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Tissue Engineering (Session Summary)

Bone tissue engineering

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The well-known limitations associated with clinical use of autografts and allografts continue to drive efforts to develop bone graft substitutes using the principles of biomaterials and tissue engineering. An important consideration in the development of bone regeneration strategies is the broad diversity of clinical problems with widely varying biological, mechanical, and structural challenges. Craniofacial reconstructions, for example, often involve highly complex geometries that suggest a conformal filling biomaterial that matches the shape of the defect. Non-united fractures are often complicated by excessive motion, fibrosis and, perhaps, infection. Spine fusion calls for a locally delivered biological solution involving osteoinductive signals, since bone must be formed where none existed previously. Large segmental bone defects, greater than three centimeters, are a particularly challenging problem for orthopaedic surgeons and require consideration of mass transport issues and the high mechanical demands under load-bearing conditions. As a result of this diversity, it is unlikely that a single approach to bone regeneration will emerge for these clinical problems.

The objectives of the Bone Tissue Engineering session were to: (i) present the current status of translational approaches to engineering bone regeneration; (ii) understand physical and chemical factors that influence cellular responses to biomaterials, and how to design biomaterial interfaces that direct cell function; and (iii) compare different strategies for bone tissue engineering involving biologic augmentation of scaffolds with osteogenic genes, proteins, or cells.

Professor Robert Guldberg gave a brief introduction on current clinical approaches to bone repair and their limitations. The current clinical gold standard for augmenting bone repair is the transplantation of fresh autologous bone. Although effective for relatively small, non load-bearing defects, autografting is limited by the small volume of available material, lack of structural integrity, and significant donor site morbidity. Allografts are available in large structural pieces and have grown in use clinically over the past twenty years but typically do not fully revascularize and remodel and are associated with a high rate of failure[1]. Recent animal studies have shown that revitalization of allografts via coating with osteogenic cells or osteoinductive genes stimulates improved revascularization, remodeling, and bone repair[2-4]. Tissue engineering approaches involving various combinations of biomaterials and biologics such as proteins, cells, and genes thus represent a very promising alternative to bone grafts for restoring the function of damaged or degenerated bone.

Numerous types of synthetic scaffolds have been fabricated and investigated in the search for effective bone graft substitutes. There are many scaffold design variables that can strongly influence the biologic response including the single or multi-phase composition of the scaffold, surface chemistry, architectural parameters such as pore size and interconnectivity, the rate of degradation and the degradation products, and the local mechanical properties of the matrix such as modulus and viscoelasticity. Structural biomaterial scaffolds that provide adequate mechanical properties must often be surface modified or combined with bioactive components to achieve the desired mix of properties.

Dramatic advances in biomaterials synthesis, biological performance evaluation, and engineering analysis have combined to create unprecedented choices for the selection of scaffold materials. Professor Guldberg introduced different strategies for biologic augmentation of biomaterial scaffolds. He emphasized the need for quantitative *in vivo* models and methods to discriminate among the many possible choices

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and combinations of growth factors, cells, and genes that may be used to promote osteogenesis[5]. Dr. Guldberg concluded with data showing differential osteogenic capacity of stem cells derived from marrow compared to those isolated from amniotic fluid and a discussion on critical factors for effective stem cell delivery, including cell survival and osteogenic programming following implantation.

As one example of a biologic augmentation strategy, Professor Paul Krebsbach presented gene therapy strategies targeted to craniofacial clinical applications. He demonstrated a significant positive effect of combining osteoinductive gene therapy with systemic parathyroid treatment. Dr. Krebsbach also emphasized the importance of testing bone regenerative strategies in clinically relevant models. He specifically introduced craniofacial defect animal models that had been pretreated with radiation to better represent the challenging local healing environment present in many cancer patients.

Professor Andrés García then addressed biomimetic interfaces designed to control cell fate in the peri-implant region. The biomimetic modification strategies centered on peptide mimetics based on motifs found in collagen and fibronectin, and *in vitro* characterization modalities stressed issues of peptide surface density, structure, and their effect on cell adhesion and differentiation[6-10]. Encouraging early stage *in vivo* results in a transcortical model demonstrated that peptide-coated implants had high bone implant contact and fixation strength.

In spite of these impressive efforts, biomaterials that precisely control the survival, proliferation, and fate of stem cell populations *in vitro* and *in vivo* are not well developed. Dr. Adam Engler mechanistically approached the effect of matrix modulus on multipotent bone-marrow derived mesenchymal stem cell differentiation. Soft matrices that mimic the mechanics in brain were neurogenic, while stiffer matrices that mimic muscle were myogenic, and comparatively rigid matrices that mimic bone proved osteogenic[11]. The results presented contribute to understanding physical effects of the cell's microenvironment on cell function and may ultimately prove important for therapeutic uses of stem cells.

The session concluded with Professor Kevin Healy stressing the need to assess the wound environment such that biomimetic materials can be specifically designed to control or redirect tissue regeneration at the molecular level. An understanding of the temporal gene and protein expression profiles during bone tissue formation was used to design hydrogel scaffolds for *in situ* bone regeneration. Specifically, the complete expression profile of matrix metalloproteinase (MMP)-related and ECM-related genes in the rat femoral ablation model was used to better design scaffolds to work temporally with the resident stem cells and MMPs naturally expressed during bone regeneration. Hydrogel scaffolds exploiting MMP-degradable cross-links and peptide adhesion motifs based on bone sialoprotein facilitated bone regeneration in the ablation model[12-14].

A lingering question that was emphasized during the various presentations is how much biological information is needed for biomaterials to become clinically attractive alternatives to existing bone graft substitutes? Although the answer to this question is unknown, one can glean from the session that there are myriad methods to achieve the same end goal of bone regeneration, and that minimal incorporation of biological components (e.g., genes, anti-sense or small interfering RNA, peptides, growth factors, and small molecular weight drugs) into biomimetic scaffolds represents the shortest path for translation into the clinic.

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