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Meeting Report: A Close Look at Teeth, Orofacial and Bone Development and Regeneration

Yitong Liu[1], **Xiaoyan Li**[1], **Dana Graves**[2], **Songling Wang**[1], **Keya Mao**[3], **Zhi Chen**[4], **Xiaoling Zhang**[5], **Bei Li**[6], **Xiaoxing Kou**[7], **Songtao Shi**[8], **Syngcuk Kim**[9], **Yi Liu**[1*] **and Shuying Yang**[8*]

[1]*Department of Periodontics and Laboratory of Tissue Regeneration and Immunology, School of Stomatology, Capital Medical University, Beijing, China*

[2]*Department of Periodontics, School of Dental Medicine, University of Pennsylvania, Philadelphia, USA*

[3]*Department of Orthopedics, Chinese PLA General Hospital, Beijing, China*

[4]*The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) and Key Laboratory of Oral Biomedicine Ministry of Education (KLOBM), School and Hospital of Stomatology, Wuhan University, Wuhan, China*

[5]*Department of Orthopedic Surgery, Xin Hua Hospital Affilliated to Shanghai Jiao Tong University School of Medicine (SJTUSM), Shanghai, China*

[6]*State Key Laboratory of Military Stomatology and National Clinical Research Center for Oral Diseases and Shaanxi International Joint Research Center for Oral Diseases, Center for Tissue Engineering, School of Stomatology, The Fourth Military Medical University, Xi'an, China*

[7]*Guanghua School of Stomatology, Sun Yat-sen University, Guangzhou, Guangdong, China*

[8]*Department of Anatomy and Cell Biology, School of Dental Medicine, University of Pennsylvania, Philadelphia, USA*

[9]*Department of Endodontics, School of Dental Medicine, University of Pennsylvania, Philadelphia, USA*

[*]**Corresponding author:** Yi Liu, Department of Periodontics, Laboratory of Tissue Regeneration and Immunology, Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, School of Stomatology, Capital Medical University, Tian Tan Xi Li, Beijing, China, Tel: +2158982685; E-mail: lililiuyi@163.com

Shuying Yang, Department of Anatomy and Cell Biology, School of Dental Medicine, University of Pennsylvania, Philadelphia, USA, Tel: +2158982685; E-mail: shuyingy@upenn.edu

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Abstract

Penn Dental Medicine Orofacial and Bone Stem Cell Research Symposium was organized by Penn Dental Medicine and hold on November 17, 2018, at Penn Wharton China Center (PWCC). This symposium marks the fourth collaborative research symposium of the Penn China Research and Engagement Fund (Penn CREF). The topics were provided by the outstanding biomedical scientists who are actively engaged in stem cell and bone research, from Penn Dental Medicine, and Chinese partner institutions, including Capital Medical University, Chinese PLA General Hospital, Fourth Military Medical University, Shanghai Jiao Tong University, and Wuhan University. The topics include: orofacial dental tissue development and regeneration; teeth development and a typical translational large animal model for orofacial tissue regeneration and functional reconstruction; Scaffold independent mesenchymal stem cells-based orofacial tissue regeneration; Orofacial Soft tissue repair and regeneration; Orofacial hard tissue formation and regeneration

Keywords: Dental medicine; Bone stem cell; Teeth; Enamel

Introduction

Teeth are complex structures composed of hard tissues like enamel, dentin, and cementum, as well as soft tissues like dental pulp. Moreover, a healthy tooth with physiological functions also needs its supportive tissues such as alveolar bone and associated periodontium, comprising both hard and soft tissues. Common diseases associated with the tooth are chronic bacterial infections like periodontitis, caries, and pulpitis. Currently, in clinic, we have a consensus that bacterial infections damage both hard and soft tissues associated with teeth, thereby posing a significant challenge in tooth regeneration due to its complex structure and limited self-healing capability [1]. Recently, we found that Mesenchymal Stem Cell (MSC)-based therapy has significantly improved tissue regeneration in preclinical models and clinical trials [2,3]. The stem cells used for tooth and periodontal regeneration could be both dental or non-dental Mesenchymal Stem Cell origin (MSCs)[4-6]. However, during oral disease or injury, the quality of regenerated tissue is influenced by damaged MSC functions, inflammatory microenvironment, and the activated host immune system. More and more studies believe that the host immune system has fundamental effects during MSC-based therapy [7]. Although stem cell implantation considerably improved tissue regeneration in animal models, the fate and function of stem cells after transplantation and the potential molecular mechanisms still need to be clarified before we widely use it for clinical applications in humans in the future [8].

As one of the oldest university-affiliated dental institutions in the USA, Penn Dental Medicine is generally recognized as a leader in the education and training of clinicians, researchers, and academicians that continue to advance the field of dental medicine. Penn Dental Medicine Orofacial and Bone Stem Cell Research Symposium in this year mark the third collaborative research symposium of the Penn China Research and Engagement Fund (Penn CREF). Launched on March 2015, Penn Dental Medicine is one of the first recipients of this award. The grant supports the development of high-level research symposia on bone, biofilm, and stem cells, as well as the expansion of conferences on the delivery of dental care to China's vast population in recent years. It is a great honor to have had Penn Dental Medicine organizes this year's symposium on 17 November at Penn Wharton China Center (PWCC) in Beijing, China. This year's symposium provided a platform for introducing new initiatives to advance ongoing research, showcase the breadth of Penn Dental Medicine's continuing

strength as a leader in the generation of new knowledge and treatment modalities to prevent orofacial and bone diseases and promote oral health. Lively discussions took place throughout the day, and this meeting report highlights some of the major findings and ideas that emerged during the symposium.

The study on teeth development and a typical translational large animal model for orofacial tissue regeneration and functional reconstruction

To understand the signal network related to tooth development and thus reduplicate the development process is one of the strategies to achieve tooth regeneration [9,10]. A group led by Professor Shuying Yang from the University of Pennsylvania School of Dental Medicine found that IFT80 is required for stem cell proliferation, odontoblast differentiation, and polarization during tooth development. They revealed that Dental Pulp Stem Cells (DPSCs) express IFT80, which is required for controlling DPSCs properties. Mice with deletion of IFT80 in odontoblast lineage show impaired molar root development and delayed incisor eruption through reduced Dental Pulp Stem Cell (DPSC) proliferation and differentiation, and disrupted odontoblast polarization. Inhibited odontoblast differentiation resulted from disrupted hedgehog (Hh) signaling pathways. While decreased DPSC proliferation is associated with impaired Fibroblasts Growth Factor 2 (FGF2) signaling caused by loss of IFT80, leading to the disruption of FGF2-FGFR1-PI3K-AKT signaling in IFT80-deficient DPSCs. These results provide the first line of evidence that IFT80 controls tooth development through influencing DPSC proliferation, differentiation, and odontoblast polarization and associated Hh and FGF/AKT signaling pathways, demonstrating IFT proteins are likely new therapeutic targets for tooth and other tissue repair and regeneration. However, compared to the mice, the miniature pig is more suitable as a model in many fields of biomedical research, for its similarities to human beings in anatomy, physiology, histology and nutrition metabolism [11-13]. Considering the oral maxillofacial region of the miniature pig is similar to that of human beings, we use miniature pigs as large animal models in dental and orofacial research [14-16]. For many years, Professor Songlin Wang from Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, Capital Medical University has focused on dental and orofacial research in miniature pigs. They have used miniature pigs for studying the development of the tooth, dental stem cells-based bio-root regeneration; periodontitis model and autologous or allogeneic dental stem cell-based therapy; osteoradionecrosis model, bisphosphonates-related osteonecrosis model, and mesenchymal stem cell-based therapy, and for gene transfer to salivary glands. They determined swine tooth developing phases (stages), constructed cDNA library, identified the specific transcriptome and cDNA profile, the specific microRNAome and expression profile in developing teeth of the miniature pig, identified the characteristic patterns about Spatio-temporal morphogenesis of successional teeth in context of their predecessor and cascade initiation of additional molars in miniature pigs. Moreover, they discovered that biomechanical stress-associated Wnt translocation regulates organ renewal rhythm in the transition to permanent teeth using miniature pig model. Their studies demonstrated that dental stem cell-based bio-root, periodontal, bone tissue regeneration, mesenchymal stem cell-based therapy for osteoradionecrosis, bisphosphonates-related osteonecrosis and gene transfer-mediated salivary function restoration have been achieved in miniature pigs at the pre-clinical level. They also developed a convenient large animal model for gene transfer to salivary glands and parotid gland irradiation damage model in miniature pigs. After AdhAQP1 or Ad-Shh delivery, the saliva flow rate in radiated glands recovered significantly. All in all, the miniature pig is a useful large animal model in dental and orofacial research, especially for typical translational medicine studies in dentistry.

Scaffold independent mesenchymal stem cells-based orofacial tissue regeneration

Some experiments showed that tissue repair and regeneration could be improved by regulating the function of MSCs [8] and the immune cells like macrophages [7]. However, the method of regulating this process, or promoting teeth regeneration *in vivo* remains unclear. The group of Professor Bei Li from Tissue Engineering Center at the Fourth Military Medical University conducted the clinical trial using autologous periodontal ligament stem cells to regenerate periodontium in patients, which may be the first regenerated organ in the clinic. According to their experiences, they believe there are two main bottlenecks in tooth regeneration, the first is the connection of periodontium and tooth, and the second is the full-length pulp regeneration. To solve these problems, key strategies to regenerate tooth should be; first, constructing pulp and regenerating pulp; second, constructing periodontal tissue and stabling the tooth; third, using biomaterials to generate hard tissue and combining pulp and periodontium to regenerate an intact tooth. They also explored the mechanism of regenerating periodontal tissue. They find epigenetic regulation and ER stress play key roles in modulating the regeneration of periodontal ligament stem cells. Moreover, they use autologous deciduous stem cells to regenerate dental pulp after implantation into injured teeth. The study suggests that deciduous stem cells are able to regenerate whole dental pulp and may be useful for treating tooth injuries due to trauma. Work by Zhi Chen from School and Hospital of Stomatology (Wuhan) explored the role of Klf4 and histone acetylation in dentinogenesis. The Wnt1-Cre; Klf4fx/fx (Klf4 cKO) mice showed significantly impaired dentin mineralization and enlarged pulp/root canal. Dmp1, Dspp, and Osx were downregulated in the odontoblasts from Klf4 cKO mice. Combinatory analysis using RNA-seq and ATAC-seq revealed that Klf4 could transcriptionally regulate odontoblastic differentiation through directly binding to the promoters of Dmp1 and Osx. Then immunoprecipitation demonstrated that KLF4 interacted with histone acetylase P300 and HDAC3. Next, ChIP analysis detected P300 and HDAC3 enrichment on the promoter region of Klf4 target genes Dmp1 and Osx. HDAC3 mainly interacted with KLF4 on day 0 of odontoblastic induction while P300 on the day 7 of induction. These spatiotemporal interactions regulated Dmp1 and Osx transcription thus regulating dentinogenesis. Taken together, these results demonstrated that Klf4 regulated Dmp1 and Osx transcription via modulation of histone acetylation which is vital to dentinogenesis.

Orofacial dental tissue development and regeneration

Over the past decades, we have treated the oral disease by "removing" the infected tissue and replacing it with a man-made prosthesis, which could not completely restore the function we need, thus reduced the quality of life. Scaffold independent mesenchymal stem cells-based tooth regeneration may provide a therapeutic alternative in the future [17,18]. Nowadays we have the consensus that there are two strategies for tooth regeneration: one is stimulating the development of tooth germ with dental/non-dental mesenchymal stem cells; the other one is to promote the regeneration of pulp, dentin, periodontal ligament, and biological root by transplanting MSCs

mixed with biomaterial scaffolds *in vivo*. Both of these strategies are currently under investigation.

Orofacial soft tissue repair and regeneration

Soft tissue repair can be briefly divided into three stages, which are the inflammation stage, extracellular matrix deposition and tissue reconstruction stage [19]. The tissue healing process is controlled by a network of multitype cells, in a spatiotemporal manner, including Mesenchymal Stem Cells (MSCs), keratinocytes, and fibroblasts [20-23]. During the soft tissue repair and regeneration process, MSCs are capable of homing and engrafting into damaged tissues, releasing trophic factors, secreting ECM and promoting neovascularization [24]; thus, they are an indispensable component of the wound healing process. The biological ability of MSCs is influenced by the microenvironment, transcription factors, growth factors, and multiple cell signaling pathways. Professor Xiaoxing Kou from the University of Pennsylvania School of Dental Medicine gave a novel talk mentioning IL-1RA secreted by MSCs accelerated the wound healing process. Mesenchymal Stem Cells (MSCs) are capable of secreting exosomes, extracellular vesicles, and cytokines to regulate cell and tissue homeostasis. However, it is unknown whether MSCs use a specific exocytotic fusion mechanism to secrete exosomes and cytokines. Professor Kou revealed that MSCs produced and secreted interleukin 1 receptor antagonist (IL-1RA) associated with sEVs to maintain rapid wound healing in the gingiva via the Fas/Fap-1/Cav-1 cascade. Besides, they also found that tumor necrosis factor-alpha (TNF-α) served as an activator to upregulate Fas and Fap-1 expression via the NF-κB pathway to promote IL-1RA release. The oral microbiome of the average adult is incredibly complex harboring about 50 to 100 billion bacteria in the oral cavity, which represent about 200 predominant bacterial species [25]. The bacteria of oral microbiome play an important role in oral health and disease or afflictions, such as dental caries, periodontal diseases, endodontic lesions, dry socket, halitosis, and odontogenic infections [26-29]. However, it is unknown whether oral microbiome affects oral stem cells and MSC mediated soft tissue repair process. Professor Yi Liu from Capital Medical University, School of Stomatology precisely indicated that the imbalance of oral microbiome led to the deficiency of mucosa/gingival stem cell, which delayed the soft tissue wound healing process. Mechanically, oral microbiome release LPS to stimulate the expression of microRNA-21 (miR-21) and then through the miR-21/Sp1/TERT pathway to maintain oral stem cells normal function and wound healing capability. After common clinic anti-biotic treatment, the oral microbiome ecology was altered which broke the homeostasis of MSCs. The results indicated for the first time, the interplay between the oral microbiome and MSC mediated soft tissue repair. Soft-tissue regeneration involves coordinated gene expression that is orchestrated by transcription factors, and FOXO1 as a member of transcription factors plays a vital role in regulating soft tissue repair [30,31]. Professor Dana T. Graves from the University of Pennsylvania, School of Dental Medicine delivered a wonderful lecture, which focused on the relationship between tissue healing and FOXO1. The data shows that keratinocytes accelerated the wound healing process in skin and gingiva by promoting fibroblast proliferation, the formation of connective tissue matrix and angiogenesis through gene regulation mediated by FOXO1. Keratinocyte specific FOXO1 deletion in K14Cre +FOXO1L/L mice reduced each of these parameters and delayed the wound healing process. Their team also found that expression of TGFβ1 and VEGFA was dependent on FOXO1 in normal wounds *in vivo*. The transcriptional activity of TGFβ1 and VEGFA in keratinocytes were induced when FOXO1 was overexpressed. Nevertheless, FOXO1 failed to induce TGFβ1 and VEGFA when exposing to high glucose *in vitro*. In contrast to TGFβ1 and VEGFA, diabetes and high glucose enhanced FOXO1 binding to the promoter DNA of MMP9, CCL20 and IL-36γ, which reinforced inflammation and caused the soft tissue healing process breakdown. The results indicated that diabetes interferes with the soft tissue wound healing by altering the expression of genes regulated by FOXO1.

Orofacial hard tissue formation and regeneration

The repair of bone fracture and skeletal tissue involves an increase in tissue volume related to the recruitment, proliferation and differentiation of stem cells that arise from the skeletal and vascular tissues [32]. Familiar with soft tissue healing, the repair of bone fracture area can be divided into four stages: inflammation, soft callus formation, hard callus formation and reconstruction stage [33]. Fracture healing is controlled by the innate and adaptive immune functions, stem cells origins and local biological enhancements [34-36].

Mesenchymal stem cell-mediated bone regeneration is an attractive option in clinical treatment due to their capability to undergo osteogenic differentiation, and local microenvironment is the key factor in bone reconstruction. Professor Xiaoling Zhang from Shanghai Jiao Tong University School of Medicine found that the high expression levels of TGFβ1 impaired the MSC-mediated new bone formation and demonstrated different TGF-β1 levels exhibited opposite effects on osteogenic differentiation and bone healing. The high level of TGF-β1 dampens BMMSC-mediated bone regeneration by activating canonical TGF-β/smad3 signaling and inhibiting Bmp2 via direct and indirect mechanisms. Besides, professor Zhang and her team also found the polarization of MSCs play an indispensable role in regulating the microenvironment and bone regeneration, but the mechanisms remain unclear. Research on related issues will set up bone regeneration microenvironment theoretical system and develop effective therapeutic targets for treating bone regeneration disabilities.

As mentioned above, transcription factor FOXO1 involved in and regulated the soft tissue repair process. However, FOXO1 modulated gene expression and promote hard tissue healing in normal conditions as well [37]. Professor Dana T. Graves showed that FOXO1 over-expression in chondrocytes *in vitro* increased VEGFA mRNA levels and VEGFA transcriptional activity, while silencing FOXO1 reduced it. Compared to control littermates in mice with long bone fracture, lineage specific deletion of FOXO1 in chondrocytes in Col2α1Cre-FOXO1L/L mice had significantly reduced CD31+ blood vessel formation and suppressed the expression of VEGFA, which demonstrated the importance of chondrocytes in endochondral bone repair. On the contrary, FOXO1 plays a negative role by increasing RANKL expression in chondrocytes under the high-glucose condition. The number of osteoclasts and the expression of RANKL were much higher in diabetes mice, which was rescued by FOXO1 ablation in chondrocytes. High glucose and advanced glycation end products stimulated FOXO1 association with the RANKL promoter and FOXO1 overexpression enhanced RANKL transcriptional activity. These results indicated that the dual functions of FOXO1 is altered by diabetes during the hard tissue repair and regeneration.

Primary cilia are membrane bounded microtubule-based organelles that emanate from basal body templates. Primary cilia exist on almost all vertebrate cells and have a variety of functions, including sensory reception and cellular signaling transduction [38,39]. Mutations of

ciliary proteins lead to ciliopathies, which manifest variable penetrance of skeletal abnormalities [38]. However, how primary cilia regulate cell alignment in bone development is unknown. Professor Shuying Yang from the University of Pennsylvania, School of Dental Medicine gave an interesting talk about the regulatory role of primary cilia in bone development. The cilia protein is synthesized in the cytosol and transferred to cilia by Intraflagellar Transport (IFT) bidirectional machinery. IFT20 maintained primary cilia count and cilia length in osteoblast precursors via Ceramide-pPKCz signaling, the deletion of IFT20 in osteoblast reduced the bone mass, strength, and stiffness. On the other hand, professor Yang found that primary cilia also present on osteoclasts, mice devoid of primary cilia in osteoclast precursors display severe osteopenia and increased osteoclast formation and activity. Cilia loss due to IFT80 deletion in osteoclast-precursors led to overactivation of the AKT/GSK3β/NFATc1 signaling pathway, driving increased osteoclast formation. All these findings suggested a novel and fundamental role of primary cilia and IFT proteins in the regulation of bone development and regeneration.

All the findings and results described above were related to the hard tissue development, repair and regeneration. For the patients with osteoporosis, Polymethyl Methacrylate (PMMA) bone cement has proven to be an effective material for improving bone strength. Although alternative materials are available and continue to be created, PMMA remains the material of choice due to its mechanical properties. However, PMMA bone cement cannot be degraded and absorbed in the human body, and may lead to long-term complications [40,41]. Recently, Magnesium Phosphate Cement (MPC) attracted more attention for its biodegradability and osteoinductive activity. Professor Keya Mao from Chinese PLA General Hospital, Department of Orthopedics focused his talk on this field and introduced the good physical and chemical properties, stable biomechanical properties, strong bond strength and biological safety of MPC, which showed a wide range of clinical application prospects.

Conclusion

The day ended with a group discussion focused on orofacial tissue repair and regeneration, which aims to accelerate the translation of basic research to clinical application. Even though we have achieved plenty of accomplishments, still considerable challenges remain that need further investigation: What are the key factors driving the permanent teeth development and eruption in humans? Why the MSCs contain limited capabilities of self-healing and regeneration? How to effectively intervene and regulate the fate of transplanted MSCs in clinic? How can we improve the biological property of the scaffold materials for regulating the fate of MSCs and enhancing the orofacial tissue regeneration? Addressing these questions is an essential prerequisite for translating basic findings in MSCs-based therapy to clinical applications. Because of the rapid progress in this field, symposium participants were in general agreement that an annual symposium should be held that brings together top scientists and clinicians to discuss the latest advancements and jointly tackle the critical unmet needs

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