include VEGF's role in inflammation and its effects on extracellular matrix metabolism. Leboy and colleagues document species disparity in BMP-2's stimulation of osteoblast differentiation from marrow stromal cells. Although BMP-2 is a potent stimulus for osteoblastogenesis in rat and mouse marrow cultures, human marrow stromal cells are far less responsive even though they are equally stimulated by dexamethasone. Original data rule out attributing the species difference to deficiency of Alk-6, to excess Msx-2, to Runx2 isoforms, or to forkhead transcription factor FKHR. It remains possible that aging accounts for some of the differences seen. In a related

paper concerning BMP for skeletal regeneration, Franceschi et al. present evidence of substantial synergies in combinatorial gene therapy to express different combinations of BMPs with or without related transcription factors. Although complex, such therapeutic strategies are designed to mimic the natural processes of bone development and postnatal repair and depend upon more precise information about the timing and magnitude of factor interactions.

The fourth group of three papers comprises examples of cell-based and scaffold approaches to tissue engineering. The work of Mina and Braut concerns the identification of donor stem cells for various components of teeth and shows that murine coronal dental pulp contains cells that can give rise to odontoblasts and osteoblasts. Specific markers are being used to track the two differentiation pathways. Feinberg and colleagues lead a program of research on engineered oral mucosal equivalents and have advanced to clinical trials. They describe the mandated regulatory processes and how their team has addressed the experimental path to expedite FDA approval. The contribution of Yannas and colleagues illustrates the important properties of collagen scaffolds to support nerve regeneration across a gap. The quality of regenerated nerves depends on pore size and cross-link density, with optimization corresponding to scaffold degradation of approximately 2–3 weeks.

This collection of reports constitutes a discovery path for integrating new basic information about regulatory genes and factors with technical approaches for enhancing repair and regeneration. Evidence shows the potential for therapies based upon factors, and/or cells, and/or scaffolds. Nevertheless, barriers to clinical applications become evident as simple concepts fail in preliminary *in vitro* and *in vivo* studies. There are likely to be multiple successful strategies, but with variable potential for transferring to different tissue applications. Approaches directed at innate repair mechanisms are the most likely to offer safe and reliable therapies, but may need to be customized for individual patients. Interactions between biologists, engineers, clinicians, and regulatory bodies will continue to drive progress in craniofacial and oral regenerative medicine.

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