9TH INTERNATIONAL

TMJ Interdisciplinary Research Meeting

April 26-27, 2024

University of Pennsylvania
Philadelphia, Pennsylvania

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Penn Dental Medicine
University of Pennsylvania

Penn Medicine
Center for Temporomandibular Joint Disease

9TH INTERNATIONAL

TMJ Interdisciplinary Research Meeting

Welcome

It is our great pleasure to welcome you to Philadelphia, USA, and the University of Pennsylvania campus for the 9th International TMJ Research Meeting (ITIRM9)!

Once again, we have reunited our friends and colleagues for another lively scientific discussion of state-of-the-art research on the temporomandibular joint (TMJ). We are pleased that this meeting continues to be an attractive venue where students as well as junior and senior level biologists, engineers, and clinicians can get together to exchange ideas, learn from one another, develop friendships, and establish collaboration.

Consistent with our theme of fostering interdisciplinary exchange, this year's program focuses on special topics such as markers and cell-based therapies, in vivo mechanics, pathophysiology of the TMJ, and bioscaffold-based functional tissue engineering.

We warmly welcome our distinguished keynote speakers, Dr. Maurizio Pacifici and Dr. Dania Tamimi. Dr. Pacifici is Director of the Translational Research Program in Pediatric Orthopaedics at the Children's Hospital of Philadelphia (CHOP) and Professor of Orthopaedic Surgery at the University of Pennsylvania. He will deliver the first keynote, "Mechanisms of Heterotopic Ossification and Possible Therapeutics." Dr. Dania Tamimi is the Principal Dentist at the oral and maxillofacial radiology private practice, *Beamreaders*. She will deliver the second keynote, "Understanding the Relationship Between the TMJ and the Airway." We are excited you are with us.

We would especially like to thank the program committee for their invaluable support in maintaining the high quality of this meeting.

Please enjoy the conference, and we look forward to an enriching exchange of knowledge and ideas.

Alejandro Almarza, PhD, *Co-Chair*
Eric Granquist, DMD, MD, *Co-Chair*

AIMS OF ITIRM9

The 9th International TMJ Interdisciplinary Research Meeting provides a forum to discuss state-of-the-art TMJ research. By bringing together leaders as well as budding investigators in our field, we hope to address challenges in the clinical management of TMJ problems affecting function, bring forth an understanding of the embryonic development of the TMJ, start to identify the primary drivers of chronic pain, and set new directions in biomechanical and biological research that hold great potential for future treatments.

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Friday, April 26

Location: Law Auditorium, Jordan Medical Education Center

1:00 pm	**Welcome and Introductions**
1:15 pm	KEYNOTE PRESENTATION **"Mechanisms of Heterotopic Ossification and Possible Therapeutics"** Maurizio Pacifici, *Children's Hospital of Philadelphia and University of Pennsylvania*
2:15 pm	**Break**
2:30 pm	**"Single Cell Mapping of Temporomandibular Joint Identifies New Treatments for Osteoarthritis and Pain"** Jian-Fu Chen, *University of Southern California*
2:45 pm	**"3D Neurovascular Mapping in the Whole Temporomandibular Joint"** Peng Chen, *Clemson University*
3:00 pm	**"Roles of Nociceptors in Post-Traumatic Hyperalgesia and Condylar Degeneration after Temporomandibular Joint Injury in Mice"** Ishraq Alshanqiti, *University of Maryland*
3:15 pm	**"Osteochondral Regeneration Through CRISPR Epigenome Editing"** Joshua Stover, *University of Pittsburgh*
3:30 pm	**"Type V Collagen Regulates the Progenitor Cell Fate and Matrix Formation of TMJ Condylar Cartilage during Postnatal Growth"** Lin Han, *Drexel University*
3:45 pm	**Break**
4:15 pm	**"Innervation of Joints and Tissue Clearing"** Alejandro Almarza, *University of Pittsburgh*
4:30 pm	**"Using a Three-Dimensional Deep Learning Approach to Generate Synthetic CT and MRI Images for Better Analysis of TMJ Structures"** Thomas Holzinger, *Medical University of Vienna*
4:45 pm	**"Towards the Integration of Nociception into Computational TMJ Modeling"** Benedikt Sagl, *Medical University of Vienna*
5:00 pm	**"TMJ and Craniofacial Function Assessment in Craniofacial Deformity Patients"** Shuchun Sun, *Clemson University*
5:15 pm	**"Mechanical and Degradational Tuning of a 3D-Printed Polycaprolactone Composite for a Mandibular Condyle Device"** Dylan Garcia, *University of Oklahoma*
5:30 pm	**Reception**
6:00 pm	**Dinner**

MORE ▶

Saturday, April 27

Location: Law Auditorium, Jordan Medical Education Center

8:15 am	**Breakfast/ Check-in**
9:00 am	**KEYNOTE PRESENTATION** **"Understanding the Relationship between the TMJ and the Airway"** Dania Tamimi, *Beamreaders*
10:00 am	**"Decellularization of Ovine Temporomandibular Joint Discs for Tissue Engineering Approaches"** Daniela Trindade, *Polytechnic of Leiria*
10:15 am	**"Ice-Free Cryopreservation of Viable Temporomandibular Joint Disc in a Pig Model"** Dustin Mueller, *Medical University of South Carolina*
10:30 am	**Break**
11:00 am	**"Molecular Events in Human Synovial Tissue of Temporomandibular Joint Osteoarthritis"** Chenshuang Li, *University of Pennsylvania School of Dental Medicine*
11:15 am	**"Acoustic Emissions (AE) in Diagnostic Analysis to Determine Temporomandibular Joint (TMJ) Implant Failure"** Jacob Eapen, *University of Illinois at Chicago*
11:30 am	**"A Single-Cell Transcriptional Atlas Reveals Resident Progenitor Cell Niche Functions in TMJ Disc Displacement"** Ruiye Bi, *West China Hospital of Stomatology, Sichuan University*
11:45 am	**"LOXL2 Deletion Promotes TMJ-OA-Like Changes, Whereas LOXL2 Overexpression Protects TMJ Fibrocartilage"** Manish Bais, *Boston University School of Dental Medicine*
12:00 pm	**Lunch**
1:00 pm	**"Investigating the Function and Mechanisms of Resident Macrophages in the Temporomandibular Joint Disc in Mice"** Han Fang, *West China Hospital of Stomatology, Sichuan University*
1:15 pm	**"Surgical outcomes of therapeutic arthroscopy using regenerative techniques for moderate to advanced TMJ disorders (Wilkes Stage 3, 4 or 5)"** Deepak Komath, *Royal Free London NHS Trust*
1:30 pm	**"CT Imaging Morphology to Predict Outcomes Following TMJ Arthroplasty"** Eric Granquist, *University of Pennsylvania* Shuchun Sun, *Clemson University*

1:45 pm	**"New Total TMJ Prosthesis System for Installation through Single Surgical Approach"** Leonard Moreira, *Instituto da Face SP*
2:00 pm	**"Precise Reconstruction of the Temporomandibular Joint Disc by Decellularized Disc-Based Prostheses"** Haozhe Chen, *West China Hospital of Stomatology, Sichuan University*
2:15 pm	**Break**
2:45 pm	**"Effect of Orthognathic Surgery on TMJ Disc Stress Distribution: Combined Multibody and Finite Element Simulation"** Farhad Ahmadi, *Clemson University*
3:00 pm	**"Intra-articular Injection of Orthobiologics for TMJ-Osteoarthritis: A Systematic Review"** Yannick Sillmann, *Harvard School of Dental Medicine, Massachusetts General Hospital*
3:15 pm	**"Decellularized Meniscus (MEND) as a Biomaterial that Supports Stem Cell Invasion and Chondrogenesis"** Hannah Bonelli, *University of Pennsylvania, Children's Hospital of Philadelphia*
3:30 pm	**"Ectopic Ossification of the Temporomandibular Joint (TMJ) in a Murine Model of Osteogenesis Imperfecta"** Joohyun Lim, *University of Delaware*
3:45 pm	**"TMJ Cartilage Stem/Progenitor Cells in Development"** Ikue Tosa, *Columbia University*
4:00 pm	**Closing Remarks**
4:15 pm	**Adjourn**

Maurizio Pacifici, PhD

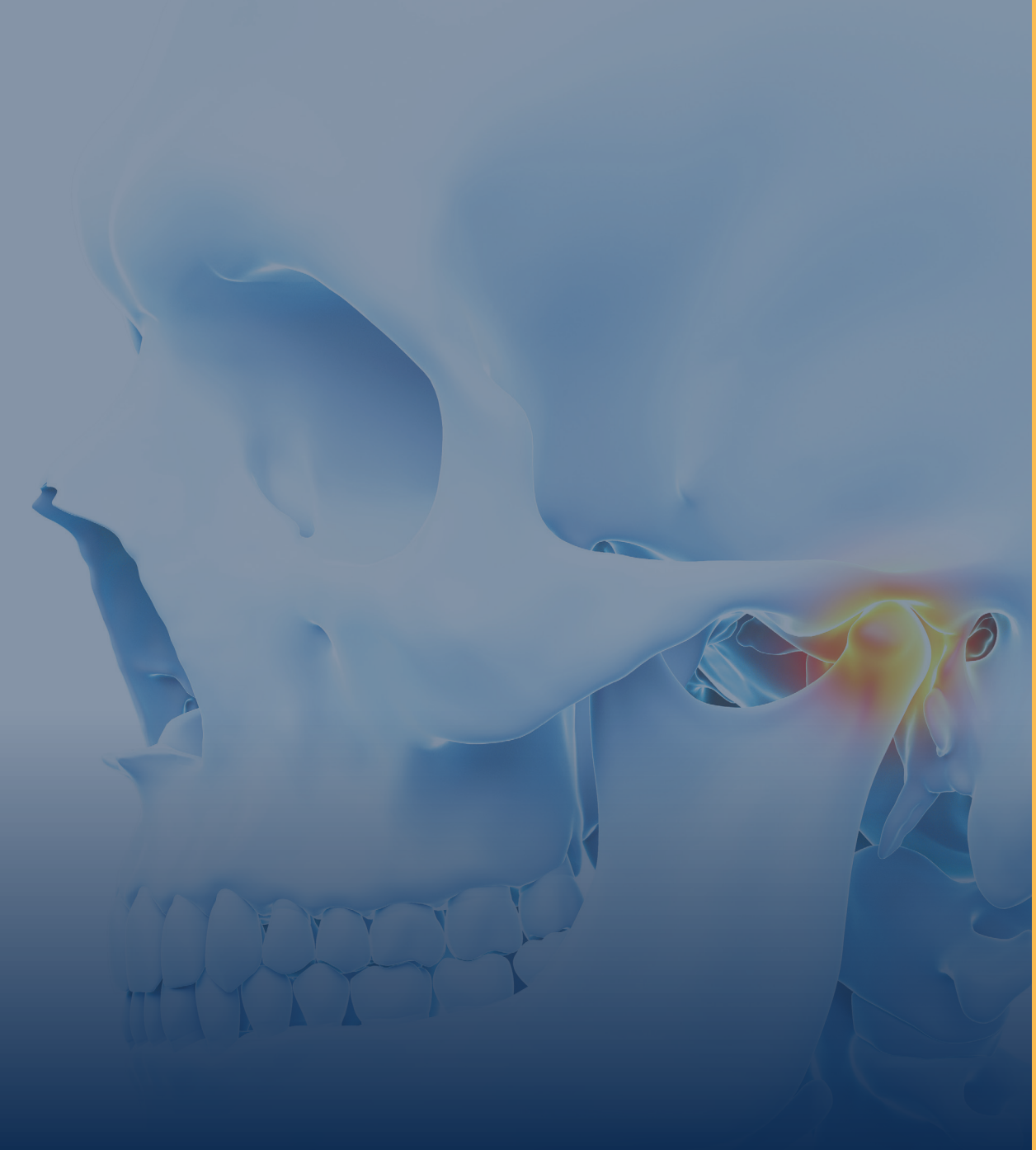
Maurizio Pacifici is Director of the Translational Research Program in Pediatric Orthopaedics at the Children's Hospital of Philadelphia (CHOP) and Professor of Orthopaedic Surgery at the University of Pennsylvania. He received a doctorate degree in Developmental Biology from the University of Rome and then joined the faculty at the University of Pennsylvania where he rose to the rank of tenured Professor. He subsequently moved to Thomas Jefferson University Medical School where he served as Director of Research in Orthopaedics and was then recruited to his current position at CHOP about 11 years ago. Dr. Pacifici's biomedical research studies focus on the cellular and molecular mechanisms that regulate skeletal development, growth and morphogenesis in fetal and postnatal life. The information deriving from these basic science research projects is used to uncover and understand the pathogenesis of congenital pediatric musculoskeletal disorders, including Multiple Osteochondroma (MO), Fibrodysplasia Ossificans Progressiva (FOP) and Achondroplasia (ACH). Combining basic developmental studies with translation and clinical studies has proven fruitful. His laboratory conceived the idea of using synthetic retinoid agonists as therapeutics against heterotopic ossification (HO) in FOP patients, leading to the identification of Palovarotene that was recently approved by the FDA. Studies are also focusing on non-genetic, acquired forms of HO that are common and still therapeutically unresolved. Dr. Pacifici has published over 170 peer-reviewed papers, and the NIH has continuously funded his biomedical research work for over three decades.

Dania Tamimi, BDS, DMSc

Dr. Dania Tamimi graduated with a dental degree from King Saud University, Riyadh, Saudi Arabia. She trained at Harvard School of Dental Medicine and earned a doctorate of medical science (DMSc) and certificate of fellowship in Oral and Maxillofacial Radiology in 2005. She is board certified by the American Board of Oral and Maxillofacial Radiology (ABOMR) and is a Fellow of the Royal College of Physicians and Surgeons (Glasgow).

She is a reviewer and an Editorial Board member for Oral Surgery, Oral Pathology, Oral Medicine and Oral Radiology (OOOO), as well as a reviewer for DMFR, Oral Radiology, Head and Neck, Angle Orthodontist and AJO-DO. She is the lead author on two textbooks: "Specialty Imaging: Dental Implants" (which has been translated to Portuguese and Russian) and "Specialty Imaging: Temporomandibular Joint and Sleep-Disordered Breathing" and a co-lead author on "Diagnostic Imaging, Oral and Maxillofacial" (translated to Spanish). She lectures nationally and internationally.

She currently runs her oral and maxillofacial radiology private practice in Orlando, Florida.



ABSTRACTS

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Effect of Orthognathic Surgery on TMJ Disc Stress Distribution: Combined Multibody and Finite Element Simulation

Ahmadi F.[1], Sun S.[1], Chen J.[1], Zhao J.[1], Hill C.[2], Almpani K.[3], Jani P.[3], Lee J.[3], Sagl B.[4], Yao H.[2]

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[3]Clinical Center, NIH
[4]University Clinic of Dentistry, Medical University of Vienna

Introduction/Rationale: This study aimed to assess the influence of orthognathic surgery on stress distribution in the temporomandibular joint (TMJ) disc using a combined Multibody Dynamics-Finite Element (MBD-FE) simulation approach. **Materials and Methods:** We developed patient-specific combined MBD-FE models comprising rigid and deformable components using the geometries segmented from CT-scan images of three patients: a class II malocclusion, a class III malocclusion, and a class I "control case." Cranium, mandible, and hyoid bone were considered rigid bodies. TMJ discs and capsules were modeled as hyperelastic bodies; articular cartilages and TMJ ligaments were simulated as elastic foundations and viscoelastic bodies, respectively. Seven muscle groups were also incorporated into the model using Hill-type actuators. Pre- and post-operation TMJ stress states of the class II and class III patients were assessed by performing maximum opening and clenching tasks enabled by the excitation of the respective muscles. Lastly, TMJ disc stress results were compared with the "control model" carrying out the same tasks. **Results:** Simulations revealed tangible alterations in stress distribution within the TMJ disc following surgical intervention. Correction of the class II malocclusion resulted in increased stress on the discs, while the class III malocclusion correction caused lower stress on the left disc and higher stress on the right disc compared to pre-operation conditions. The TMJ discs' stress distribution in class II and III cases after surgery showed more conformity with the control case regarding the magnitude and region. **Conclusion:** Our study indicates that orthognathic surgery affects TMJ disc biomechanics, aligning it more with the control case. Further, MBD-FE simulation is a powerful tool in quantitatively assessing the surgery's success and could potentially be used for surgical planning in the future. **Clinical Significance:** MBD-FE simulation showed that orthognathic surgery influenced TMJ disc biomechanics and highlighted the potential of quantitative surgical assessment. It also has potential for scalability across TMJ SYMPHONY Consortium sites.

NIH, R01 DE021134, P20 GM121342, U01 DE031512, R34 DE033593

Innervation of Joints and Tissue Clearing

Alejandro Almarza[1], Kyle Allen[2], Rob Caudle[3], Yenisel Cruz-Almeida[4], Juliane Rolim[5], Mairobys Socorro[5], Janak Gaire[2], Alan Watson[6]

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Roles of Nociceptors in Post-Traumatic Hyperalgesia and Condylar Degeneration After Temporomandibular Joint Injury in Mice

Alshanqiti I.[1], Son H.[2], Shannonhouse J.[2], Jiaxin H.[3], Parastooei G.[3], Ro J.[3], Kim Y.[2], Man-Kyo C.[3]

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Introduction/Rationale: Trauma to temporomandibular joints (TMJ) is a risk factor to increase the incidence of TMD. TMJ trauma also causes increased degeneration of TMJ structure, leading to TMJ osteoarthritis (TMJOA). The causal relationships between TMJ pain and TMJ degeneration among patients have been controversial. It is also clinically significant to know if treatment of pain could alter the progress of TMJ degeneration, or vice versa. Recent studies suggest that nociceptive afferents modulate bone remodeling through multiple mechanisms, and it is feasible that nociceptive nerves projected to TMJ not only mediate nociception but also modulate TMJ structures. However, the roles of nociceptive afferents on TMJ pain and TMJ degeneration are not known. **Materials and Methods:** To address this question, we used the forced mouth opening (FMO) model in mice. Pain-like behaviors and in vivo Ca2+ imaging in trigeminal ganglia were performed to assess pain and nociception. MicroCT and histological assays were performed to assess TMJ degeneration. To manipulate nociceptor function, we performed chemogenetic silencing using an inhibitory designer receptor exclusively activated by designer drugs. **Results:** Repeated forced mouth opening beyond physiological limitation produced long-lasting pain-like behaviors, consistent with clinically relevant pain conditions (i.e., spontaneous pain, mechanical pain, and function-evoked pain). Trigeminal ganglia neurons showed increased spontaneous and evoked responses, supporting peripheral sensitization underlying the post-traumatic hyperalgesia. FMO also caused thinning of condylar cartilage and the degeneration of subchondral bone, supporting that FMO can produce both post-traumatic hyperalgesia and TMJ condylar degeneration. Chemogenetic silencing of the TRPV1-lineage afferents attenuated spontaneous pain-like behaviors but not mechanical hyperalgesia on skin overlying TMJ. Silencing of nociceptors modestly decreased FMO-induced subchondral bone degeneration without impact on cartilage degeneration. **Conclusion:** TRPV1-lineage afferent fibers mediate post-traumatic spontaneous pain but are not a primary contributor to condylar degeneration following TMJ injury. **Clinical Significance:** Post-traumatic pain level may not be associated with TMJ degeneration in patients. Treatment of pain by targeting nociceptors may not impact TMJ degeneration.

NIH, NIDCR, R01DE031477; R35DEDE030045

LOXL2 Deletion Promotes TMJ-OA-like Changes, Whereas LOXL2 Overexpression Protects TMJ Fibrocartilage

Bais M.[1], **Raut R.**[1], **Choudhury C.**[1], **Cheyleann Del Valle-Ponce D.**[1], **Chakraborty A.**[1], **Almarza A.**[2], **Grinstaff M.**[3], **Pushkar M.**[4]

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Introduction/Rationale: Temporomandibular joint (TMJ) disorders affect 5%–12% of the United States population and no FDA-approved drug. Our study has reported for the first time that Lysyl oxidase like-2 (LOXL2) has an anabolic effect on the cartilage in vitro and in vivo. It is expressed in regenerating cartilage during fracture healing and in human TMJ cartilage. We showed that LOXL2 promotes anabolic responses in TMJ cartilage of a chondrodysplasia (Cho/+) mouse model and protects against degenerative changes. LOXL2 enriches gene sets related to extracellular matrix remodeling, proteoglycan deposition, and aggrecan gene expression in human TMJ chondrocyte implants in nude mice. The goal of the study is to evaluate the novel function of LOXL2 using genetic and trauma-induced TMJ-OA mice models and goat cartilage. **Materials and Methods:** Loxl2 floxed (fl) mice were obtained from collaborators and crossed with Acantm(IRES-CreERT2) or Acan-CreERT2 mouse line (Jax # 019148) to generate (Acan-Cre;Loxl2fl/fl). Six-month-old Acan-Cre;Loxl2fl/fl mice divided into two groups (n=8/ condition; M/F), injected with intraperitoneal Tamoxifen or vehicle. These mice were sacrificed after 4 months, followed by TMJ histology and RNAseq. Importantly, LOXL2 overexpression upregulates anabolic proteins (COL2A1, SOX9, ACAN), and the epigenetic regulator lysine-specific demethylase 6B (KDM6B) in human chondrocytes. Conversely, TMJ cartilage-specific LOXL2 knockout mice showed a reduction of ACAN and KDM6B. Next, we established a unilateral anterior crossbite (UAC) model where the metal braces are applied to the tooth to dislocate the right TMJ. These mice were intra-articularly injected with Ad5-LOXL2 or Ad5-Empty(n=8/condition; M/F) and sacrificed after 2 months. LOXL2 expression correlated with single-cell RNAseq data published by another group. Finally, freshly isolated primary goat condylar cartilage cells were transduced with AD5-LOXL2 and IL1 to study the protective effect on cartilage. **Results:** TMJ cartilage-specific LOXL2 knockout mice (Acan-Cre;Loxl2fl/fl) showed a reduction of ACAN and proteoglycan expression, enriching gene sets related to IL1β and MMP13 expression in RNAseq. The loss of LOXL2 promotes TMJ-OA-like changes. The gain of function in the UAC model showed that LOXL2 promotes anabolic responses in TMJ cartilage, restores the damaged cartilage and bone, and protects against degenerative changes. Moreover, re-analysis of single-cell RNA sequencing data of 3-day to 16-week-old mice showed a progressive decrease in the LOXL2 expression with age and traumatic injury to condylar cartilage. In addition, LOXL2 inhibits IL-1-induced NF- κB signaling in chondrocytes, which is supported by our phospho-flow cytometry analysis of LOXL2 and IL1 treated goat TMJ cells, where phosphorylation levels of NF- κB were induced upon IL1 treatment and restore to a relatively normal state when treated along with LOXL2. **Conclusion:** The genetic deletion of LOXL2 in TMJ cartilage attenuates aggrecan and collagen crosslinking and promotes IL-1β- NF-B -MMP13, leading to progressive TMJ-OA-like changes, whereas gain of LOXL2 restores it. **Clinical Significance:** There are limited treatment options for TMJ cartilage regeneration, and no FDA-approved drug is available. Identifying role of LOXL2's mechanism could provide its role in future translation.

NIH, NIDCR, R01 DE031413

A Single-Cell Transcriptional Atlas Reveals Resident Progenitor Cell Niche Functions in TMJ Disc Displacement

Bi R., Yang X., Li H., Zhan Y., Fang H., Zhu S. Zhu S.

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Introduction/Rationale: This study aimed to investigate cell identity and transcriptional changes of the mouse temporomandibular joint (TMJ) disc during postnatal growth, aging and in disc injury. **Materials and Methods:** The unbiased transcriptome-wide scRNA-seq analysis was performed on 39111 cells from mice at different postnatal stages (3d, 3w, 16w, 78-82w). Analysis results were validated using in situ RNA hybridization, immunohistochemistry, flow cytometry and in vivo TMJ disc injury model and lineage tracing model. **Results:** Our data show that TMJ disc cells are composed of 9 distinct clusters that can be divided into 4 principal cell types: fibroblasts, endothelial cells, macrophages and mural cells. Fibroblasts are found to exhibit heterogeneity which cells could be divided into chondrogenic and non-chondrogenic clusters. These 2 different fibroblast clusters display distinct expression features during postnatal growth and aging. Notably, we find for the first time that resident mural cells are the source of the progenitors in TMJ discs. The pseudotime trajectory shows that the mural cell cluster has 2 lineage fates: self-renewal or multidirectional differentiation toward functional cells. In the mural cells, the NOTCH3 and THY1 signaling pathways are ubiquitously active at all stages, coupled with specific gene expression of Notch3 and Thy1, indicating the critical role of these molecules in disc mural cell characteristics. As an example, THY1+ cells in TMJ discs show markedly stronger stem cell characteristics than THY1- disc cells in vitro and ex vivo. In addition, lineage tracing in vivo shows that the Myh11-CreER+ mural cell lineage proliferates and migrated to coordinate angiogenesis during disc injury but gradually loses its progenitor characteristics and ultimately becomes Sfrp2+ non-chondrogenic fibroblasts instead of Chad+ chondrogenic fibroblasts. **Conclusion:** We reveal multiple insights into the coordinated development of TMJ disc cells and are the first to describe the resident progenitor cell during TMJ disc injury. **Clinical Significance:** The panoramic sketch of TMJ disc cells at single cell resolution brings new insight for potential biological diagnosis and target biotherapy of the TMJ disc related diseases.

Decellularized Meniscus (MEND) as a Biomaterial that Supports Stem Cell Invasion and Chondrogenesis

Bonelli H.[1], Smith K.[1], Klessel S.[1], Dana R.[2], Gehret P.[1], Gottardi R.[1]

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Introduction/Rationale: Juvenile idiopathic arthritis is the most common chronic arthritis in children, of which 87% experience temporomandibular joint osteoarthritis (TMJOA). Tissue engineering repair of the TMJ is an active area of research that leverages materials like collagen, gelatin, hyaluronic acid, and extracellular matrix (ECM)-based scaffolds. These approaches are often combined with mesenchymal stem cells (MSCs), a key reparative player in bone marrow aspirate applications and a frequently used cell source for cartilage repair because of their high proliferation rate and accessibility. However, these approaches have poor mechanics and often result in non-uniform matrix secretion. To overcome this, we developed a decellularized porcine meniscus (MEND) scaffold. In this work, we show that MEND can be recellularized by MSCs and investigate whether MEND possesses any intrinsic pro-chondrogenic potential. **Materials and Methods:** We seeded MEND with varying MSC densities and chondrogenesis in MEND was compared to that in 3D hydrogels of methacrylated type I collagen (ColMA) and methacrylated gelatin-hyaluronic acid (GelMA/HAMA) via histological analysis (Alcian Blue), GAG assay, and RT-qPCR for key chondrogenic genes (SOX9, ACAN, COL2A1, COL1A1). **Results:** We successfully re-seeded MEND with MSCs, achieving high cell viability and a uniform distribution throughout the scaffold. Cell-seeded constructs after chondrogenesis showed uniform matrix deposition compared to pellet culture (positive control), ColMA, and GelMA/HAMA. Gene expression confirmed robust chondrogenesis of MSCs in MEND. **Conclusion:** Unlike when seeded in MEND, MSCs in pellets and hydrogels experienced inconsistent chondrogenesis with disordered matrix secretion. Analysis of differentiation across constructs by RT-qPCR and biochemistry demonstrated that MEND supports chondrogenesis comparably to other materials, proving its suitability as a scaffold for cartilage repair. **Clinical Significance:** MEND's fibrocartilaginous nature and ability to also support hyaline-type regeneration provides a scaffold with promising compositional similarities to condylar articular cartilage which could be used for TMJ repair.

NIH, T32-AR007132

Precise Reconstruction of the Temporomandibular Joint Disc by Decellularized Disc-Based Prostheses

Chen H., Zhu S., Jiang N.

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Introduction/Rationale: To address the absence of effective clinical treatments for temporomandibular joint (TMJ) disc-related diseases, we have engineered a novel prosthesis. **Materials and Methods:** This prosthesis is constructed using a decellularized natural disc, which has been reinforced with laser-drilled modifications and polycaprolactone. This design not only replicates the innate morphology of the TMJ disc but also closely matches its structural, biomechanical, and biological properties. This approach represents a significant advancement in the development of prosthetic solutions for TMJ disc-related pathologies. **Results:** The developed prosthetic construct exhibited excellent biocompatibility, safety, and immunological tolerance, as evidenced by in vitro assessments and in vivo experiments using a rat subcutaneous model. Upon six months of implantation in an allogeneic rabbit model for TMJ disc reconstruction, the prosthesis preserved its structural integrity, maintained the orientation of collagen fibers, and retained its mechanical properties. Additionally, it ensured the stability of the joint structure and effectively prevented damage to the articular cartilage and bone. Furthermore, an "upgraded" version of the disc prosthesis, derived from decellularized porcine discs, was implanted into a goat model for TMJ disc reconstruction. This xenograft prosthesis, which closely emulates the strength and viscoelastic properties of a natural TMJ disc, successfully restored the structural and functional integrity of the TMJ for a duration of up to 20 weeks. This highlights the potential of this prosthesis as a promising solution for TMJ disc reconstruction, offering significant clinical relevance. **Conclusion:** The outcomes of this study underscore the translational potential of utilizing allogeneic or xenogeneic decellularized disc prostheses in the treatment of advanced TMJ disc-related diseases. **Clinical Significance:** This approach highlights a viable pathway for clinical application, demonstrating the feasibility of such prostheses in effectively addressing complex TMJ disc-related diseases.

Single cell mapping of temporomandibular joint identifies new treatments for osteoarthritis and pain

Chen J., Jariyasakulroj S.

Center for Craniofacial Molecular Biology, University of Southern California

Introduction/Rationale: Temporomandibular joint (TMJ) osteoarthritis (OA) is characterized by the joint degeneration with synovitis, cartilage remodeling, and subchondral bone destruction along with the potential orofacial pain. Disease driving mechanisms are poorly understood and effective treatments are not yet available. **Materials and Methods:** Here we established an inflammatory TMJOA mouse model via intra-articular injection of CFA (Complete Freund's Adjuvant). We performed single-cell transcriptomic profiling in TMJ followed by the validation using tissue clearing, 3D imaging, multiplex immunodetection, and functional genetics. **Results:** TMJOA mice exhibited cartilage remodeling, bone loss, synovial inflammation, and orofacial pain, recapitulating hallmark symptoms in patients. We found: 1) two blood capillary vessels, 2) multiple transcriptionally distinct, anatomically discrete, and functionally different fibroblast subsets that are expanded in TMJ OA along pain development, and 3) a distinct population of tissue-resident macrophages that form an internal immunological barrier at the synovial edge adjacent to the lining fibroblast subsets and physically seclude the joint. Lastly, genetic and pharmacological studies in TMJ function coupled with hydrogen injection identified multiple therapeutic strategies for mitigating TMJOA symptoms and orofacial pain. **Conclusion:** We revealed cellular diversity and functions in painful TMJOA. **Clinical Significance:** Our studies identified new treatment strategies for mitigating TMJOA and pain.

3D Neurovascular Mapping in the Whole Temporomandibular Joint

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Introduction/Rationale: Temporomandibular disorders (TMDs) cause pain and dysfunction. However, the underlying disease mechanism remains elusive. Neurovascular networks are critical to temporomandibular joint (TMJ) disease development, particularly TMJ inflammation and pain. Given spatial heterogeneity at the joint and tissue levels, a comprehensive study of the TMJ 3D neurovascular network is crucial to investigate tissue or region-specific mechanisms and build an integrated mechanistic understanding of the TMJ. However, a 3D atlas of TMJ neurovascular structure is nonexistent. This study sought to map the 3D neurovascular networks in the TMJ using advanced tissue clearing and large-field light-sheet imaging techniques. **Materials and Methods:** Fresh mice, rats, and pig TMJs were harvested and fixed in 10% formalin solutions. Following decalcification, deep permeabilization, decolorization, and delipidation, samples were immunostained for blood vessels (CD31) and nerve fibers (CGRP and NF200), then cleared and imaged using a light sheet microscope. **Results:** TMJ samples were successfully cleared and imaged. TMJ 3D neurovascular structure was spatially heterogeneous in all studied species, with higher neurovascular density in the posterior regions vs. anterior regions. In the prg4 knockout mouse, a known model with progressive joint damage, we identified 3D degenerative changes in multiple TMJ components. In the forced mouth-opening mouse model of TMJ injury, neurovasculature was regressed in the TMJ's anterior region but was increased in the internal side of the condylar neck. **Conclusion:** We developed a complete method to stain, clear, and map the 3D TMJ neurovascular network at the whole joint level across multiple species. This method allows us to quantitatively assess 3D TMJ neurovascular changes along disease progression and following therapy, therefore advancing TMD mechanistic studies and treatment development. **Clinical Significance:** This valuable 3D information will support the endeavor of our TMJ SYMPHONY Consortium to reveal the structure-function-pain relationship and its interplay with the neurovascular network, and assist in developing new treatment strategies for TMDs.

National Institutes of Health, R34DE033593 (HY, JSL), U01DE031512 (HY, JSL), R01DE021134 (HY), P20GM121342 (HY), R01DE031477 (MC), R35DE030045 (MC)

Acoustic Emissions (AE) in Diagnostic Analysis to Determine Analysis to Determine Temporomandibular Joint (TMJ) Implant Failure

Eapen J.[1], **Keaty B.**[2], **Sun Y.**[2], **Tonghao Z.**[2], **Xu C.**[2], **Ozevin D.**[2], **Mercuri L.**[3], **Mathew M.**[1]

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Introduction/Rationale: Alloplastic temporomandibular joint (TMJ) implants are viable solution for end-stage pathology [1], however, failures due to screw loosening were found recently in TMJ implants[2]. Inspired by the application of acoustic emission (AE) in detecting early deformations in hip implants [3], we hypothesize that AE can detect screw loosening in TMJ implants. **Materials and Methods:** The experiments were conducted on a customized TMJ simulator equipped with AE, bite force and displacement sensors, along with a Stryker Ti6Al4V TMJ implant featuring eight screws. Four screws (S1, S2, S7, and S8) were selected based on prior studies [4], while the rest remained fixed. To simulate different TMJ implant conditions (fully functional, partially functional, and failed), four groups were established: (i) All fixed screws as the control group, (ii) S1 and S2 loosened by 360°, (iii) S7 and S8 loosened by 360°, and (iv) all four screws loosened. Throughout the experiment, the TMJ moved at 1 Hz with a 10N bite force. **Results:** The displacement was detected to be approximately 22mm ffl 2%. AE signals were collected for hit driven data and amplitude for all groups, where Group (ii) exhibited higher peaks than (i), Group (iii) showed higher peaks than (i) but lower than Group (ii), and Group (iv) demonstrated the highest amplitude. The results indicate that loosened screws show higher AE signals than tightened ones, which may be attributed to the mechanical and tribological interactions occurring at the interface between the screw and the bone. **Conclusion:** AE signals of the loosened screws were detected and compared to the fixed ones, suggesting that AE might be a potential tool to detect early screws loosening in TMJ implants. **Clinical Significance:** Timely detection of early screw loosening is crucial to prevent complications. This project revolutionizes TMJ implant monitoring through non-invasive and real-time monitoring AE technology, which may improve patient outcomes and advance TMJ implant diagnostics.

UIC Honors College, UIC Honors College

Investigating the Function and Mechanisms of Resident Macrophages in the Temporomandibular Joint Disc in Mice

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Introduction/Rationale: In synovial joints, the resident macrophages were found mainly distributed in the synovial membrane, and were considered to contribute to the immune response of the chronic inflammatory diseases such as osteoarthritis (OA). Our recent studies using scRNA-Seq analysis identified the disc resident macrophage (DRM) in the temproromandibular joint (TMJ) disc of mice. In this current study, we aims to further explore the cellular origin, distribution and transduction of the disc resident macrophages (DRM) in TMJ homeostasis and injury in mice. **Materials and Methods:** The distribution of DRM in the disc was identified by immunohistochemistry of Lyz/ F4/80 /CD115. The flow cytometry was performed using mouse disc tissues at different postnatal growth stages (3 d, 3 w, 17 w, 78 w). Surgically induced disc injury mouse model was established, and animals was sacrificed at 3 d, 1 w, 2 w, 4 w after model generation. The transmission electron microscopy (TEM) detection was used to observe microstructural changes of DRM in the disc injury area. The Lyz2-CreER; Rosa-tdTomatofl/fl mice were used for DRM lineage tracing. Finally, analyzing the intercellular interaction of DRM in Lyz2-CreER; Rosa-tdTomatofl/fl disc injury model by single-cell sequencing. **Results:** Immunohistochemistry analysis showed that DRM were mainly located near the anterior/posterior disc attachment. Flow cytometry analysis showed that there were abundant CD206+ DRM at the neonatal stage (2.2ff10.9% at 3 d), and were stably expressed at a relatively low level at later stages from 3 weeks to 78 weeks. In the disc injury mouse model, the number of Lyz+ DRM were dramatically increased near the injury area. Linage tracing showed that these newly formed Lyz+ DRM were partially derived from the original RFP+/Lyz2+ DRM, which cells proliferated and migrated from the disc-retrodiscal junction to the injury area. Interestingly, we found another RFP-/Lyz2+subpopulation accumulated in the disc injury area, suggesting the transcriotional transduction of non-DRM to DRM-like cells in disc injury. Furthermore, the scRNA-Seq analysis showed that the transcriptional patterns were distinct between RFP+/Lyz+ DRM and RFP-/Lyz+ Non-DRM. This finding suggested a incomplete transformation of Non-DRM toward DRM in dsic injury. Single-cell sequencing has revealed cell-to-cell communication and transdifferentiation between DRM and fibroblasts in TMJ disc injury sample. **Conclusion:** In conclusion, we for the first time verified the existence of a novel DRM population in TMJ disc, characterized by highly expressed Lyz2. In disc injury, DRM potentially cooperate with fibroblasts near the injury area for the disc injury process and repair. **Clinical Significance:** The identification of DRM provide a new clinical vision of therapy target tfor TMJ inflammatory diseases.

Mechanical and Degradational Tuning of a 3D-Printed Polycaprolactone Composite for a Mandibular Condyle Device

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Introduction/Rationale: Our group previously designed and evaluated a mandibular condylar joint replacement device in a goat model, developed as a potential future alternative to total joint replacement for select patient populations. Here, validation for a three-dimensional printed (3DP) polycaprolactone-hydroxyapatite (PCL-HAp) condylar implant was evaluated with in vitro degradation and compression mechanical testing. **Materials and Methods:** PCL filament with 20% w/w HAp powder was fabricated using a Filabot filament extruder (Filabot Ex6, Barre, VT). The filament was fed into the Cura Lulzbot TAZ 6 3D and extruded 5 mm3 cubes with orthogonal crosshatch porous internal architecture. Two groups (i.e. 0% Pure PCL, 20% PCL-HAp) of eight 3DP cubes were submersed into 0.25 M or 5M sodium hydroxide (NaOH) solution for one month for accelerated in vitro degradation. Five, groups (i.e., 0% Pure PCL, 20%, 30%, 40%, and 50% PCL-HAp) of eight 3DP cubes were evaluated under uniaxial compression to obtain the modulus and yield stress. **Results:** After 2 weeks, 20% PCL-HAp cubes showed 100% weight loss. The yield stress of PCL was increased by 30-76% with the addition of HAp ($p < 0.0001$). Unexpectedly, the stiffness of pure PCL was 19-41% greater than those of PCL with HAp ($p < 0.0001$). **Conclusion:** The 20% PCL-HAp displayed a higher degradation rate compared to pure PCL during the early submersion phase. A slightly faster degradation time is desired based on high amounts of PCL-HAp (20%) remaining after the previous 6-month goat study. The device must maintain mechanical stability during the bone regeneration phase. Therefore, work is ongoing to determine a composition with faster degradation while maintaining mechanical integrity for the initial period post-implantation. **Clinical Significance:** Understanding the mechanical integrity and degradation of PCL-HAp condylar implants is crucial for their clinical effectiveness and safety, ensuring optimal outcomes for select patients.

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CT Imaging Morphology to Predict Outcomes Following TMJ Arthroplasty

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Type V Collagen Regulates the Progenitor Cell Fate and Matrix Formation of TMJ Condylar Cartilage during Postnatal Growth

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Introduction/Rationale: The TMJ condylar cartilage has a unique bi-layered structure with a collagen I-dominated fibrocartilage layer covering the collagen II-rich hyaline layer. Currently, there is limited knowledge on the molecular activities regulating the formation and degeneration of condylar cartilage, which limits the development of regeneration strategies for treating its degeneration. This study aims to elucidate the role of collagen V, a regulatory minor collagen, in the postnatal growth of condylar cartilage. **Materials and Methods:** Cartilage-specific deletion of Col5a1 gene was induced in Col5a1- Col5a1f/f/AcanCreER (cKO) model at 4 weeks of age. Morphological, structural and biomechanical phenotypic changes were evaluated via histology, μCT, SEM and AFM-nanoindentation at 5 and 8 weeks. RNAscope and IF imaging were applied to validate the reduction of collagen V and assess changes in cell proliferation (Ki-67). **Results:** In condylar cartilage, we found high expression of Col5a1 in the fibrous layer of control mice, and validated its reduction in cKO mice. At 5 weeks of age, cKO condylar fibrous layer developed increased cell density, aberrant cell clustering and increased Ki-67 activities. At 8 weeks, loss of collagen V resulted in decreased hyaline layer thickness with reduced sGAG staining. On condylar surface, loss of collagen V resulted in thickening of collagen fibrils and reduced modulus. Meanwhile, there was an ectopic overgrowth of subchondral bone at the posterior end. Therefore, given that the progenitors in fibrous layer is essential for condylar formation, our results suggest that collagen V could mediate the fate and proliferation of these progenitors, and in turn, regulate condylar matrix elaboration during postnatal growth. **Conclusion:** Our results highlight collagen V as an essential matrix constituent that is crucial for regulating progenitor cell activities and matrix establishment during TMJ maturation. **Clinical Significance:** This study enables a path for using collagen V as a potential candidate for improving progenitor/stem cell-based regenerative therapies.

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Using a Three-Dimensional Deep Learning Approach to Generate Synthetic CT and MRI Images for Better Analysis of TMJ Structures

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Introduction/Rationale: In order to diagnose or to plan treatments concerning the temporomandibular joint (TMJ) computed tomography (CT) and magnetic resonance imaging (MRI) are commonly used medical imaging methods and provide complementary information. However, most centers routinely only use one modality, while the other is used sparsely in special cases. Thus, an approach that would allow a modality conversion from CT to MRI and conversely could strengthen diagnostic workflows without any additional image acquisition. While such algorithms exist for other joint systems, there is a lack of research for the TMJ. Therefore, our aim was to develop a deep learning (DL) approach to perform a bidirectional modality conversion focusing on the TMJ structures. **Materials and Methods:** Our approach is based on a cycle-consistent generative adversarial network (cycleGAN). Moreover, the network was designed to be trained on 3D volumes. This has the advantage of preserving spatial consistency among slices and saves memory. Afterwards, those volumes will be used for further image analysis such as segmentation of the TMJ structures. **Results:** The generated synthetic volumes showed good quality for further processing. Both synthetic CT (sCT) and MR (sMR) were able to illustrate relevant TMJ components. sCT and sMR allowed for the successful delimitation of hard and soft tissue structures, respectively. We will present a quantitative analysis of conversion performance at the conference. **Conclusion:** Our study shows that a 3D cycleGAN approach can successfully create sCT and sMR volumes. This allows for an easier assessment or segmentation of relevant TMJ tissues, even if only data from one modality were initially collected. This approach opens up new potential applications such as the fast and easy creation of patient-specific biomechanical models. **Clinical Significance:** This DL approach allows to enhance the data acquisition of CT and MRI concerning the TMJ by generating the missing complementary modality, which has the potential to improve diagnostic workflows.

Surgical Outcomes of Therapeutic Arthroscopy Using Regenerative Techniques for Moderate to Advanced TMJ Disorders (Wilkes Stage 3, 4 or 5)

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Introduction/Rationale: Temporomandibular joint (TMJ) arthroscopy procedures allow diagnosis and therapy of joints with degenerative changes and can be used as a predictive tool for patients who may require temporomandibular total prosthetic joint replacements (TJR) in the future. Whilst it is well documented that early stage arthritis responds well to arthroscopy and arthrocentesis, there is limited published data on outcomes of surgical management of more advanced TMJ arthritis. This study sought to assess both quantitative and qualitative outcomes 6 months after arthroscopic surgery, employing regenerative techniques in patients diagnosed with moderate to advanced temporomandibular joint disease. **Materials and Methods:** Retrospective data was collected of patients who underwent therapeutic (Level – 2) arthroscopy combined with regenerative medicine techniques between 2019-2023 at Royal Free Hospital, London, UK. The inclusion criteria were that of patients having been through the Royal Free London TMJ protocol and clinical and radiological Wilkes's score of greater than or equal to 3. All patients underwent radiofrequency ablations, disc reduction, and intra discal injection of platelet rich plasma (PRP). Quantitative and qualitative measures of pre and post-operative pain score measured on a visual analogue scale (VAS) diet scores (1-10 – 10 being normal texture and 1 being liquid alone) and mouth opening (interincisal distance limited by pain measured in mm) were evaluated. Arthroscopic findings were correlated with MRI Wilkes staging and clinical staging. **Results:** 126 therapeutic arthroscopies with intra articular and intra discal PRP injections was carried out. The mean age was 40.5 years, with 82% of whom were female. 64.0% of patients reported a significant improvement in their pain score ($p = 0.001$). 54.4% demonstrated an improvement in their mouth opening ($p = 0.001$) and diet scores improved in 81.3% of patients ($p = 0.001$). 16 (12.6%) patients were planned for a TMJ replacement following arthroscopic confirmation of end stage TMJD. 8 (6.3%) cases were listed for further arthroscopies. **Conclusion:** The surgical intervention for cases of TMJ arthritis, confirmed both radiologically and clinically, demonstrates favourable outcomes in patients presenting with moderate to advanced disease (Wilkes stages 3, 4, and 5). This study, which incorporates intra-articular/intra-discal injections with platelet-rich plasma (PRP) has proven to be efficacious. **Clinical Significance:** The synergy of regenerative medicine and advanced arthroscopic technologies has markedly enhanced clinical outcomes.

Molecular Events in Human Synovial Tissue of Temporomandibular Joint Osteoarthritis

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Introduction/Rationale: Temporomandibular joint osteoarthritis (TMJOA) is a debilitating degenerative disease with high incidence and deteriorating quality of patient life. However, compared to relatively well-studied large joints, the underlying cellular and molecular mechanism behind the degenerative disorders of TMJ is not well understood, which significantly hindered the development of TMJOA-specific treatment strategies. **Materials and Methods:** In the current study, we analyzed a published transcriptome dataset of human TMJ synovial tissue from 5 donors with reducible anterior disc displacement and 5 donors with TMJOA. Data analyses were performed on the Galaxy platform. Quasi-likelihood F-tests (ANOVA-like analysis) were achieved to identify DEGs. Genes with a fold change of more than 2 and a false discovery rate of less than 0.05 were assigned as DEGs. The pathway enrichment of identified DEGs was performed against the STRING network. **Results:** 360 DEGs (250 upregulated, 110 downregulated) were identified in the human TMJOA synovial tissue compared to the tissue from patients with reducible anterior disc displacement. Pathway enrichment of the upregulated DEGs showed the activation of "primary immunodeficiency," "systemic lupus erythematosus," "Th1 and Th2 cell differentiation," "Th17 cell differentiation," and "Osteoclast differentiation". While downregulated DEGs enriched "dilated cardiomyopathy," "calcium signaling pathway," "cGMP-PKG signaling pathway," "Apelin signaling pathway," and "cAMP signaling pathway." **Conclusion:** By utilizing the only publicly available human tissue dataset associated with TMJOA, the current study confirmed that OA is an inflammatory disease. In addition, the synovium tissue plays a significant role in TMJOA with the dysregulation of immune responses. Last but not least, the current study indicated that during TMJOA, the synovium tissue has reduced elasticity which may further contribute the joint stiffness. **Clinical Significance:** TMJ disorders affect 5-12% of the population and are associated with an annual cost estimated at $4 billion. The current study revealed the molecular events in the synovium tissue which may provide insights into novel treatment targets for TMJOA.

Ectopic Ossification of the Temporomandibular Joint (TMJ) in a Murine Model of Osteogenesis Imperfecta

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Introduction/Rationale: The mandibular condyle cartilage (MCC) in the temporomandibular joint (TMJ) is formed and maintained through complex crosstalk between resident cell populations and their extracellular matrix (ECM) microenvironment. Whereas the articular cartilage expresses high levels of type II collagen, the MCC surface consists of fibrous connective tissue that is abundant in collagen I. Previous studies have shown that chondrogenic or osteogenic differentiation of condyle resident progenitor cells is regulated by a unique progenitor niche created within the fibrous surface layer. However, the mechanisms by which type I collagen modification and cross-linking regulates niche signaling and progenitor cell fate in the mandibular condyle remains unclear. Fkbp10 belongs to a family of FK506-binding proteins and is essential for the conversion of telopeptide lysine to hydroxylysine in procollagen I and, hence, collagen cross-linking. Loss of function mutations in FKBP10 in human patients cause Osteogenesis Imperfecta with or without joint contractures. Still, the role of Fkbp10 in mandibular joint development and homeostasis is poorly understood. **Results:** Here, we show that conditional removal of Fkbp10 in the superficial layer and periosteum of TMJ causes abnormal bone formation in the MCC and periosteum. In addition, loss of Fkbp10 induces progressive joint degeneration and ectopic bone formation in TMJ, indicating phenotypic overlap with temporomandibular joint osteoarthritis (TMJ-OA). Further, ectopic osteogenesis in Fkbp10-deficient mice is associated with dysregulated Wnt signaling. **Conclusion:** Together, our results indicate that defects in collagen I modification and cross-linking in the mandibular condyle microenvironment causes abnormal bone formation that may be driven by aberrations in niche signaling. Thus, our studies may provide additional insight into the mechanisms of postnatal TMJ development and pathophysiology.

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New Total TMJ Prosthesis System for Installation through Single Surgical Approach

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Introduction/Rationale: Temporomandibular joint prostheses are indicated for reconstruction of this joint in cases of osteoarthritis in advanced stage of joint tissue degeneration, in fractures with destruction of the total mandibular condyle, in cases of tumors and condylar deformities to restore the patient's joint function. . In this context, the aim of this abstract is to present a new TMJ prosthesis design, using the same gold standard materials and with biomechanical stability, but with smaller shape and size, allowing its installation by a single surgical approach. From its inception, the design of each prosthesis is unique in that the shape of the jaw bone, the zygomatic arch and the physiological conditions are individual to each patient. However, design features are replicated in all designs, such as planning of implant fixation holes, joint geometry and dimension, fitting of different components, and sizing to allow single-incision access during surgery, providing less surgical trauma and consequent faster patient recovery. **Materials and Methods:** The model was developed through finite elements evaluation. **Results:** A TMJ prosthesis system through single surgical approach. **Conclusion:** A new TMj prosthesis with the aim of reducing surgical trauma. **Clinical Significance:** a new TMJ Prosthesis generation, through a single approach and reduced surgical trauma

Ice-Free Cryopreservation of Viable Temporomandibular Joint Disc in a Pig Model

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Introduction/Rationale: Approximately 30% of the 35 million Americans with temporomandibular joint (TMJ) disorders experience TMJ disc degeneration and mechanical dysfunction, and ~10% of these cases require surgery. Utilizing a TMJ disc allograft in replacement surgery holds promise because its properties resemble those of the native tissue. However, its potential remains largely unexplored due to its limited donor graft availability and preservation issues. This study aimed to develop a vitrification method to preserve viability, tissue structure and mechanical properties. **Materials and Methods:** Based on model simulation and validation using micro-computed tomography (μCT) imaging to predict effective CPA penetration, three protocols (2-, 3-, and 4-hour) were used for the vitrification studies by loading a 55% CPA solution (VS55). Fluorescence live/dead imaging and metabolic analysis were used to assess cell viability. Histological, biochemical, and mechanical methods were used to determine the integrity and mechanical strength of the ECM. **Results:** Three protocols (2-, 3-, and 4-hour) for loading a 55% vitrification solution (VS55) were used for vitrification due to effective CPA penetration in the range of 70-90%. Live/dead imaging demonstrated significantly higher viability in 3-hour vitrified samples (~80%) compared to slow-frozen (~40%), 2-hour (~60%) and 4-hour (50%) samples ($p<0.001$). Metabolic analysis showed that the 3-hour vitrified samples achieved 100% of metabolic activity after a 2-day incubation post-warming compared to the fresh, while slow-frozen, 2-, and 4- hour samples achieved ~35%, 78%, and 75%, respectively. Minimal changes in ECM structure were observed between 3-hour and fresh tissues through the histological analysis. Additionally, there was no significant difference in equilibrium modulus between 3-hour and fresh samples. **Conclusion:** The work presented in this project will assist in the establishment of a vitrification procedure for the long-term storage of donor TMJ discs. **Clinical Significance:** Through our exploration of vitrification, we expect to significantly improve the prospects of successful donor TMJ disc transplantation in the future.

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Towards the Integration of Nociception into Computational TMJ Modeling

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Introduction/Rationale: Computational modeling of TMJ biomechanics can give valuable insights into the effect of various variables on TMJ loading, which cannot be measured in vivo due to patient safety restrictions. While biomechanics are important for the investigation of various TMDs, like osteoarthritis or disc degradation, they currently neglect the potential impact of altered loading schemes on a patient's nociception. Nociception, and in consequence pain, is arguably one of the most important outcomes for the patient. Hence, this abstract presents a workflow that allows for the inclusion of nociceptive signals into computational modeling. **Materials and Methods:** Our modeling approach combines a computational model of the masticatory region with microscopic images of the nerve ending distribution throughout the TMJ disc, by establishing a map of nociceptive reception over the TMJ disc tissue. Previous literature on mechanotransduction is used to establish a computational method creating a "nociceptive signal" depending on tissue strain as well as nerve ending distribution. **Results:** To showcase the approach, we have developed a proof-of-concept model. Moreover, we used a full model of the human masticatory region to show the effect of different idealized nerve ending distributions during muscle-driven clenching. Lastly, we will present a preliminary mouse TMJ computer model with the nociceptive signal computed from data mapped directly from the same specimen, highlighting the future application of our approach. **Conclusion:** This abstract presents a highly novel method for the inclusion of nociceptive signaling information into biomechanical computer simulations. By leveraging microscopy data, we have successfully incorporated realistic nerve ending distributions into our proof of concept and will highlight future fields of application at the conference. **Clinical Significance:** The presented approach has great potential to investigate novel risk factors for TMD nociception and could help to gather a better understanding of nociceptive processes in TMD patients.

Intra-articular Injection of Orthobiologics for TMJ-Osteoarthritis: A Systematic Review

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Introduction/Rationale: Temporomandibular joint (TMJ) osteoarthritis (OA) involves degeneration and chronic inflammation, causing pain and functional limitations. When conservative treatments fail, minimally invasive procedures like TMJ arthrocentesis and hyaluronic acid (HA) injections are implemented. Orthobiologics, such as blood-derived products and stem cell therapy, have gained attention for their potential in tissue healing. This study aimed to answer the question: Is there a difference in the treatment effect between the injection of orthobiologics compared to the injection of HA or saline for TMJ-OA? **Materials and Methods:** A search, following the PRISMA guideline, was conducted. Eligibility criteria included randomized controlled trials with a follow-up period of ≥6 months. Databases (MEDLINE/PubMed, Web of Science, Embase, Cochrane Library) were searched until June 10th, 2023. **Results:** A total of nine studies were included. Platelet-rich plasma (PRP) and cell-derived products showed potential benefits in reducing TMJ pain and improving maximum mouth opening, with some studies reporting significant differences compared to the injection of HA or saline arthrocentesis. **Conclusion:** This review suggests a potential but inconsistent benefit of orthobiologics in treating TMJ-OA. The evidence is not uniformly supportive, and the heterogeneity among the included studies may influence the observed effects. Cell-derived orthobiologics seem promising, while PRP couldn't show conclusive evidence of superiority over HA injections. Future research should address methodological limitations and aim for homogeneity in the production of orthobiologics to provide a more reliable assessment of orthobiologics. **Clinical Significance:** TMJ-OA has clinical significance as it often leads to pain, impacting daily activities such as eating and speaking. Orthobiologics are gaining recognition for their potential in promoting tissue regeneration and inflammation reduction. In the context of TMJ-OA, these injections offer a minimally invasive approach to alleviate symptoms, enhance joint function, and potentially slow the progression of the disease, providing a promising adjunct to traditional treatments.

Osteochondral Regeneration Through CRISPR Epigenome Editing

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TMJ and Craniofacial Function Assessment in Craniofacial Deformity Patients

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Introduction/Rationale: Patients with craniofacial deformities exhibit unique morphological features and compromised TMJ function. Orthognathic surgery seeks to modify these morphological abnormalities with the hope of improving functional outcomes. However, the effect of these surgical alterations on TMJ and craniofacial function is not well understood. Our study aims to understand the impact of abnormal craniofacial morphology on TMJ and craniofacial function, and assess how orthognathic surgery influences the function, thereby advancing our knowledge of the craniofacial system structure-function relationship. **Materials and Methods:** Employing a novel chair-side TMJ and craniofacial functional assessment system, we captured mandibular movement, masticatory muscle electromyography, and bite force in craniofacial deformity patients both before and after orthognathic surgery. Our assessments included various oral tasks, such as maximum opening and closing, along with static and dynamic biting. To address hard-to-measure parameters like joint force and disc stress, we integrated direct measurements with advanced biomedical imaging techniques, creating computational models for a comprehensive analysis of TMJ and craniofacial mechanics. **Results:** Our preliminary findings indicate distinct kinematic patterns across craniofacial deformity classes. For instance, Class II asymptomatic females demonstrated greater central incisor displacement and rotation, with increased variability during lateral tasks, while Class III patients exhibited reduced kinematic symmetry. Bite force capacity analysis revealed compromised control capacity in these patients, which is improved following surgery. Furthermore, computational modeling showed that surgical interventions affect TMJ disc stress differently depending on the deformity type. **Conclusion:** These findings confirm that mandibular morphology determines TMJ and craniofacial functions, and highlight that orthognathic surgery has the potential to improve TMJ and craniofacial function. Similar approaches can be applied in different patient cohorts to study other TMJ and craniofacial symptoms. **Clinical Significance:** The successful implementation of this novel chair-side functional assessment system in the NIDCR Clinical Center demonstrates its user-friendliness and potential for multi-center data collection and analysis through the TMJ SYMPHONY Consortium.

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TMJ Cartilage Stem/Progenitor Cells in Development

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Introduction/Rationale: The temporomandibular joint (TMJ) is a site for mandibular growth and is crucial for dental occlusion, speaking, and chewing. It is well known that stem cells have an important role in TMJ health and diseases. Previously, we identified Lgr5-Cre as a marker for progenitors which differentiate into disc, perichondrium, periosteum, and bone in the TMJ. However, cartilage stem/progenitor cells and their markers are still unknown. Our objective is to define cartilage stem/progenitor cells critical for TMJ development and homeostasis. **Materials and Methods:** To identify TMJ cell populations in the mandibular condyles during development, we performed scRNA-seq analyses using E16.5 C57BL6 mice. To explore the localization for potential stem/progenitor cells, we performed in situ hybridization for each developmental stage. To confirm their contribution to TMJ development and homeostasis, we performed linage tracing experiments with Cre/loxP system. **Results:** Histological analysis showed that the polymorphic zone harbors cartilage progenitor cells in the TMJ. scRNA-seq analysis using TMJ condyles from E16.5 showed Lgr5-expressing perichondrium/disc cell populations (5) and Acan-expressing cartilage cell populations (6). Trajectory analysis suggested that Thy1+ and Gli1+ populations are immature compared to other cartilage cell populations in E16.5 TMJ. In situ hybridization analysis showed that Gli1+ and Thy1+ cells were localized in the polymorphic zone. We confirmed their contribution to TMJ development and homeostasis by using Gli1-CreERT; tdTomato mice and Thy1-Cre; tdTomato mice. **Conclusion:** We found that Gli1+ and/or Thy1+ cells are cartilage progenitor cells critical for TMJ development and tissue homeostasis. Our on-going studies aim to determine key signal pathways which determine TMJ stem cell fate. **Clinical Significance:** Minimally invasive cell-based therapies that prevent TMJ degeneration or promote repair are not available clinically. Therapeutic strategies that harness resident stem/progenitor cells to repair and maintain adult musculoskeletal tissue homoeostasis could be a minimally invasive cell-based treatment option.

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Decellularization of Ovine Temporomandibular Joint Discs for Tissue Engineering Approaches

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Introduction/Rationale: Temporomandibular joint disc (TMJd) pathologies require effective therapeutic approaches for restoring the loss of fibrocartilaginous tissue. Animal-derived tissues are ideal for tissue engineering (TE) applications as they mimic the native environment. However, for safe clinical application, it is necessary to carry out decellularization techniques to eliminate cellular content while preserving the extracellular matrix (ECM). **Materials and Methods:** Different protocols were applied to ovine TMJd to evaluate the adequate decellularization method: sodium dodecyl sulphate (SDS) and Triton X-100 in 3 concentrations (0.1, 0.5, and 1% w/v). The addition of 1 and 3 cycles of freeze-thaw were also tested. Cellular content, sulphated glycosaminoglycans (GAGs) and soluble collagen were quantified by PicoGreenTM, dimethylmethylene blue assay and Sirius red method, respectively. Mechanical compression tests were also performed. **Results:** As the detergent percentages increased, SDS samples presented greater cell removal, being more pronounced with the application of 1 freeze-thaw cycle. Within SDS samples, the favourable result in terms of cell content removal-ECM maintenance relation was achieved the combining 1 cycle with 0.1% SDS. However, there was still 56% of cells within in a matrix of 40% collagen and 55% sGAGs. As for the Triton samples, 1 freeze-thaw cycle did not significantly impact cell removal or ECM maintenance. On the other hand, 3 freeze-thaw cycles was substantial, as cell loss was 70%, while collagen and sGAGs remained at 80% and 48%, respectively. Regarding compressive performance, 1 freeze-thaw cycle with 1% resulted in an increase in Young's modulus by 34% for SDS and 29% for Triton, while for 3 cycles with 0.1% Triton the stiffness remained unchanged. **Conclusion:** A preliminary decellularization method has been established, where 3 freeze-thaw cycles with 0.1% Triton presented a good outcome. **Clinical Significance:** This protocol is crucial for TE strategies in total disc replacement or partial disc regeneration with the development of minimally invasive therapeutics.

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