June 14-15, 2018

Temporomandibular Joint Disorder TMJ Bioengineering Conference



Portofino Hotel and Marina 260 Portofino Way Redondo Beach, CA 90277

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Welcome!

It is indeed our pleasure to welcome you to Los Angeles, CA, for the Sixth Temporomandibular Joint Bioengineering Conference (TMJ6)!

Once again, we have reunited our friends and colleagues for another lively scientific discussion of state-of-the-art research on the 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 friendship and establish collaboration.

Consistent with that theme, this year's program focuses on special topics with accompanying keynote speakers such as markers and cell based therapies, in vivo mechanics, pathophysiology of the TMJ, and bioscaffold based functional tissue engineering.

We would especially like to thank our generous sponsors, the program committee, and our organizers, Diane Turner and Michele Leahy; all of your support is an integral part of maintaining the high quality of this meeting.

Please enjoy the conference!

With our very best wishes.

Sincerely, Alejandro Almarza, PhD Michael Detamore, PhD Boaz Arzi, DVM, DAVDC, DEVDC

General Information

Aims of the Symposium

The *TMJ Bioengineering Conference* 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 challenging problems in clinical management of TMDs, and set new directions in biomechanical and biological research that hold great potential for the future.

Organizing/Program Committee

Advisory Board

Alejandro Almarza – Chair Michael Detamore Boaz Arzi Kyriacos Athanasiou Lou Mercuri

Instructions to Presenters

I. Podium Presenters

The time for presentations will be limited, in favor of more time for discussion. Therefore, the speakers and moderators have been asked to limit the number of slides as well as to adhere to the time allotted for each presentation.

Important Notes:

All speakers are asked to check-in with the projectionist and the session moderators 15 minutes before the start of session in which they will present.

For 15 minute time slots

10 min. presentations each immediately followed by a 5 min. discussion. Maximum **15 PowerPoint slides** for computer presentation.

Note: In view of time and the large number of talks, there will be no opportunity to use your personal computer or load your PowerPoint file during the symposium.

Conference Sponsor



http://www.tmjconcepts.com/

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http://www.ccr.pitt.edu

Featured Keynote Speakers



Ali Khademhosseini, PhD, is the Levi Knight Professor of Bioengineering, Chemical Engineering and Radiology at the Univeristy of California-Los Angeles (UCLA). He is the Founding Director of the Center for Minimally Invasive Therapeutics at UCLA as well as an Associate Director of the California NanoSystems Insitute. He joined UCLA in Nov. 2017 from Harvard University where he was Professor of Medicine at Harvard Medical School (HMS) where he directed the Biomaterials Innovation Research Center (BIRC), a leading initiative in making engineered biomedical materials. He is recognized as a leader in combining micro- and nano-engineering approaches with advanced biomaterials for regenerative medicine applications. In particular, his laboratory has pioneered numerous technologies and materials for controlling the

architecture and function of engineered vascularized tissues. He has authored ~500 journal papers (Hindex > 96, >34,500 citations) and 60 books/chapters. In addition, he has delivered 300+ invited/keynote lectures. Dr. Khademhosseini's interdisciplinary research has been recognized by over 40 major national and international awards. He is a recipient of the Presidential Early Career Award for Scientists and Engineers, the highest honor given by the US government for early career investigators. In 2011, he received the Pioneers of Miniaturization Prize from the Royal Society of Chemistry (RSC) for his contribution to microscale tissue engineering and microfluidics. In 2016, he received the Sr. Scientist Award of Tissue Engineering and Regenerative Medicine Society -Americas Chapter (TERMIS-AM) and in 2017 he received the Clemson Award of the Society for Biomaterials. He is also a fellow of the American Institute of Medical and Biological Engineering (AIMBE), Biomedical Engineering Society (BMES), Royal Society of Chemistry (RSC), Fellow of the Biomaterials Sciences and Engineering (FBSE) and American Association for the Advancement of Science (AAAS). Currently he serves on the editorial board of numerous leading journals as well as an Associate Editor for ACS Nano (IF: 13.3) and a member of NIH BTSS study section. He received his Ph.D. in bioengineering from MIT (2005), and MASc (2001) and BASc (1999) degrees from University of Toronto both in chemical engineering. Read more at: http://www.tissueeng.net/



Nadim James Hallab, PhD, is a Professor in the Dept of Orthopedics, Dept of Immunology, and Dept of Anatomy and Cell Biology at Rush University. He is a researcher of orthopedic implant biocompatibility and immunology, and teaches Biomaterials at the graduate level. Dr Hallab's research is focused on understanding (1) how implant debris is produced and what forms it takes and (2) how this debris is interacts with the innate and adaptive immune systems. The long term goal of this lab is to improve implant performance by minimizing the biologic impact of implant debris.

Dr Hallab research involves the study of implant degradation and quantifying and predicting biologic reactivity to soluble and particulate implant debris:

Primary Research Areas:

1) Immune reactivity to implant debris, from both an adaptive (T-cell) and innate (macrophage) perspective,

2) Implant connections (modular junctions) and implant fretting corrosion, metal release and metal-protein complex formation,

3) Peri-implant cell toxicity responses to implant degradation products such as metals,

4) Study of how material surfaces can be used to control immune and cell function such as bone deposition.



TMJ Conference Schedule at a Glance

CONFERENCE LOCATION:

Portofino Hotel and Marina (Bayside Room)

260 Portofino Way

Rendondo Beach, CA 90277

DAY 1 – Wednesday, June 13, 2018

7:00 PM WELCOME RECEPTION

Location: Portofino Hotel and Marina - Baleen Lounge

DAY 2 - Thursday, June 14, 2018

- 8:00 AM BREAKFAST, REGISTRATION, CHECK-IN
- 8:40 AM Opening Ceremony, Welcome & Announcements Alejandro Almarza
- 9:00 AM KEYNOTE SPEAKER Ali Kahdemhosseini, PhD

"Enabling Precision Medicine through Engineering"

10:00 AM BREAK

PODIUM SESSION 1: ANIMAL MODELS

10:30 AM The Temporomandibular Joint of the Domestic Dogs (Canis Lupus Familiaris) in Health and Disease

Lin A, Vapniarsky N, Cissell DD, Verstraete FJM, Ting V, Hatcher DC, Arzi B

10:45 AM Microstructural Changes in the TMJ Bone and Cartilage are Preceded by Early Upregulation of Joint Hypoxia and Catabolic Factors After TMJ Loading that Causes Pain in the Rat

Sperry MM, Troche HR, Winkelstein BA, Granquist EJ

11:00 AM Temporomandibular Joint Pathology in Wild Carnivores in the Western United States

Verstraete FJM, Arzi B

11:15 AM Regional and Disease-related Differences in Properties of the Equine Temporomandibular Joint Disease

Guerrero Cota JM, Leale DM, Arzi B, Cissell DD

11:30 AM NETWORKING (POSTER) SESSION

Transzygomatic coronoidectomy and autologous fat placement as a treatment for pseudoankylosis of the temporomandibular joint - *Tekin U, Koçyiğit D, Özgül O*

Strategy of Temporomandibular Joint Cartilage Regeneration via Endogenous Progenitor Cell Homing - Seol D, Song I, Kendrick DE, Chiphet K, Lehman A, Salem AK, Martin JA,

Shin K

In Vivo Regenerative Potential of Various Soft Polymeric Scaffolds in Osteochondral TMJ Defects - *Chin AR, Taboas J, Gao J, Wang, Y, Almarza AJ*

Orofacial Pain Assessment of Rats with Bite-Raising Splints - *Li W, Pineda-Fairas JB, Gold MS, Almarza AJ*

12:00 PM Lunch

PODIUM SESSION 2: TMJ TISSUE ENGINEERING PART 1

| 1:00 PM | Fabrication of a Polycaprolactone and Hyaluronic Acid Multi-Material Regenerative Condylar Implant for Temporomandibular Joint Reconstruction |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| | Wyman OM, Detamore M, Srcuri LG |
| 1:15 PM | TMJ Condylar Cartilage Regeneration by Scaffold-free MSC-Chondrocyte Cell Sheet: An Invitro Study |
| | Hu Y, Zhou G, He D |
| 1:30 PM | Subcutaneous Implantation of Collagen Scaffolds with MSCs: The Effect of Angiostatin |
| | Helgeland E, Pedersen T, Mustafa K, Rosén A |
| | |

10:00 AM BREAK

PODIUM SESSION 3: BIOMECHANICS

| 2:45 PM | BREAK |
|---------|-------------------------------------------------------------------------------------------------------------------------|
| | Sagl B, Piehslinger E, Kundi M, Schimd-Schwap M, Stavness I |
| 2:30 PM | A Novel Combined Rigid Body – Finite Element Model for the Investigation of Temporomandibular Joint Loads |
| | Mesnard M, Teschke M, Supernak M, Duarte R, Ramos A São Leopoldo Mandic School of Dentistry - Campinas -SP- Brazil |
| 2:15 PM | Characterization of the Trabecular Bone Tissue within the Mandible Ramus - An Innovative Method and Statistical Results |
| | Colombo V, Gallo LM, Gonzalez YM, Iwasaki LR, Markovà M, Nickel JC |
| 2:00 PM | Characteristics of the TMJ Biomechanical Environment During Function |

PODIUM SESSION 4: TOTAL JOINT PROSTHETICS

3:00 PM Serum Metal Levels in Maxillofacial Reconstructive Surgery Patients. A Pilot Study

Mercuri LG, Miloro MJ, Skipor AK, Jacobs JJ, Sukotjo C, Mathew MT

3:15 PM Bespoke 3D Printed TMJ Prosthesis

Fink S, Dimitroulis G

3:30 PM A New Concept and Design for an Alloplastic Total TMJ Prosthesis Using PEEK LT1 20% BA

Genovesi W, Comenale IC, Lordes Pereira W, Faro GG

3:45 PM BREAK

PODIUM SESSION 5: TMJ TISSUE ENGINEERING PART 2

4:00 PM Scaffold-free Cartilage Cell Sheet Combined with Bone-phase BMSCs-scaffold Regenerate Osteochondral Construct in Mini-pig Model

Wang Y, Kumta P, Almarza AJ Wang F, Hu Y, He D, Zhou G, Ellis E

4:15 PM In vivo Modeling and Repair of TMJ disc Thinning and Partial Perforation Using Scaffold-free Biomimetic Tissue-engineered Implants

Vapniarsky N, Huwe LW, Arzi B, Houghton MK, Wong ME, Wilson JW, Hatcher DC, Hu JC, Athanasiou KA

4:30 PM Hydrogels for controlled drug delivery and tissue engineering of the Temporomandibular Joint Stem Cell Derived Methods for Temporomandibular Joint Regeneration

Ruscitto A, Embree M

4:45 PM CLOSE

6:00 PM DINNER

DAY 3 – Friday, June 15, 2018

- 8:00 AM BREAKFAST, REGISTRATION, CHECK-IN
- 8:30 AM KEYNOTE SPEAKER Nadium James Hallab, PhD

"Biologic Responses to TJR Implant Debris: What are Innate and Adaptive Immune Responses to TMJR Implant Debris and why do they matter?"

9:30 AM BREAK

PODIUM SESSION 1: CLINICAL STUDIES

10:00 AM PRP in the Therapy of TMD. Evaluation of Pain in 5 Years Follow-up Study

Machon V, Levorova J

10:15 AM EMG Measurements Reveal Abnormal Masticatory Muscle Coordination in TMD Patients

Tartaglia G, Gizdulich A, Silva R, Connelly ST

10:30 AM Is General Joint Hypermobility Associated with Altered Extra Cellular Matrix Proteinn Concetrations in the Temporomandibular Joint?

Ulmner M, Sugars R, Weiner CK, Lund B

10:45 AM BREAK

PODIUM SESSION 2: DEVELOPMENTAL BIOLOGY

- 11:00 AM
 Hormone-Receptor-MMP Axis in the Targeted Degradation of TMJ Fibrocartilage and Subchondral Bone

 Kapila S

 11:30 AM
 On the Way to the Progenitor Cells of Mandibular Condylar Cartilage: Zone-Specific Microarray Study

 Basudan AM, Yang YQ
- **11:45 AM** Regulation of Bone Morphogenetic Protein 2 in the Postnatal Growth and Development of the Mandibular Condylar Cartilage

O'Brien MH, Dutra EH, Chen P-J, Yadav S

12:00 PM Lunch

PODIUM SESSION 3: TREATMENTS AND DIAGNOSTICS

1:00 PM Outcome Analysis of Mandibular Patient Specific Joint Replacements in TMJ Ankylosis and Tumor: A Prospective Analysis

Mehrotra D

1:15 PM Temporomandibular Joint Chondromatosis: A Difficult Diagnosis

Levorova J, Machon V

1:30 PM Estrogen Inhibits Lubricin Expression in Baboon TMJ Synovial Cells

Perez L, Phelix CF, Millican L, Lebaron RG

1:45 PM BREAK

PODIUM SESSION 4: BIOLOGIC TREATMENTS

2:15 PM The Effects of Long Term Intermittent Administration of PTH on the Mandibular Condylar Cartilage and Subchondral Bone

Dutra Eh, O'Brien MH, Chen P-J, Yadav S

2:30 PM Articular Disc: Development Guided Regeneration

Weekes K, Pal D, Pryce B, Lam P, Schweitzer, Johnstone B

2:45 PM The Effects of Parathyroid Hormone and Bisphosphonate on the Cartilage and the Subchondral Bone of the Temporomandibular Joint

Chen P-J, Dutra Eh, O'Brien MH, Yadav S

- 3:30 PM DISCUSSION OF DAY'S TOPICS
- 3:30 PM CLOSING REMARKS

TMJ Bioengineering Conference Abstracts

PODIUM SESSION 1: ANIMAL MODELS

10:30 AM The Temporomandibular Joint of the Domestic Dogs (Canis Lupus Familiaris) in Health and Disease

Lin A, Vapniarsky N, Cissell DD, Verstraete FJM, Ting V, Hatcher DC, Arzi B

This study aimed to characterize the histologic, biomechanical and biochemical properties of the temporomandibular joint (TMJ) of the domestic dog in health and disease. In addition, we sought to identify structure-function relationships and to characterize TMJ degenerative lesions that may naturally be found in this species. The TMJ (n=20) from fresh cadaver heads (n=10) of domestic dogs were examined by cone beam computed tomography, macroscopically, and microscopically. The TMJ discs were also evaluated for their mechanical and biochemical properties. Furthermore, if TMJ arthritic changes were found, joint pathological characteristics were described and compared to healthy joints. Five dogs demonstrated macroscopically normal fibrocartilaginous articular surfaces and fibrous discs and 5 dogs exhibited degenerative changes that were observed either in the articular surfaces or the discs. In the articulating surfaces, these changes included erosions, conformational changes and osteophytes. In the discs, degenerative changes were represented by full thickness perforations. Histologically, pathologic specimens demonstrated fibrillations with or without erosions, subchondral bone defects and subchondral bone sclerosis. Significant anisotropy in the TMJ discs was evident upon histology and tensile mechanical testing. Specifically, the discs were significantly stiffer and stronger in the rostrocaudal direction compared with the mediolateral direction. No significant differences were detected in compressive properties of different disc regions. Biochemical analyses showed high collagen content and low glycosaminoglycan (GAG) content. No significant differences in biochemical composition, apart from GAG, were detected among the disc regions. GAG concentration was significantly higher in the central region as compared to the posterior region. The TMJ of the domestic dog exhibit similarities but also differences compared to other mammals with regards to structurefunction relationships. The TMJ articular surfaces and the disc exhibit degenerative changes as seen in other species including perforation of the disc as seen in humans. The degenerative changes had effects on mechanical and biochemical properties of the TMJ components. Translational motion of the TMJ does occur in dogs but is limited and may be rudimental

10:45 AM Microstructural Changes in the TMJ Bone and Cartilage are Preceded by Early Upregulation of Joint Hypoxia and Catabolic Factors After TMJ Loading that Causes Pain in the Rat

Sperry MM, Troche HR, Winkelstein BA, Granquist EJ

Abnormal joint overloading through parafunctional habits or disc dislocations is a risk factor for temporomandibular osteoarthritis (TMJ OA) pain. Patients with long-standing TMJ OA often exhibit pain, functional remodeling of joint tissues, and variable degrees of synovial inflammation. However, the time course and initiators of TMJ pain remain poorly understood. In this study, TMJ bone microstructure was assessed in an IACUC-approved rat model of repeated TMJ loading (3.5N; 1hr/day for 7 days) at days 8 (n=3) and 15 (n=6) and compared to normal (n=7). Increased trabecular spacing was detected in the superficial, posterior TMJ by microCT on both days compared to normal (one-way ANOVA; p<0.05). Yet, thickness of the subchondral bone decreased only on day 15 compared to normal (one-way ANOVA; p=0.0004). Previous experiments using the Mankin score suggest that cartilage is not damaged at day 8 but is moderately degenerated at day 15. Despite delayed changes in bone and cartilage structure, hypoxia and catabolic factors do increase on day 8, in the same time that both evoked and spontaneous pain are detected. Collectively, these findings suggest that the early hypoxia and inflammation after painful TMJ loading may contribute to the structural changes that are evident later while pain persists.



11:00 AM Temporomandibular Joint Pathology in Wild Carnivores in the Western United States

Verstraete FJM, Arzi B

The skulls of large numbers of pinnipeds, mustelids, ursids, felids and canids occurring in the western United States, were examined for the presence of bony changes associated with osteoarthritis of the temporomandibular joint (TMJ-OA). The museum specimens were sourced from wild populations. The occurrence of TMJ-OA ranged from 0-63.5%. The most commonly affected species included the California sea lion (63.5%), walrus (60.5%) and American black bear (50.0%). Conversely no TMJ-OA was found in the California bobcat and gray fox. Severe TMJ-OA, likely to have been associated with impaired function, was found in the southern sea otter (4.1%). In addition, osteochondritis dissecans-like lesions of the TMJ were also found among the species. While the etiology and pathophysiology of TMJ-OA in wildlife remains unknown, it was concluded that is some species TMJ-OA may contribute to morbidity and mortality.

11:15 AM Regional and Disease-related Differences in Properties of the Equine Temporomandibular Joint Disease

Guerrero Cota JM, Leale DM, Arzi B, Cissell DD

Naturally occurring temporomandibular joint (TMJ) disorders are increasingly recognized in animals, including horses. While the TMJ disc plays a major role in TMJ disorders in people, little is known about its role in TMJ pathology in animals. This study characterizes differences in properties of equine TMJ discs associated with age, disc region, and presence of TMJ osteoarthritis (OA). Discs were dissected from both TMJ's of sixteen horses euthanized for reasons unrelated to this study. Each joint was grossly evaluated and scored as normal, mild OA, or severe OA. Samples from the rostral, caudal, lateral, central, and medial regions of the disc were subject to compressive testing, quantitative biochemistry, and histology. Samples from the lateral, central, and medial region were tested for tensile properties in the rostro- caudal and mediolateral directions. We found that the equine TMJ disc is highly anisotropic, and its glycosamino-glycan (GAG) content and compressive stiffness with increasing age. While equine TMJ disc properties are generally similar to other herbivores, greater compressive stiffness throughout the disc and greater GAG content in its rostral region suggest that mechanical demands on the TMJ disc differ between horses and other species. Importantly, a region-specific decrease in compressive stiffness was observed associated with joint disease and corresponded to cartilage erosions in the underlying condylar surface.



Figure 1 Scatter plot of compressive relaxation modulus (ER) at 20% strain for discs from horses with asymmetrical TNU osteoarthritis. Within each disc region, points labeled "0" represent samples from normal or mildly osteoarthritic joints and points labeled "1" represent samples from joints with worse osteoarthritis. Matched samples from opposite joints within the same horse are connected by solid lines. ER was significantly decreased in the caudal region of discs from joints with greater osteoarthritis (p=0.006).

11:30 AM NETWORKING (POSTER) SESSION

Transzygomatic coronoidectomy and autologous fat placement as a treatment for pseudoankylosis of the temporomandibular joint - *Tekin U, Koçyiğit D, Özgül O*

Temporomandibular joint (TMJ) pseudoankylosis is rare and characterized by limited mouth opening, resulting from an extrinsic condition of the joint leading to fusion between the coronoid process and zygomatic or maxillary bone. Pseudoankylosis can be congenital or acquired; the majority of cases are associated with trauma. Once diagnosed, treatment consists of surgical excision of coronoid process via intraorally or extraorally followed by vigorous jaw movement exercises. Autologous fat transplantation is useful adjunct procedure to minimize the occurrence of excessive joint fibrosis, heterotopic calcification and reankylosis. In this study, treatment of a patient with pseudoankylosis has been successfully performed by applying transzygomatic coronoidectomy and autologous fat placement.

Strategy of Temporomandibular Joint Cartilage Regeneration via Endogenous Progenitor Cell Homing - Seol D, Song I, Kendrick DE, Chiphet K, Lehman A, Salem AK, Martin JA, Shin K

Degeneration of cartilage surfaces in temporomandibular joint (TMJ) disorders cause pain and disability and seldom resolve spontaneously; thus, there is a need to develop regenerative therapies. We have developed a promising strategy to stimulate cartilage regeneration via endogenous progenitor cell homing. The strategy can be achieved by adding chemotactic factors (white blood cell (WBC) lysates or microvesicles (MVs)) in hydrogel that improve progenitor cell recruitment, and a notch inhibitor (DAPT) in microspheres that enhances migrated cell differentiation into chondrocytes. Migrating cells repopulated scratch- or impact-injured areas and WBC lysates (higher than 8.3 million cells/ml) and MVs (100x concentrated medium) significantly increased the number of migrated cells. DAPT treatment in the culture medium induced the formation of morphologically chondrocyte-like cells and hyaline cartilage matrix by migrated progenitor cells. For sustained release, DAPT was encapsulated in PLGA microspheres and release profile demonstrated an initial burst release (20%) in first 24 hours, followed by a sustained release until 2 months. The combination of hydrogel with chemotactic factor(s) and DATP-microspheres will be applied for ex vivo TMJ cartilage defect in near future. In conclusion, our cell homing strategy has a great potential for the regeneration of TMJ cartilage.

In Vivo Regenerative Potential of Various Soft Polymeric Scaffolds in Osteochondral TMJ Defects - *Chin AR, Taboas J, Gao J, Wang, Y, Almarza AJ*

See Poster

Orofacial Pain Assessment of Rats with Bite-Raising Splints - Li W, Pineda-Fairas JB, Gold MS, Almarza AJ

See Poster

PODIUM SESSION 2: TMJ TISSUE ENGINEERING PART 1

1:00 PM Fabrication of a Polycaprolactone and Hyaluronic Acid Multi-Material Regenerative Condylar Implant for Temporomandibular Joint Reconstruction

Wyman OM, Detamore MSrcuri LG

In patients suffering from end-stage temporomandibular joint (TMJ) disorders, TMJ reconstruction using autologous or alloplastic implants are often required for symptomatic relief and improved function. We have developed a combinational 3D printer for TMJ tissue engineering of the condyle that incorporates natural materials such as devitalized cartilage (DVC), demineralized bone (DBM), hydroxyapatite (HAp), and pentanoate-functionalized hyaluronic acid (PHA) with polycaprolactone (PCL) to create anatomically precise patient- specific condylar implants. Four integration methods were developed to test interface adhesion between PHA and PCL and a unique fabrication paradigm was developed to harness capillary action to homogenously distribute cells. Rat mesenchymal stem cells (rMSCs) were seeded onto the fabricated gradient constructs and allowed to culture for 6 weeks. Results were analyzed after 2, 4, and 6 weeks using micro-computed tomography (µCT) to assess and quantify mineralization. Osteocalcin expression was tracked during the experiment and correlated to rMSC differentiation within the constructs. A statistically significant increase in mineralization was observed via µCT for the groups containing 30% HAp or 10% DBM compared to controls. Greater Saf-O staining was apparent for the group containing 10% DVC compared to controls.

1:15 PM TMJ Condylar Cartilage Regeneration by Scaffold-free MSC-Chondrocyte Cell Sheet: An Invitro Study

Hu Y, Zhou G, He D

Condylar cartilage lesions caused by temporomandibular joint osteoarthritis (TMJOA) are mostly irreversible for the limited repair and regeneration capacity of chondrocyte. To date, many studies of TMJ cartilage regeneration focus on scaffold-based tissue engineering, which may lead to some adverse effects after scaffold insertion and then degradation. Herein, we used a scaffold-free cell sheet technology to regenerate condylar cartilage by combining bone marrow stromal cells(BMSC) with condylar chondrocytes. Through high density co-culture of these two cells at different ratio(Chondrocyte:BMSC=10:0,7:3,5:5,3:7,0:10), hyaline, homogeneous condylar cartilage cell sheets with elasticity were obtained with appropriate proportion.

Comparison of chondrogenesis between different groups were using histological staining, immunofluorescence technique, Q-PCR and some quantitative test to evaluate tissue formation, related protein and gene expression. The results showed that after 3 weeks chondrogesis by micro-environment induction, the 10:0 and 7:3 group can form ideal cartilage cell sheets. And the 7:3 group showed the best cartilage formation from different aspects. Fluorescin lentivirus infection was also used to trace BMSC-chondrocyte interaction model and extracellular matrix(ECM) formation of cell sheet. We found that double-burst stimulation by chondrogenesis culture solution and chondrocyte interaction can make MSC differentiate to chondrocyte, then formed a better cell sheet. In short,Our research proved the feasibility and great potential of MSC-chondrocyte cell sheet to regenerate condylar cartilage. Futuremore, in-vivo animal model studies are needed to investigate this co-culture cell sheet to regenerate TMJ condylar cartilage in-situ.

1:30 PM Subcutaneous Implantation of Collagen Scaffolds with MSCs: The Effect of Angiostatin

Helgeland E, Pedersen T, Mustafa K, Rosén A

Aim : To investigate the effect of the angiogenesis inhibitor, angiostatin, on fibrocartilage formation in an ectopic rat-model. **Material and methods:** Collagen type-I scaffolds were divided into four groups: (i) scaffold only, (ii) scaffold + BMSCs, (iii) scaffolds + angiostatin and (iv) scaffolds + angiostatin + BMSCs. Cell were harvested from femur of six donor rats. One construct from each group was randomly, subcutaneously implanted on dorsum of Lewis rats (n=24). Time points for harvest of constructs were 2 and 8 weeks. The implanted discs were analyzed radiographically (µCT), and expression of selected biomarkers for inflammation and vascularization were analyzed with RT-PCR. The morphology of the constructs were analyzed with histology and immunofluorescence quantification (CD31 and COL-I) will be performed. **Results:** After 8 weeks, group (ii) and (iv) showed similar radiographic density to the native TMJ rat discs. Biomarkers for inflammation (LL-1a and LL-1b) and vascularization (CD31) were significantly down-regulated in constructs functionalized with angiostatin, after 2 weeks. Scaffolds in group (i) and (ii) were considerably degraded after 8 weeks implantation. **Conclusion:** Angiostatin is effective in preventing blood vessel formation and decrease the level of inflammatory markers. The scaffold biomaterial degraded too rapidly for fibrocartilage formation *in vivo*.

PODIUM SESSION 3: BIOMECHANICS

2:00 PM Characteristics of the TMJ Biomechanical Environment During Function

Colombo V, Gallo LM, Gonzalez YM, Iwasaki LR, Markovà M, Nickel JC

The study of mechanical origins of TMJ breakdown requires precise acquisition of the biomechanical environment. Dynamic loading areas can be characterized by parameters sensitive to oral tasks and to factors such as gender and diagnostic group. These parameters are *e.g.* associated with the energy density spent in the TMJ (mJ/mm³). We analyzed in a cross-sectional study (191 TMJs in 59 f and 45 m, 20-40y) the general behavior irrespectively of diagnostic groups of the mediolateral stress-field excursion ΔD , its average velocity *v*, volume *Q* and aspect ratio *a/h*. The latter reflects the degree of penetration of the stress-field area into the disc fibrocartilage. Overall, in jaw opening/closing and contralateral laterotrusion, significant gender differences were found (p≤0.01), females having smaller Q (134±86 vs. 161±95 mm³) and *a/h* (2.3±0.9 vs. 2.5±1.1). ΔD (3.1±1.7 mm) and *v* (4.3±2.2 mm/s) did not differ. For the whole sample, in contralateral laterotrusions *a/h* was smaller by 0.2±0.7 and ΔD larger by 1.6±2.3 mm than in jaw opening/closing (p<0.05 resp. 0.001). Results suggest gender differences in TMJ loading due to anatomy as well as different articular surface incongruities due to the nature of mandibular movements. Supported by NIDCR (R01 2DE016417). No conflicts of interest.

2:15 PM Characterization of the Trabecular Bone Tissue within the Mandible Ramus - An Innovative Method and Statistical Results

Mesnard M, Teschke M, Supernak M, Duarte R, Ramos A São Leopoldo Mandic School of Dentistry - Campinas -SP- Brazil

Characterizing the geometry of the bone tissues within the mandible ramus using an image processing method allows the design / selection of mandibular components for a temporomandibular joint (TMJ) prosthesis and, in the same time, setting the location of bone resection or surgical screws. On the other hand, procedures available to build 3D models based on CT scans and dedicated software are often time consuming and do not provide the surgeon a quick response to prepare the intervention and select a prosthetic concept. The study built and evaluated an innovative image processing method to characterize the geometry of cancellous and cortical bone tissues [1]. The first results highlighted the accuracy and the reproducibility of the method and the slight influence of the user's skills. They also pointed out strong intra- and inter-individual variations [2]. Therefore, in the TMJ prosthesis design process, it seems necessary to integrate these fluctuations as well as specific pathological situations and to design modular or customized components easy to select and to use. To

evaluate these fluctuations, the statistical phase of the study analyzes a sample of 92 patients. Five parameters were selected to characterize the distribution of the cancellous bone tissue between the condyle and the mandibular nerve and foramen.

[1] DUARTE R., DELOS V., RAMOS A., TESCHKE M. & MESNARD M. Development of a relevant image processing method to characterize the distribution of tissue within a bone structure / Journal of Computer Science & Systems Biology - vol.8 - 2015 - p.199-202. [2] RAMOS A., NYASHIN YI. & MESNARD M. Influences of geometrical and mechanical properties of bone tissues in mandible behaviour - Experimental and numerical predictions / Computer Methods in Biomechanics and Biomedical Engineering - vol.20, n°9 - 2017 - p.1004-14.

2:30 PM A Novel Combined Rigid Body – Finite Element Model for the Investigation of Temporomandibular Joint Loads

Sagl B, Piehslinger E, Kundi M, Schimd-Schwap M, Stavness I

Temporomandibular joint disorders (TMD) are among the most prevalent human syndromes. Due to the complexity of the masticatory system, the development of TMD is not fully understood. Investigations of joint loads could lead to a better understanding of TMD. Hence, this project aims to use a novel biomechanical model of the masticatory region for the investigation of temporomandibular joint (TMJ) loads. CT data of a healthy person were acquired to create detailed models of the bony structures of the masticatory region. Additionally, MRI scans using a special TMJ imaging sequence were performed to acquire a high-resolution representation of the soft tissue structures of the TMJ for different static postures. The maxilla and mandible were represented as rigid bodies and jaw muscles were modeled using Hill-type line-based muscle models. This project aims to use the combined Rigidbody/FEM model to gather insight into the mechanisms that underlie pathologies of the TMJ. Moreover, the model created for this project could be used for a variety of studies that investigate the effects of different movement or force patterns on the loading of different TMJ structures.

PODIUM SESSION 4: TOTAL JOINT PROSTHETICS

3:00 PM Serum Metal Levels in Maxillofacial Reconstructive Surgery Patients. A Pilot Study

Mercuri LG, Miloro MJ, Skipor AK, Jacobs JJ, Sukotjo C, Mathew MT

Purpose: The aim was to assay metal concentration in the serum of the patients with dental implants, orthognathic surgery metal fixation plates and screws, and total temporomandibular joint replacement devices. **Materials and Methods:** Thirty patients were identified. Sixteen patients (9 males/8 females), average age 44 (19-79) years old, were divided into 3 groups (Groups 1: orthognathic patients; Group 2: TMJ TJR patients; and Group 3: dental implant patients). A control group consisted of volunteers never implanted with any metallic devices. Blood samples for serum metal analysis were obtained and analyzed in accordance with the standardized collection protocol utilized at Rush University Department of Orthopaedic Surgery. **Results:** All control patients had below the normal reference range for all the markers measured. Among the orthognathic patients, only one patient showed a high level of cobalt in the serum; whereas for TMJ TJR patients, two patients indicated a higher value of cobalt and chromium. For dental implant patients, one subject indicated a high level of titanium, and another indicated a both a high level of titanium as well as chromium. None of the subjects exhibited serum metal levels for the metal studied equal to those found in patient with orthopedic joint devices.

3:15 PM Bespoke 3D Printed TMJ Prosthesis

Fink S, Dimitroulis G

3D printing has revolutionized medical device development, allowing for rapid prototyping and innovative new designs. In Melbourne, Australia, Dr George Dimitroulis, working with the University of Melbourne, has developed a 3D printed temporomandibular joint (TMJ) total joint replacement system. This is the first of its kind; a print-to-order TMJ prosthesis which can be either patient-sized or fully custom, designed utilizing CAD-CAM technologies and produced using additive and subtractive manufacturing. Dr Dimitroulis, founder of the biotech start-up company, OMX Solutions, has, along with several other Australian Maxillofacial surgeons, implanted over 80 TMJ devices so far with good outcomes and up to 3 years follow- up. The presentation will focus on the digital workflow process from ordering of the device to implantation together with a brief overview of the development history, engineering, clinical outcomes and benefits/advantages of this new device. The TMJ device has been TGA approved for the Australian market and efforts are now being made to introduce the device to a global market through CE marking and FDA approval.

3:30 PM A New Concept and Design for an Alloplastic Total TMJ Prosthesis Using PEEK LT1 20% BA

Genovesi W, Comenale IC, Lordes Pereira W, Faro GG

The TMJ (temporomandibular joint) is a complex joint, with distinct anatomical and functional characteristics, difficult to treat. Many authors, from the early twentieth century, reported techniques for TMJ reconstruction, aiming at returning its shape and ideal function. Many prototypes have been developed in pursuit of the ideal prosthesis, which adheres to the principles of biomechanics and biocompatibility, with good long-term performance and lower cost. Based on 10 years of experience (1990 to 2000) with 125 patients who underwent TMJ reconstruction using full custom prosthesis in gold (unilateral and bilateral), with a new design and shorter than the prosthesis found in the market. A new surgical technique was performed, less traumatic, than used by others urgeons in the world. Because of the high cost of gold alloys, ensued in search of a suitable material, to follow the ideal characteristics. Among the new materials, highlights PEEK LT1 20% Ba, is a polymer derived from petroleum (Invibio, UK) thermoplastic biocompatible, inert and high stability and resistance. Successfully used as the material of choice for orthopedic implants and spine. This study demonstrates the feasibility of a custom prosthesis in PEEK LT1 20% Ba, with protocol development for TMJ reconstruction.

PODIUM SESSION 5: TMJ TISSUE ENGINEERING PART 2

4:00 PM

Scaffold-free Cartilage Cell Sheet Combined with Bone-phase BMSCs-scaffold Regenerate Osteochondral Construct in Mini-pig Model

Wang Y, Kumta P, Almarza AJ Wang F, Hu Y, He D, Zhou G, Ellis E

Tissue-engineered biological condyles provide a promising approach for end-stage osteoarthritis to reconstruct normal physiological structure and function of the temporomandibular joint (TMJ). However, lack of successful tissue-engineered biological condyles in large animal model restricts clinical transference. Scaffold-free cartilage cell sheets do not contain any biodegradable polymeric material which potentially risks local nonspecific inflammatory reactions. It consists of chondrocytes from autogenous auricular cartilage and cartilage extracellular matrix excreted by chondrocytes in vitro. In this study, we used scaffold- free cartilage cell sheets covering bone marrow mesenchymal stem cells- Polycaprolactone/ Hydroxyapatite (BMSCs-PCL/HA) scaffolds (cell sheet group, N=8) transplanted subcutaneously and intramuscularly in mini-pigs. In contrast, autogenous chondrocytes were seeded on polyglycolic acid/ polylactic acid (PGA/PLA) scaffolds for 4 and 12 weeks in-vitro pre-cultivation. They were used as a cartilage-phase composition covering BMSCs-PCL/HA scaffolds which served as bone-phase composition, then the entirety (biphase scaffold group, N=8) was transplanted subcutaneously into mini-pigs. After 12 weeks, the harvested explants were examined histologically (hematoxylin and eosin, safranin-O/fast green, COL I, COL II). The cartilage layer was evaluated for thickness, glycosaminoglycan (GAG) quantitation, total collagen quantitation and Young's modulus. The biphase scaffold group failed in regeneration because of local nonspecific inflammation led by residual and degradation products of the PGA/PLA scaffold, while the cell sheet group regenerated a healthy osteochondral construct with a mature cartilage layer and closely integrated subchondral structure. The GAG quantitation, total collagen quantitation and Young's modulus of regenerated cartilage was more close to those of the natural condylar cartilage. Collectively, these results indicated that cartilage cell sheets combined with bonephase BMSCs-PCL/HA scaffolds had the potential to be an ideal approach for biological condylar regeneration.

4:15 PM In vivo Modeling and Repair of TMJ disc Thinning and Partial Perforation Using Scaffold-free Biomimetic Tissue-engineered Implants

Vapniarsky N, Huwe LW, Arzi B, Houghton MK, Wong ME, Wilson JW, Hatcher DC, Hu JC, Athanasiou KA

The treatments currently available for the patients with TMJ disc disease are palliative rather curative. Recent advancements in the developments of animal models for the study of TMJ and tissue engineering of biomimetic tissues of substantial size without exogenous scaffold provide a promising platform to explore regenerative solutions for the TMJ disc disease. In this study, we developed an innovative surgical method, modeling disc thinning with partial perforation in a minipig. We designed and tested a surgical technique for the implantation and stabilization of the engineered tissue *in situ* and tested *in vivo* the efficacy of the tissue engineered constructs to regenerate induced disc defects. The morphological, histological, biomechanical, and biochemical examinations at 2 and 8 weeks after implantation indicated that implantation of tissue-engineered constructs promoted disc integrity and regeneration. Implantation resulted in 4.4 times more complete defect closure than in the empty defect controls, as quantified by histological evaluation. This occurred in conjunction with mild lymphocytic response around the implants. The treatment induced the formation of repair tissue (fibrous connective tissue) that filled the defect and was 3.4-fold stiffer in tension than similar tissue in empty defect controls. Tensile mechanical testing determined that the leaflets of the defects containing tissue-

engineered implants fused 3.2-fold more efficiently than defects with no implants. The safety and efficacy of this approach pave the way toward clinical treatment of TMJ disc pathology.

4:30 PM Hydrogels for controlled drug delivery and tissue engineering of the Temporomandibular Joint Stem Cell Derived Methods for Temporomandibular Joint Regeneration

Ruscitto A, Embree M

DAY 3 – Friday, June 15, 2018

8:30 AM KEYNOTE SPEAKER - Nadium James Hallab, PhD

"Biologic Responses to TJR Implant Debris: What are Innate and Adaptive Immune Responses to TMJR Implant Debris and why do they matter?"

PODIUM SESSION 1: CLINICAL STUDIES

10:00 AM PRP in the Therapy of TMD. Evaluation of Pain in 5 Years Follow-up Study

Machon V, Levorova J

In years 2011-13, 65 patients underwent intra-articular PRP administration after an ineffective arthroscopic lavage. These patients were Wilkes IV and V. Of these patients 47 accepted 5 years follow up of controls. During the 5-year follow-up, a pain-relieving effect was observed (1 month after surgery at 85%, 5 years after surgery in 48% of patients). Overall, PRP was unsuccessful in 7% of patients, with another 45% of patients experiencing recurrence of pain within 5 years. In particular, patients with degenerative changes have been affected. A major factor in the failure of PRP was the duration of pain before beginning of TMJ therapy.

10:15 AM EMG Measurements Reveal Abnormal Masticatory Muscle Coordination in TMD Patients

Tartaglia G, Gizdulich A, Silva R, Connelly ST

EMG was used to assess masticatory muscle activity in a group of 5 TMD patients whose disease was surgically indicated and compared to a group of healthy adults while both groups performed 15s unilateral gum chewing. A wireless electromyographic analyzer (BTS instruments, Milan Italy) was used to measure standardized activities of the masseter and temporalis muscles. The statistical analysis comparing the two groups revealed that in addition to dissimilar chewing frequency, a bivariate analysis was performed on the simultaneous differential of the right-left masseter (Δ M) and temporalis (Δ T) activity (Lissajous's plot) to analyze the coordination pattern. Symmetry indices, effort-related parameters and intra-group variability were also assessed. The outcomes showed that TMD patients had different muscular coordination in comparison to healthy adults, probably due to the neuromuscular impairment on the diseased side resulting in less muscular recruitment. Additionally, it was found that there was an accentuated divergence in muscular activation between the two chewing sides, suggesting the existence of a temporary preferred side of mastication, likely induced by an asymmetric acute state of TMJ functionality. Overall, these control data provide a reference database for the assessment of patients, either children or adults, with functionally altered stomatognathic conditions.

10:30 AM Is General Joint Hypermobility Associated with Altered Extra Cellular Matrix Protein Concentrations in the Temporomandibular Joint?

Ulmner M, Sugars R, Weiner CK, Lund B

Aim: To analyze synovial tissue (ST) samples on patients with internal derangement (ID) regarding concentrations of extra cellular matrix (ECM) components in relation to general joint hypermobility (GJH)

versus normal joint mobility (NJM). **Material and methods:** Consecutive patients at Karolinska University Hospital, Stockholm, Sweden, with either chronic closed lock (CCL) or painful clicking (PC) and scheduled for arthroscopy or discectomy were included. Joint hypermobility was determined using the Beighton score (≥ 4 or more indicating GJH). Protein was extracted from ST samples harvested from the posterior attachment. Multiplex analysis were made for detection of ECM proteins Lumican, Collagen 1 and 4, Syndecan-1 and 4, Tenascin C, Fibronectin, Aggrecan, and Fibroblast Activating Protein. Two-sample Wilcoxon rank-sum tests and a quantile regression model with joint mobility diagnosis as the dependent variable and sex, age and ID as independent variables were performed. **Results:** A total of 42 patients were analyzed, 14 GJH and 28 NJM, see table 1 and 2.

Table 1. Age, gender and distribution in relation to diagnose.

| | Mean age (range) | Sex (Female/Male) | PC (percent) | CCL (percent) |
|-----|------------------|-------------------|--------------|---------------|
| GJH | 32.6 (19-55) | 14 /0 | 6 (43) | 8 (57) |
| NJM | 44.7 (20-69) | 24 /4 | 11 (39) | 17 (61) |

Table 2. Protein concentrations.

| ECM protein | GJH mean conc. (pg/ml) | NJM mean conc. (pg/ml) | GJH (p-value) | ID (p-value) | Age (p-value) | Sex (p-value) |
|----------------|---------------------------|---------------------------|------------------|-----------------|------------------|------------------|
| Lumican | 394687.8 | 415484.8 | 0.661 | 0.002 * | 0.418 | 0.674 |
| Collagen 1 | 3097.3 | 4587.4 | 0.603 | 0.101 | 0.209 | 0.849 |
| Collagen 4 | 1148.3 | 1649.4 | 0.936 | 0.009 * | 0.266 | 0.601 |
| Syndecan 1 | 303.6 | 270.4 | 0.830 | 0.054 | 0.723 | 0.566 |
| Syndecan 4 | 102.0 | 77.7 | 0.738 | 0.426 | 0.500 | 0.462 |
| FAP | 1749.4 | 1908.0 | 0.953 | 0.038 * | 0.388 | 0.618 |
| Tenascin C | 1759.8 | 2824.2 | 0.910 | 0.274 | 0.114 | 0.849 |
| Fibronectin | 196777.0 | 338635.6 | 0.177 | 0.220 | 0.205 | 0.757 |
| Aggrecan | 610.2 | 401.4 | 0.374 | 0.016 * | 0.520 | 0.512 |

FAP, Fibroblast Activating Protein. P<0.05 *.

Conclusion: No significant differences of ECM concentrations between GJH and NJM were found. ID diagnosis (PC, CCL) showed significant differences on ECM concentration indicating that deterioration of the TMJ in CCL is more decisive for ST ECM concentrations than GJH.

PODIUM SESSION 2: DEVELOPMENTAL BIOLOGY

11:00 AM Hormone-Receptor-MMP Axis in the Targeted Degradation of TMJ Fibrocartilage and Subchondral Bone

Kapila S

There are no current long term graft replacements for the TMJ following discectomy. Extracellular matrix (ECM) scaffolds have shown potential as a graft replacement in the canine TMJ model. In this study, we evaluated the biochemical and biomechanical remodeling of ECM scaffolds implanted in a porcine model following discectomy at 1, 3, and 6 months post implantation. Three month old female pigs received unilateral implantation of an ECM scaffold following discectomy. Scaffolds were resected at 1, 3, and 6 months post implantation and compared to age-matched native controls. Hydroxyproline, glycosaminoglycan (GAG), and DNA assays were performed to determine biochemical content. Unconfined stress relaxation compressive testing and uniaxial tensile testing were performed to determine the compressiv We have previously shown that estrogen and relaxin contribute to the targeted degradation of TMJ disc fibrocartilage in parallel with the induction of matrix metalloproteinases (MMP) -9 and -13. Here, using cell culture and in vivo studies, we identified the receptors and the role of MMP- 9 and -13 in relaxin- and estrogen-mediated TMJ fibrocartilage and subchondral bone matrix degradation. We identified that estrogen signals via ER□ but not ER□, and relaxin via RXFP1 but not RXFP2 in the induction of MMP-9 and -13 by their respective hormones. Estrogen

and relaxin also caused significant increase in TMJ subchondral bone porosity by about 80% and 60%, respectively. Relaxin treatment in the absence of MMP-9 and MMP-13 did not contribute to increased subchondral bone porosity, whereas estrogen caused an increase in porosity in MMP-9-null mice similar to that in WT mice but not in MMP-13-null mice. None of the hormones caused any adverse changes in the articular subchondral bone or sub-epiphyseal trabecular bone of the femur. Indeed, the latter site displayed the characteristic effects of estrogen on osteoclasts, the osteochondral junction and surfaces of mineralized spicules were heavily stained for TRAP-positive multinucleated cells, presumably osteo/chondroclasts. Consistent with relaxin's known effects in increasing osteoclast differentiation, relaxin-treated mice showed increased subchondral osteo/chondroclasts but these were fewer than those observed with estrogen. In conclusion, estrogen and relaxin specifically compromise the integrity of TMJ condylar subchondral bone possibly through enhanced matrix degradation and increased osteo/chondroclastic activity that requires MMP-9 or MMP1-3 or both proteinases. (Supported by K02 DE00458 and R01 DE018455)

11:30 AM On the Way to the Progenitor Cells of Mandibular Condylar Cartilage: Zone-Specific Microarray Study

Basudan AM, Yang YQ

Combining Laser-Capture microdissection (LCM), which allows precise cells procurement from heterogeneous tissues, and microarray analysis (MAA), which allows analyzing the expression of thousands of genes simultaneously, enables accurate large-scale studies. The objective of this study is to identify potential genes that distinguish the cells of mandibular condylar cartilage (MCC) zones from each other and from the articular chondrocytes. **Materials/Methods and Results:** LCM-RNA samples were collected from femoral condylar cartilage (FCC) and MCC zones; fibrous (FZ), proliferative (PZ), mature (MZ), and hypertrophic (HZ) zones individually using SD rat. Microarray hybridization using Affymetrix GeneChip Rat Genome 230 2.0 Array was performed. Data mining allowed creating **7 gene subsets** that identified 127 differentially expressed genes mostly obvious between FCC and all MCC zones. Nevertheless, strong differences were also identified within the MCC. The strongest upregulation was observed for the Crabp1 gene in FZ as compared to HZ and FCC, while the most downregulated gene was Clec3a in FZ relative to FCC. **Conclusion:** Comprehensive evaluation of genome-wide expressions using combined LCM/MAA approach revealed robust gene expression differences, supporting the hypothesis that differential gene expression exists between articular chondrocytes and MCC cells on one hand, and among the four MCC zones on the other hand.

11:45 AM Regulation of Bone Morphogenetic Protein 2 in the Postnatal Growth and Development of the Mandibular Condylar Cartilage

O'Brien MH, Dutra EH, Chen P-J, Yadav S

Objectives: BMP2 plays an important role in cartilage growth and development. Paradoxically, elevated levels of BMP2 leads to degeneration and osteoarthritis of cartilage. Our aim was to determine the effects of BMP2 loss of function on the cartilage and subchondral bone of the TMJ. **Methods:** Three-week-old mice with floxed BMP2 and Cre-expression specific to the aggrecan (ACAN) promoter, and their Cre-negative littermates, were injected with tamoxifen (75 µg/kg body weight) for 5 days. The animals were euthanized at 3 and 6 months following treatment with tamoxifen. They were injected with alizarin complexone and calcein before euthanization. EdU was injected at 2 days and 1 day before euthanization. **Results:** In mice at each age group the conditional deletion of BMP2 led to a break in the cartilage integrity, with a decrease in cartilage thickness and matrix synthesis. There was a significant increase in TRAP and alkaline phosphatase staining with deletion. **Conclusion:** In summary, deletion of BMP2 in ACAN-expressing cells during postnatal development can lead to cartilage breakdown and early development of osteoarthritis.

PODIUM SESSION 3: TREATMENTS AND DIAGNOSTICS

1:00 PM Outcome Analysis of Mandibular Patient Specific Joint Replacements in TMJ Ankylosis and Tumor: A Prospective Analysis

Mehrotra D

Purpose: Autologous grafts or alloplastic stock prosthesis have been commonly used for reconstruction of temporomandibular joint (TMJ) post-arthrectomy in cases of TMJ ankylosis. However, they have their own specific problems, like second surgical site, donor site morbidity with grafts or inappropriate fit with a stock prosthesis. This investigation sought to determine the outcome of patient specific alloplastic replacement of the

mandibular joint. Materials and Methods: This was a prospective study in cases of TMJ ankylosis or mandibular pathology who were given patient specific TMJ alloplastic metallic mandibular condular replacement and glenoid fossa reconstruction with ultra high molecular weight polypropylene. Dermal fat graft was interposed in between the reconstructed glenoid fossa and condylar head. Patients were followed for a year by direct questioning, clinical observation (pain scores, mouth opening and diet:1 scored for liquid, 2 semisolids and 3 solid diet) radiographic examination, and measurements of quality of life to find out the subjective, functional, psychological and social aspects of TMJ ankylosis. Results: Patient specific joint replacements provided both form and function in all patients. Their diet score improved gradually within first 3 months and remained stable later. Their quality of life improved in the post operative phase and they were very satisfied with their overall performance. Conclusions: Patient specific joint replacements for mandibular condyle and glenoid fossa in TMJ ankylosis cases provide good outcome with a minimal complications.

1:15 PM Temporomandibular Joint Chondromatosis: A Difficult Diagnosis

Levorova J, Machon V

Chondromatosis is a benign metaplastic process of synovial joints with relatively rare occurrence in temporomandibular joint. Clinical signs are non-specific and similar to other internal TMJ diseases and therefore might be overlooked. Diagnosis is based on imaging methods (CT, MRI) or arthroscopic findings. Malignant transformation of this lesion is rare. Possible similar macroscopic appearance to chondrosarcoma might cause underestimation of the situation. On the other hand, histopathology of chondromatosis shows signs that are normal in chondrosarcoma - a malignant mesenchymal tumor with poor prognosis, although it is still benign metaplastic process. Surgical therapy of chondrosarcoma is radical and involves many possible complications. Treatment and prognosis of both entities (chondromatosis and chondrosarcoma) are completely different and diagnosis of chondromatosis or chondrosarcoma will fundamentally influent the patient's life. Authors present difficulties in diagnostics of chondromatosis on case reports.

1:30 PM Estrogen Inhibits Lubricin Expression in Baboon TMJ Synovial Cells

Perez L, Phelix CF, Millican L, Lebaron RG

Background: TMJ disorders affect a significant portion of the USA population, with the majority of those seeking treatment being women of childbearing age. Owing to this prominent sexual dimorphism it has been postulated that sex hormones influence TMJ homeostasis. Lubricin (PRG4) is a secreted molecule that promotes articular joint lubrication. We have previously identified estrogen response elements in promoter regions of human and baboon lubricin genes, which was a basis for the hypothesis that estrogen modifies TMJ cell lubricin expression. Purpose: To investigate estrogen's influence on lubricin expression in TMJ synovial cells. Methods: Female baboon TMJ synovial cells were isolated and expanded in culture. hTERT expression immortalized the cells. Quantitative PCR was used to measure lubricin gene transcripts in estrogen-treated and non-treated cells. Results: Estrogen significantly inhibited lubricin transcript expression when compared to transcript expression non- treated cells. Conclusions: This result is consistent with the hypothesis that estrogen has a negative influence on TMJ joint lubrication that has been proposed to lead to TMJ disorders. The outcome extends our understanding of gender-specific craniofacial biology, opening new pathways for novel therapeutics and treatments and successful regeneration of injured tissues.

PODIUM SESSION 4: BIOLOGIC TREATMENTS

2:15 PM The Effects of Long Term Intermittent Administration of PTH on the Mandibular Condylar

Cartilage and Subchondral Bone

Dutra Eh, O'Brien MH, Chen P-J, Yadav S

Objective: To characterize the long-term effects of intermittent PTH (I-PTH) on the mandibular condylar cartilage (MCC) and subchondral bone of the temporomandibular joint (TMJ), in vivo and in vitro. Methods: For the in vivo experiments, 24 10-week-old mice (C57BL/6J) were divided into two groups: (1) I-PTH (n=12): subcutaneous daily injection of PTH; (2) Control Group (n=12): subcutaneous daily injection of saline solution. Experiments were carried out for four weeks. Mice were injected with calcein, alizarin complexone and EdU before euthanasia. For the in vitro experiments, primary chondrocyte cultures from the MCC of 6-week-old mice (C57BL/6J) were treated with I-PTH for 14 days. Results: There was a significant increase in bone volume, tissue density, mineral deposition, TRAP activity, cell proliferation and cartilage thickness in the I-PTH treated mice when compared to control group. In addition, immunohistochemistry revealed that I-PTH administration led to a significant increase in expression of VEGF and RUNX2, and to a decreased expression

of SOST, MMP13 and ADAMS. QPCR analysis of the I-PTH treated chondrocytes revealed significantly increased lubricin and significantly decreased SOST, Col10, IHH and BMP2. **Conclusion:** The anabolic effects mediated by I-PTH at the MCC may be correlated with decreased chondrocyte differentiation.

2:30 PM Articular Disc: Development Guided Regeneration

Weekes K, Pal D, Pryce B, Lam P, Schweitzer, Johnstone B

The articular disc (AD) is classically described as fibrocartilage with lateral pterygoid (LP) tendon attachments. However, a long-forgotten description postulated the LP tendon becomes 'pinched' between the mandibular condyle and articular eminence resulting in the articular disc structure. Since understanding the origin and composition of this tissue is essential for developing regenerative strategies for AD pathologies, we used modern molecular tools to reexamine this forgotten hypothesis. Using ScxGFP and Pax7RosaT reporter mice we found that Scx is expressed continuously from the myotendinous junction of the LP through the AD and posterior attachments. We also found tendon markers at the gene and protein level in mature rhesus macaque ADs, with minimally expressed chondrogenic markers. We then isolated clonable, expandable progenitor cells from the AD that can undergo trilineage differentiation, and exhibit tendon/fibrocartilage features in vitro. A tendon origin for the disc suggests novel strategies for tissue engineering, which we are now exploring using the clonable, highly expandable AD progenitor cells.

2:45 PM The Effects of Parathyroid Hormone and Bisphosphonate on the Cartilage and the Subchondral Bone of the Temporomandibular Joint

Chen P-J, Dutra Eh, O'Brien MH, Yadav S

Objective: To study the effects of simultaneous injections of the intermittent PTH (I- PTH) and alendronate on the mandibular condylar cartilage and the subchondral bone in a preclinical mice model. **Materials and Methods:** We used 3 to 4 weeks old triple transgenic reporter mice (Col1a1 X Col2a1 X Col10a1). Mice were divided into 4 groups (n=8); Group 1: Control (injected with saline); Group 2: I-PTH (injected with PTH daily); Group 3: Alendronate (injected with alendronate three times a week) and Group 4: I- PTH + Alendronate (injected with I-PTH + alendronate). Mice were injected with alizarin and calcein bone labels and EdU (proliferation marker) before euthanization. **Results:** MicroCT and histological analysis revealed that the 3 experimental groups presented with significantly increased bone density, mineralization, cell proliferation and proteoglycan distribution in comparison to the control group, but there was no apparent difference among the experimental groups. **Conclusion:** Our study suggests that the effects of alendronate at the mandibular condylar cartilage are similar to the effects of I- PTH. However, the effects of simultaneous injections (I-PTH + Alendronate) are more pronounced in the subchondral bone.