Temporomandibular Joint Disorder

TMJ Bioengineering Conference

University Club

University of Pittsburgh
123 University Place
Pittsburgh, PA

May 18-19, 2022

TMJ Bioengineering Conference - 7

May 18 -May 19, 2022

University Club
University of Pittsburgh
Pittsburgh, PA, USA

W e l c o m e!

It is indeed our pleasure to welcome you to Pittsburgh, USA, for the seventh Temporomandibular Joint Bioengineering Conference (TMJ7)!

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 the program committee, and Diane Turner; 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
Michael Detamore
Boaz Arzi

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 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.

Conference Organizer

Alejandro Almarza – Chair

Program Committee

Alejandro Almarza
Michael Detamore
Boaz Arzi

Advisory Board

Kyriacos Athanasiou
Lou Mercuri

Instructions to 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.

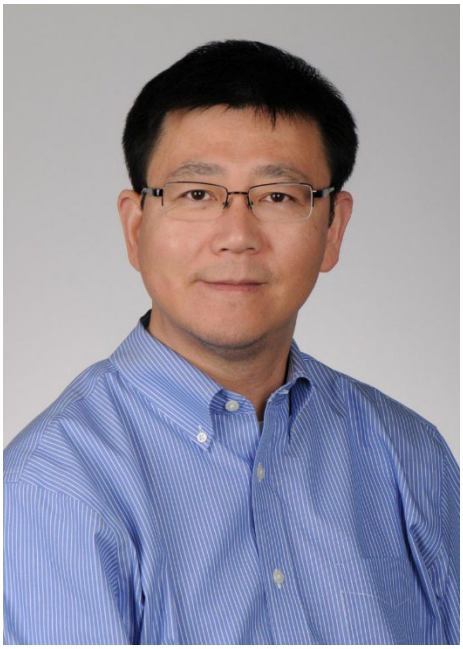
Important Notes:

- 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 **10 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.

Featured Keynote Speakers



Hai Yao, PhD, is the Ernest R. Norville Endowed Chair and Professor of Bioengineering at Clemson University and Professor of Oral Health Sciences at the Medical University of South Carolina (MUSC). He serves as the associate department chair for the Clemson-MUSC Joint Bioengineering Program. He is also the PI and director of the NIH Center of Biomedical Research Excellence (COBRE) for Translational Research Improving Musculoskeletal Health (TRIMH) at Clemson and the NIH Dental T32 Research Training Program at MUSC. His research focuses on the biomechanical function, degeneration, and regeneration of musculoskeletal systems, including the temporomandibular joint (TMJ). His lab has established multiscale TMJ models, which integrate joint morphology and kinematics, tissue mechanics, cell metabolism, and genetics, to facilitate earlier diagnosis and management of temporomandibular disorders (TMDs). He served on the committee for the most recent TMD study report from the National Academies of Sciences, Engineering, and Medicine (March 2020, PMID: 32200600). Hai received his PhD in Biomedical Engineering from the University of Miami and his postdoctoral training in Musculoskeletal Bioengineering at the Georgia Institute of Technology

Laura Iwasaki, DDS, MSc, PhD, is Professor and Chair of the Department of Orthodontics at Oregon Health & Science University School of Dentistry. Her research focus is the biomechanics of the human craniomandibular complex with special interests in the TMJ, jaw muscles, and tooth movement. Her responsibilities include clinical and didactic teaching and research supervision in the advanced education, dental and graduate programs. Dr. Iwasaki received her formal education in Canada at the Universities of British Columbia, Alberta and Manitoba. She is a Diplomate of the ABO and has been involved in the clinical practice of orthodontics for several decades.

TMJ Bioengineering Conference Agenda

May 18-19, 2022 (Pittsburgh, PA)

CONFERENCE LOCATION: University Club
University of Pittsburgh

May 18, 2022

8:00 AM BREAKFAST, REGISTRATION, CHECK-IN

8:40 AM **OPENING REMARKS**

9:00 AM **KEYNOTE SPEAKER -** Hai Yao, PhD

10:00 AM **BREAK**

ANIMAL MODELS

10:15 AM Intra- and Extra-articular Features of Temporomandibular Joint Ankylosis in the Cat

Vapniarsky N, Zavodovskaya R, Garcia T, Verstraete FJM, Hatcher DC, Arzi B
School of Veterinary Medicine, University of California, Davis

10:30 AM TMJ gap arthroplasty for treatment of intra- and extraarticular ankylosis in cats

Arzi B, Verstraete FJM
Department of Surgical and Radiological Sciences, School of veterinary medicine, university of California, Davis, USA

10:45 AM The Diagnostic Yield of Cone - Beam Computed Tomography for Degenerative Changes of the Temporomandibular Joint in Dogs

Vapniarsky N, McKay RM, Hatcher D, Carr N, Chen S, Verstraete FJM, Cissell DD, Arzi B
School of Veterinary Medicine, University of California, Davis, California

11:00 AM **BREAK**

SURGICAL AND DIAGNOSTIC TECHNIQUES

11:15 PM Are All Alloplastic Total TMJ Replacement Devices the Same?

Mercuri LG, Neto MQ, Pourzal R, Radice S, Hall D, Mathew M
Department of Orthopaedic Surgery, Rush University Medical Center Chicago, Illinois USA

11:30 PM Platelet rich plasma and Hyaluronic acid blends for Treatment of temporomandibular joint osteoarthritis

Ayman H, Abdelkader H
Al-Azhar University, Department of oral and maxillofacial surgery

11:45 PM Accuracy of fit analysis of the patient-specific Groningen temporomandibular joint prosthesis

Merema BJ, Kraeima J, van Bakelen NB, Witjes MJH, Spijkervet FKL
Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen

12:00 PM Logarithmic approach for treatment of condylar hyperplasia

Ashraf M., Mohamed M, and Abdelaziz B
Azhar Univrsity, Oral and Maxillo Facial Department; Ahram Canidian University (ACU)

12:15 PM **Lunch Break**

1:15 PM **OPEN FORUM –** Affiliation, format and name of conference

2:15 PM **BREAK**

TISSUE ENGINEERING AND REGENERATION

2:30 PM Treating small perforations of the temporomandibular joint disc using tissue-engineered implants

Donahue RP[1], Bielajew BJ[1], Vapniarsky N[2], Heney CM[3], Arzi B[4], Hu JC[1], Athanasiou KA[1]
1: Department of Biomedical Engineering, Henry Samueli School of Engineering, University of California, Irvine
2: Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis
3: Dentistry and Oral Surgery Service, School of Veterinary Medicine, University of California, Davis
4: Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis

2:45 PM Dynamic Self-Regenerating Cartilage (dSRC) from rabbit auricular chondrocytes for temporomandibular joint (TMJ) repair. A proof-of-concept study

Guastaldi FPS[1], Matheus HR[1], Hadad H[1], Redmond RW[2], Randolph MA[3]
1: Massachusetts General Hospital, Harvard School of Dental Medicine, Department of Oral and Maxillofacial Surgery
2: Massachusetts General Hospital, Harvard Medical School, Wellman Center for Photomedicine
3: Massachusetts General Hospital, Harvard Medical School, Division of Plastic and Reconstructive Surgery

3:00 PM Treatment of large perforation defects of the temporomandibular joint disc using tissue-engineered implants

Donahue RP,[,1] Bielajew BJ,[*,1] Vapniarsky N,[2] Heney CM,[3] Arzi B,[4] Hu JC,[1] Athanasiou KA[1]*
* Equal contribution
1: Department of Biomedical Engineering, Henry Samueli School of Engineering, University of California, Irvine
2: Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis
3: Dentistry and Oral Surgery Service, School of Veterinary Medicine, University of California, Davis
4: Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis

3:15 PM Stem cell-based therapies for regeneration of condylar defects: a systematic review of pre-clinical models of the temporomandibular joint

Matheus HR[1], Ozdemir SD[1], Hadad H[1], Guastaldi FPS[1]
[1]Massachusetts General Hospital, Harvard School of Dental Medicine, Department of Oral and Maxillofacial Surgery

3:30 PM Integrated Osteochondral Biomaterial for Mandibular Condyle Regeneration

Nedrelow D[1], Rassi A[2], Ajeeb B[1], Kiyotaki E[1], Huebner P[2], Williams W[3], Ritto F[4], Detamore MS[1]
1: Stephenson School of Biomedical Engineering, University of Oklahoma, Norman, OK
2: School of Industrial and Systems Engineering, University of Oklahoma, Norman, OK
3: Department of Comparative Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK
4: Department of Oral and Maxillofacial Surgery, University of Oklahoma Health Sciences Center, Oklahoma City, OK

3:45 PM **BREAK**

4:00 PM **OPEN FORUM –** Need for regenerative therapies?

5:00 PM **CLOSE**

6:00 PM **DINNER**

May 19, 2022

8:30 AM **BREAKFAST**

9:00 AM **KEYNOTE SPEAKER** – Laura Iwasaki, DDS, MSc, PhD

Mechanobehavior Scores for Prediction of TMJ Growth & Degeneration

10:00 AM **BREAK**

BIOMECHANICS

10:15 AM The effect of bolus stiffness on TMJ loading and muscle excitation during unilateral chewing

Sagl B,[1] Schmid-Schwap M,[1] Piehslinger E,[1] Rausch-Fan X,[1] Stavness I[2]
1: University Clinic of Dentistry, Medical University of Vienna
2: Department of Computer Science, University of Saskatchewan

10:30 AM Orthognathic surgery significantly increases TMJ normal stresses

Nickel JC,[1] Glovsky T,[1] Stumpos M,[2] Liu Y,[3] Liu H,[1] Iwasaki LR[1]
1: Oregon Health & Science University, School of Dentistry, Department of Orthodontics
2: Private Practice, Denver Colorado
3:East Tennessee State University, Department of Epidemiology and Statistics

10:45 AM NG2/CSPG4 localizes to fibroblast like synoviocytes during TMJ OA

Insel O[1], Israel H[1], Mercuri LG[2], Reed DA[3], Reeve G[1]
1: Weill Cornell Medicine, Division of Oral and Maxillofacial Surgery, New York, NY

2: Rush University Medical Center, Department of Orthopaedic Surgery, Chicago, IL
3: University of Illinois Chicago, Department of Oral Biology, Chicago IL

11:15 AM Decreased drinking time in rats splinted – chronic pain model

Almarza AJ, Trbojevic S, Li W, Gold M
University of Pittsburgh

11:30 AM **OPEN FORUM –** Funding from NIH (ODCS vs SBSR and SBDD)

12:00 PM **Lunch Break**

BIOLOGY AND TISSUE CHARACTERIZATION

1:00 PM Distinct Activities of Type V Collagen in TMJ Articular Disc versus Condylar Cartilage during Joint Growth and Remodeling

Chandrasekaran P,[1] Alanazi A,[1] Kwok B,[1] Mauck RL,[2] Dyment NA,[2] Koyama E,[3] Han L[1]
1: Drexel University, Philadelphia, PA; 2University of Pennsylvania, Philadelphia, PA;
2: The Children's Hospital of Philadelphia, Philadelphia, PA.

1:15 PM Profiling the molecular heterogeneity of mandibular condylar cartilage with single-cell RNA-sequencing

Reed DA,[1] Banks J,[1] Shin J,[1] Drnevich J[2]
1: University of Illinois Chicago, Department of Oral Biology, Chicago IL
2: University of Illinois Urbana-Champaign, Roy J. Carver Biotechnology Center, Urbana IL

1:30 AM MMP levels in TMJ tissues of surgical patients with TMJ disorders track pain scores more than radiological damage scores

Ita M, Ghimire P, Granquist E, Winkelstein B
Dept. of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania

1:45 PM Exosome-Based Lineage-Specific Differentiation for Tissue Regeneration

Ganesh V[1,2]; Gomez-Contreras P[1]; Fredericks D[1]; Petersen E[1]; Martin J[1,2]; Shin K[3]; Cucco C[4]; Seol D[1,3]
1: Orthopedics and Rehabilitation, University of Iowa, Iowa City, IA, USA
2: Roy J. Carver Biomedical Engineering, University of Iowa, Iowa City, IA, USA
3: Orthodontics, University of Iowa, Iowa City, IA, USA
4: Endodontics, University of Iowa, Iowa City, IA, USA

2:00 PM Methodology to quantify collagen subtypes and crosslinks: application in minipig cartilages

Bielajew BJ, Hu JC, Athanasiou KA
University of California, Irvine, Department of Biomedical Engineering

2:15 PM **CLOSING REMARKS**

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10:00 AM **BREAK**

ANIMAL MODELS

10:15 AM Intra- and Extra-articular Features of Temporomandibular Joint Ankylosis in the Cat

Vapniarsky N, Zavodovskaya R, Garcia T, Verstraete FJM, Hatcher DC, Arzi B
School of Veterinary Medicine, University of California, Davis

Background: Temporomandibular joint (TMJ) ankylosis is an uncommon clinical entity in human and veterinary medicine. However, the condition is severely debilitating and is life-limiting if not treated. This study sought to characterize the intra- and extra-articular features of naturally occurring TMJ ankylosis in cats.

Methods: TMJs from client-owned cats (n = 5) that underwent bilateral TMJ gap arthroplasty were examined and compared with TMJs from healthy, age-matched feline cadavers (n = 2) by cone-beam computed tomography (CBCT), micro-computed tomography (µCT) and histologically.

Results: Features of bilateral intra- and extra-articular ankylosis compounded by degenerative joint lesions were identified radiographically and histologically in all affected cats. Features of TMJ 'true' ankylosis included variable intracapsular fibro-osseous bridging, degeneration of the disk and the articular surfaces, narrowing of the joint space and flattening of the condylar process of the mandible. Extra-articular features of TMJ ankylosis included periarticular bone formation and fibro-osseous bridging between the mandible, zygomatic arch and coronoid process. In addition, subchondral bone loss or sclerosis, irregular and altered joint contours and irregularly increased density of the medullary bone characterized the degenerative changes of the osseous components of TMJ.

Conclusion: Complex radiological and histological features of both ankylosis and pseudoankylosis were identified that clinically manifested in complete inability to open the mouth.

10:30 AM TMJ gap arthroplasty for treatment of intra- and extraarticular ankylosis in cats

Arzi B, Verstraete FJM
Department of Surgical and Radiological Sciences, School of veterinary medicine, university of California, Davis, USA

Background: Temporomandibular joint (TMJ) ankylosis is defined as fibrous or bony fusion of the TMJ and can be intraarticular, extraarticular or both. In complex situations, both TMJ ankylosis and

pseudoankylosis may occur, requiring special surgical considerations, planning and execution.

Objectives: To report the surgical planning, technique and outcome of bilateral gap arthroplasty for extensive naturally occurring TMJ ankylosis in cats.

Samples and design: Client-owned cats (n=7) were examined clinically and surgical planning included the use of CBCT and 3D printed models. Gap arthroplasty was performed using a piezosurgical unit and included zygomectomy, coronoidectomy and condylectomy as well as fossectomy.

Results: in all cats, gap arthroplasty was performed without surgical complications and resulted clinically acceptable mouth opening was achieved in all cases. However, a noticeable mandibular instability was observed. Follow-up demonstrates acceptable quality of life and no recurrence of ankylosis.

Conclusion: TMJ gap arthroplasty in cats is indicated in cases of severe intra- and extraarticular ankylosis. Diagnostic imaging by means of CBCT and 3D printing are essential for precise surgical planning. The use of piezosurgical unit allows for safe and precise bone incisions. Clinically, mandibular instability still allows for eating and drinking. Future efforts are aimed at TMJ replacement for mandibular stabilization following gap arthroplasty.

10:45 AM The Diagnostic Yield of Cone - Beam Computed Tomography for Degenerative Changes of the Temporomandibular Joint in Dogs

Vapniarsky N, McKay RM, Hatcher D, Carr N, Chen S, Verstraete FJM, Cissell DD, Arzi B
School of Veterinary Medicine, University of California, Davis, California

Objective(s): This study aimed to correlate cone-beam computed tomography (CBCT) findings of naturally occurring degenerative temporomandibular joint disease (DTJD) of dogs with gross and histopathologic changes.

Study Design: Temporomandibular joints (TMJ) (n=38) from fresh cadaver heads of asymptomatic dogs (n=19) were examined radiologically, macroscopically, and microscopically. Association of CBCT- detected DTJD changes with gross and histological findings were statistically evaluated via kappa statistics and ordinal logistic mixed-effects models.

Results: The radiological changes observed on CBCT included joint space narrowing, subchondral/cortical bone change (i.e., erosions or lysis), osteophytes, and subchondral bone sclerosis. Upon macroscopic evaluation, the surface area of cartilage defects and osteophytes comprised less than 10% of the total articular surface area in the majority of specimens. Histopathologic changes comprised splitting and degeneration of the fibrous cartilage layers, subchondral bone exposure, subchondral bone sclerosis, focal subchondral bone lysis, and occasional cell death. Subchondral sclerosis was the most prevalent finding radiographically and histologically with a fair to excellent agreement. Importantly, the more severe the TMJ degenerative changes, the higher the agreement between CBCT and histology.

Conclusion: Based on the correlative results of statistical analysis, CBCT was found to be a suitable modality to evaluate DTJD.

11:00 AM **BREAK**

SURGICAL AND DIAGNOSTIC TECHNIQUES

11:15 PM Are All Alloplastic Total TMJ Replacement Devices the Same?

Mercuri LG, Neto MQ, Pourzal R, Radice S, Hall D, Mathew M
Department of Orthopaedic Surgery, Rush University Medical Center Chicago, Illinois USA

Purpose:
The purpose of this study was to demonstrate that the manufacturing process and alloy microstructure of metallic temporomandibular joint replacement (TMJR) components have a direct effect on the implant's electrochemical and mechanical properties.

Materials and Methods:
A total of 26 wrought titanium alloy (Ti6Al4V) and cobalt chrome molybdenum (CoCrMo) alloy TMJR devices were analyzed using scanning electron microscopy coupled with electron backscatter diffraction and energy dispersive x-ray spectroscopy. Electrochemical studies were performed in simulated joint fluid utilizing a three-electrode cell.

Results:
The results of this study showed that wrought Ti6Al4V alloys were superior with respect to the corrosion rate, repassivation potential, and the capacitance values compared to 3D printed Ti6Al4V alloys. This study also demonstrated that TMJR implant CoCrMo alloys can vary with respect to microstructure within ASTM specifications.

Conclusions:
One important conclusion from this study was that, with the introduction of additively manufactured joint replacement components, critical evaluation of 3D printed Ti6Al4V implant alloy microstructure is essential to prevent potential premature implant failures. Further, just as with the medications prescribed, it behooves the surgeon to understand the differences in biologic and physiologic responses to these materials when prescribing which device to implant in a specific case.

11:30 PM Platelet rich plasma and Hyaluronic acid blends for Treatment of temporomandibular joint osteoarthritis

Ayman H, Abdelkader H
Al-Azhar University, Department of oral and maxillofacial surgery

Purpose: Increasing evidence has supported the use of PRP combined with HA for the treatment of knee osteoarthritis and effectively promote the proliferation of chondrocytes and improve cartilage repair. The specific aim of this study was to provide another potential TMJ-OA treatment option that uses a single injection of HA/PRP blends at a maximal dose of 2 mL according to the maximal volume of the joint space. Patients & methods: The patients were randomly assigned to 1 of the following 3 groups: Group I: the patients treated by arthrocentesis followed by single HA injection. Group II: the patients treated by arthrocentesis followed by single PRP injection. Group III: the patients treated by arthrocentesis followed by single of PRP/HA blends injection. The outcome variables were maximum non-assisted (voluntary) mouth opening (MVMO), joint sounds, and pain index scores. Other variables, including patient age and gender, were evaluated in relation to the outcomes. Descriptive and bivariate statistics were computed, and the P value was set at .05. Results: PRP/HA blends showed significant improvement over PRP or HA injection ($P<0.005$). Conclusion: based on the results of the current study; we recommended a single injection of 2ml of HA/PRP Mix.

11:45 PM

Accuracy of fit analysis of the patient-specific Groningen temporomandibular joint prosthesis

Merema BJ, Kraeima J, van Bakelen NB, Witjes MJH, Spijkervet FKL
Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen

Background: For patients with severe temporomandibular joint (TMJ) dysfunction, a total joint replacement of the TMJ using a prosthesis can be indicated. Surgical accuracy of custom TMJ total joint replacement (TMJ-TJR) devices is important in order to correctly translate preoperatively predicted function outcome, wear and biomechanical behaviour. In a cadaver test series the surgical accuracy of the Groningen-TMJ-TJR (G-TMJ-TJR) device based on 3D-deviation, placed through fixated intra-operative surgical guides, was less than 1mm.

Objectives: Validate the accuracy of placement of the custom G-TMJ-TJR in the clinical setting. We hypothesise that a virtual surgical plan (VSP) in combination with guided placement of the G-TMJ-TJR can be followed comparably accurate in patients as in our earlier study in cadavers.

Methods: All patients who received a G-TMJ-TJR between December 2017 and December 2019 are included in this study. Each patient received a VSP-based custom G-TMJ-TJR according to the validated workflow. The accuracy was analysed based on postoperative CBCT data.

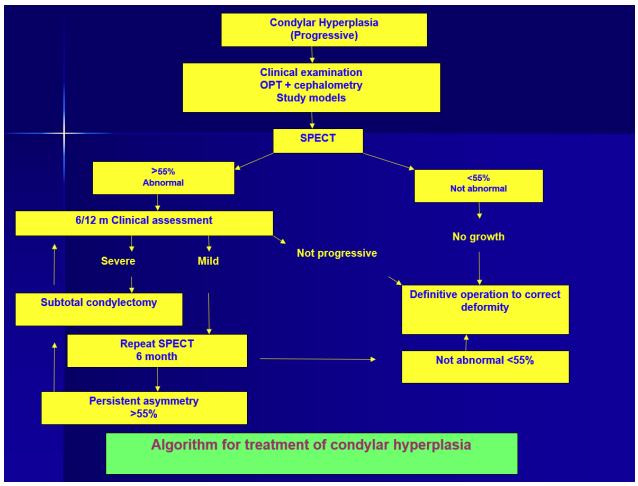
Results: All 10 prostheses could be inserted using routine preauricular and retro-mandibular surgical approaches. Analysis showed an average 3D-deviation of 1.12mm (sd:0.45) for all fossa- and mandibular components.

Conclusion: The custom G-TMJ-TJR can be applied safe and accurate in a clinical setting.

12:00 PM

Logarithmic approach for treatment of condylar hyperplasia

Ashraf M., Mohamed M, and Abdelaziz B
Azhar Univrsity, Oral and Maxillo Facial Department; Ahram Canidian University (ACU)



Condylar Hyperplasia is an uncommon malformation of the mandible involving change in the size and morphology of the condylar head and neck. Condylar hyperplasia is a well-documented cause of facial asymmetry. It is a unilateral self-limiting disease that usually develops in the teens and early 20s. The male: female ratio is said to be 1: 1 but some studies have shown that it is more common in girls.
The deformity and disharmony that occur secondary to condylar hyperplasia, in its two forms of presentation: Hemimandibular elongation – Hemimandibular hyperplasia. Hemimandibular Hyperplasia (HH) is characterized by a three-dimensional enlargement of one side of the mandible terminating exactly at the symphysis of t he affected side. It is common in childhood. Hemimandibular Elongation (HE) is a horizontal displacement of the mandible towards the unaffected side, and horizontal rami lie on the same level. It usually occurs in the older patient.
Etiology: Authors have debated whether intrinsic or extrinsic factors regulate the growth of the condyle.
• Local circulatory problems.
• Trauma.

• Hormonal disturbance.
• Cartilaginous exostosis.
• Genetic factor.
Diagnosis:
• Clinical examination.
• Study models, wax bite.
• Radiological examination (PA,Panorama).
• Cephalometric analysis.
• Scintigraphy (SPECT).
• Histological examination.


12:15 PM **Lunch Break**

1:15 PM **OPEN FORUM –** Affiliation, format and name of conference

2:15 PM **BREAK**

TISSUE ENGINEERING AND REGENERATION

2:30 PM Treating small perforations of the temporomandibular joint disc using tissue-engineered implants

Donahue RP[1], Bielajew BJ[1], Vapniarsky N[2], Heney CM[3], Arzi B[4], Hu JC[1], Athanasiou KA[1]
1: Department of Biomedical Engineering, Henry Samueli School of Engineering, University of California, Irvine
2: Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis
3: Dentistry and Oral Surgery Service, School of Veterinary Medicine, University of California, Davis
4: Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis

The objective of this work was to heal small disc perforation defects in the temporomandibular joint (TMJ) discs of minipigs. Self-assembled implants were created with mechanical properties on par to those of native tissue and implanted in 3mm diameter through-and-through defects. After 24 weeks in vivo, all implant-treated discs exhibited complete fill of the perforation defects. Implant-treated discs had repair tissue that was 3.4-times stronger, 8.9-times more resilient, and 6.2-times tougher under uniaxial tension when compared to untreated controls. Repair tissue of implant-treated discs exhibited more collagen type I (indicative of mature native TMJ discs) and less collagen type III (indicative of scar tissue), compared to controls. No systemic differences in blood biochemistry or complete blood count parameters were detected after implant treatment when compared to preoperative values, and no systemic issues were identified during necropsy. Immunogenically, implants were well-tolerated, exhibiting diminishing local immune cell response over time. Over 24 weeks, the excised implants remodeled toward a biochemical makeup similar to the native disc. Overall, tissue-engineered implants resulted in 1) no adverse systemic or local effects, 2) regeneration toward the mechanical and biochemical properties of native disc, and, ultimately, 3) exceptional healing of TMJ disc perforations.

2:45 PM

Dynamic Self-Regenerating Cartilage (dSRC) from rabbit auricular chondrocytes for temporomandibular joint (TMJ) repair. A proof-of-concept study

Guastaldi FPS[1], Matheus HR[1], Hadad H[1], Redmond RW[2], Randolph MA[3]
1: Massachusetts General Hospital, Harvard School of Dental Medicine, Department of Oral and Maxillofacial Surgery
2: Massachusetts General Hospital, Harvard Medical School, Wellman Center for Photomedicine
3: Massachusetts General Hospital, Harvard Medical School, Division of Plastic and Reconstructive Surgery

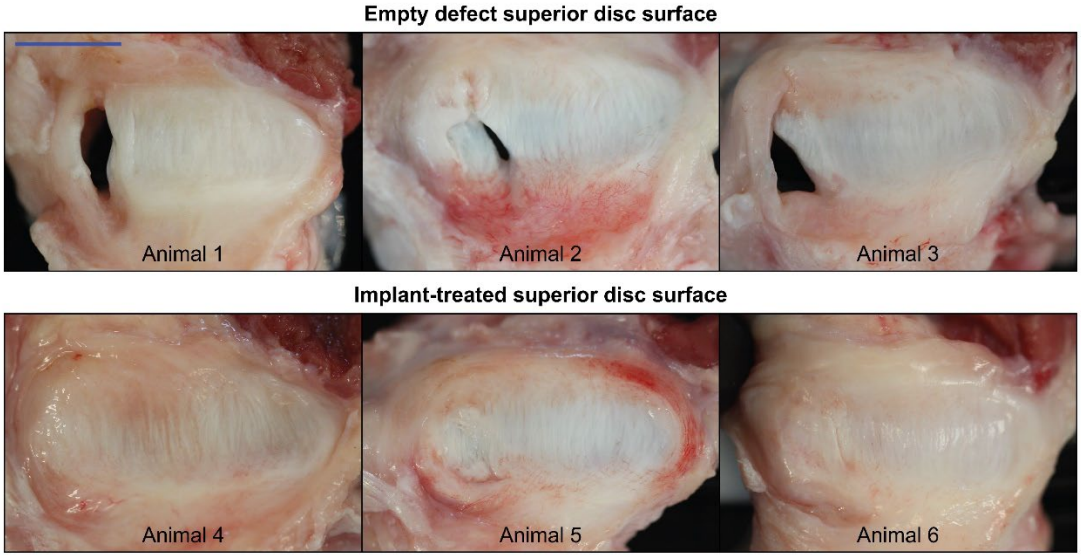
The development of regenerative solutions that focus on the TMJ condyle cartilage has the potential to impact the lives of many. The aim of this study was to evaluate new cartilage matrix using dSRC for TMJ repair. To form the dSRC, freshly harvested rabbit ear chondrocytes were placed into sealed 15-mL polypropylene tubes and cultured on a rocker at 40 cycles per minute for 14 days at 37°C. dSRC samples after 2, 4 and 8 weeks of in vitro culture and samples of native articular cartilage were stained with H&E to evaluate chondrocyte density. Safranin O (glycosaminoglycan: GAG) staining and Toluidine blue (proteoglycan) staining were also performed to assess the biochemical composition of the neomatrix. Consistent formation of dSRC matrix in vitro, in the form of sheets or pellets, after 2, 4 and 8 weeks was observed. H&E staining shows a high cell density in dSRC compared to native cartilage that decreases with increased time in vitro as the matrix matures toward that of native cartilage. All dSRC groups demonstrated intense staining with Safranin O (high GAG production), and intense staining with Toluidine blue (greater proteoglycan content). The viability and successful matrix formation from dSRC was demonstrated in vitro.

3:00 PM

Treatment of large perforation defects of the temporomandibular joint disc using tissue-engineered implants

Donahue RP,[,1] Bielajew BJ,[*,1] Vapniarsky N,[2] Heney CM,[3] Arzi B,[4] Hu JC,[1] Athanasiou KA[1]*
* Equal contribution
1: Department of Biomedical Engineering, Henry Samueli School of Engineering, University of California, Irvine
2: Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis
3: Dentistry and Oral Surgery Service, School of Veterinary Medicine, University of California, Davis
4: Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis

Temporomandibular joint (TMJ) disc pathologies include both disc thinning and perforation. Motivated by prior work demonstrating healing of small 3mm diameter disc thinning and perforation defects, the objective of this study was to heal large 6mm diameter perforations in the minipig TMJ disc. Minipigs underwent surgeries using the intralaminar fenestration surgical technique for the creation of large perforations in the lateral aspect of the TMJ disc and received self-assembled, tissue-engineered implants. After 8 weeks, empty defects (controls) did not heal, exhibiting through-and-through holes in the discs, while discs treated with implants displayed repair tissue fill (see figure) that was mechanically robust. Empty defects had a significantly higher unhealed defect perimeter compared to implant-treated discs. For the implant-treated discs, repair tissue metrics under uniaxial tension approached native tissue values of unoperated, contralateral controls; Young's modulus, ultimate tensile strength, resilience, and toughness reached 81.2%, 79.2%, 64.5%, and 64.4% of native tissue values, respectively. Tissue-engineered implants are poised to provide exceptional healing for those patients suffering from TMJ disc perforations.



3:15 PM

Stem cell-based therapies for regeneration of condylar defects: a systematic review of pre-clinical models of the temporomandibular joint

Matheus HR[1], Ozdemir SD[1], Hadad H[1], Guastaldi FPS[1]
[1]Massachusetts General Hospital, Harvard School of Dental Medicine, Department of Oral and Maxillofacial Surgery

This review aimed to assess the potential of stem cell-based therapies for the regeneration of cartilage/osteochondral defects in the mandibular condyle. PubMed, Web of Science, Cochrane Library, and the grey literature were searched for publications of pre-clinical experiments evaluating therapies applying stem cells for the regeneration of temporomandibular joint (TMJ) cartilage/osteochondral defects in the condyle. The search strategy was complemented by a manual search in the reference list of review studies and relevant journals in the field. Bias was assessed by means of the SYRCLE's risk of bias tool for animal experimental studies. Four articles were included in the qualitative synthesis. The potential of human adipose-derived regenerative cells (hADRCs), mesenchymal stromal cells, mesenchymal stem cells (MSCs), and human umbilical cord matrix MSCs was evaluated. Stem cells were carried onto scaffolds in three studies, and just one of them evaluated the influence of the scaffold alone (control). Higher rate of subchondral bone remodeling, and improved cellularity and biochemical patterns were observed when stem cells were used. Complete closure of the defects with cartilaginous tissue was reported in all studies. Stem cells alone or within a carrier may be a promising therapeutic approach for regenerating condylar cartilage/osteochondral defects in the TMJ.

3:30 PM Integrated Osteochondral Biomaterial for Mandibular Condyle Regeneration

Nedrelow D[1], Rassi A[2], Ajeeb B[1], Kiyotaki E[1], Huebner P[2], Williams W[3], Ritto F[4], Detamore MS[1]

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Bioinert implants are prescribed for temporomandibular joint replacement; but for growing patients, there is a need for regenerative biomaterials that can not only restore joint function, but also allow normal growth to occur and maintain facial symmetry. Our lab is evaluating a patient-specific 3D-printed regenerative scaffold for region-specific cartilage and bone regeneration in a goat TMJ model. For bone regeneration, we fabricated a 20 wt% hydroxyapatite (HAp) polycaprolactone (PCL) filament that can be 3D-printed with fused deposition modeling. For cartilage, our implants featured a chondroinductive hydrogel composed of polyethylene glycol diacrylate (PEGDA, 20 wt%), functionalized hyaluronan (PHA, 4 wt%) and devitalized cartilage (DVC, 15 wt%). The implant's condyle featured a porous structure to enhance resorption and bone growth in addition to geometric undercuts for hydrogel retention. Six 3-5 year-old Female Spanish cross goats were evaluated for six months, three of which had implants with a hydrogel, and three did not. Preliminary micro CT data and gross morphology on subjects that have reached termination suggested ectopic bone growth adjacent to the condyle and along the ramus. In addition, moderate bone resorption was observed under the implant along the posterior border of the mandible. Further assessments of bone and cartilage tissue growth with histology, immunohistochemistry, and optical coherence tomography is scheduled for completion in Spring 2022.

3:45 PM **BREAK**

4:00 PM **OPEN FORUM –** Need for regenerative therapies?

5:00 PM **CLOSE**

6:00 PM **DINNER**

May 19, 2022

8:30 AM **BREAKFAST**

9:00 AM **KEYNOTE SPEAKER** – Laura Iwasaki, DDS, MSc, PhD

Mechanobehavior Scores for Prediction of TMJ Growth & Degeneration

Iwasaki LR,[1,2] Gonzalez Y,[2] Gallo L,[3] Liu Y,[4] Liu H,[1] Yao H,[5] Nickel JC[1,2]
1: Oregon Health & Science University
2: University at Buffalo
3: LMG Engineering GmbH
4: East Tennessee State University
5: Medical University of South Carolina/Clemson University

10:00 AM **BREAK**

BIOMECHANICS

10:15 AM The effect of bolus stiffness on TMJ loading and muscle excitation during unilateral chewing

Sagl B,[1] Schmid-Schwap M,[1] Piehslinger E,[1] Rausch-Fan X,[1] Stavness I[2]
1: University Clinic of Dentistry, Medical University of Vienna
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Mastication is the motor task with the highest muscle activations of the jaw region and increased loading of the temporomandibular joint (TMJ) is often associated with temporomandibular disorders (TMD). Consequently, TMJ mechanics during chewing are potentially relevant in TMD treatment. TMD self-management guidelines suggest eating soft food to reduce TMJ pain and since TMJ loading cannot be measured in vivo, our in silico study aims to investigate the effect of bolus stiffness on TMJ loading.

We previously investigated the effect of bolus stiffness on TMJ loading using a fixed muscle activation pattern. In the presented study, we expand our work to use a forward-dynamics tracking approach to investigate the effect of bolus stiffness on TMJ loading as well as masticatory muscle activation. We ran tracking simulations, using an idealized mandibular chewing motion, to simulate unilateral chewing with a bolus size of 10mm and stiffness values of 0.5, 1 and 2MPa. First results show a clear connection between food stiffness and increased muscle activation as well as TMJ loading.

Overall, the presented study aims to collect biomechanical reasoning behind the clinically motivated food choice guidelines for patients with TMD pain. This could potentially help to validate or improve the current guidelines.

10:30 AM Orthognathic surgery significantly increases TMJ normal stresses

Nickel JC,[1] Glovsky T,[1] Stumpos M,[2] Liu Y,[3] Liu H,[1] Iwasaki LR[1]
1: Oregon Health & Science University, School of Dentistry, Department of Orthodontics
2: Private Practice, Denver Colorado
3:East Tennessee State University, Department of Epidemiology and Statistics

Objective: To test the effects of maxillo-mandibular osteotomies on temporomandibular joint (TMJ) normal stresses.

Approach: According to IRB and STROBE guidelines, pre- and post-surgery CBCT images were collected from males (n=19) and females (n=30), average age of 32.0 (± 15.2) years. 3D anatomical data were derived from images and used in a numerical model, which predicted TMJ loads and

masticatory muscle forces based on an objective of minimization of muscle effort. Regression analyses tested for correlations between TMJ normal stress, muscle forces, and surgical changes in anteroposterior position and angulation of occlusal plane. Principal Component Analyses (PCA) identified masticatory muscle forces that accounted for changes in TMJ loads.

Results: It was found that TMJ stresses increased by ≥ 20% for unilateral posterior and laterally directed biting forces on canines when arch length increased by ≥ 5 mm, and occlusal plane angulation increased by ≥ 5º (0.47 ≤ R2 ≤ 0.52). PCA showed that post-surgery changes in muscle forces had moderate (ipsilateral, $\lambda = 4.59$; $\eta 2 = 0.071$) to large (contralateral, $\lambda = 1.49$; $\eta 2 = 0.31$) effects on TMJ normal stresses.

Conclusions: Linear and angulation changes in occlusal plane increase normal stresses through altered masticatory muscle activation patterns.

10:45 AM NG2/CSPG4 localizes to fibroblast like synoviocytes during TMJ OA

Insel O[1], Israel H[1], Mercuri LG[2], Reed DA[3], Reeve G[1]
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2: Rush University Medical Center, Department of Orthopaedic Surgery, Chicago, IL
3: University of Illinois Chicago, Department of Oral Biology, Chicago IL

Degenerative and primary arthritis in the temporomandibular joint (TMJ) is a whole-joint disorder affecting cartilage, articular disc, and synovium. The intimal lining of the synovium is composed of fibroblast-like synoviocytes (FLS). This population of cells is implicated in the pathophysiology of hyperplastic synovitis, commonly associated with TMJ arthropathy. Neuron-glial antigen 2 (NG2/CSPG4) is a transmembrane proteoglycan regulating the cell stress/injury response of TMJ cartilage. This study evaluates NG2/CSPG4 in healthy and diseased FLSs in the TMJ synovium using both preclinical and clinical samples. Preclinical TMJ OA was induced in skeletally mature c57 BL6/J mice using surgical destabilization of the joint (UIC ACC 20-068). Clinical samples were collected by synovial biopsy during arthroscopy treatment of TMJ arthropathy (20-11022980 WC/2021-0456 UIC). Samples were evaluated by H&E staining and immunohistochemistry. FLS cells were identified by immunostaining for ICAM1 and ICAM1-NG2/CSPG4 colocalization was calculated. NG2-ICAM colocalization is spatially restricted to the intimal lining of the synovium in TMJ OA tissue from the preclinical samples (n=3/3). NG2-ICAM1 colocalization is also observed in a restricted cell population in the clinical synovial biopsy sample (n=1/3). Taken together, these data indicate that NG2/CSPG4 may be a biomarker for FLS activation during the early stages TMJ OA.

11:15 AM Decreased drinking time in rats splinted – chronic pain model

Almarza AJ, Trbojevic S, Li W, Gold M
University of Pittsburgh

11:30 AM **OPEN FORUM –** Funding from NIH (ODCS vs SBSR and SBDD)

12:00 PM **Lunch Break**

BIOLOGY AND TISSUE CHARACTERIZATION

1:00 PM

Distinct Activities of Type V Collagen in TMJ Articular Disc versus Condylar Cartilage during Joint Growth and Remodeling

Chandrasekaran P,[1] Alanazi A,[1] Kwok B,[1] Mauck RL,[2] Dyment NA,[2] Koyama E,[3] Han L[1]
1: Drexel University, Philadelphia, PA; 2University of Pennsylvania, Philadelphia, PA;
2: The Children's Hospital of Philadelphia, Philadelphia, PA.

In the temporomandibular joint (TMJ), condylar cartilage contains a collagen I-dominated fibrocartilage layer covering the collagen II-rich hyaline layer, while the disc consists of only fibrocartilage. Regeneration of the two units is challenged by limited understanding of molecular mechanisms that govern the formation of their extracellular matrices. This study delineated the activities of type V collagen, a regulatory fibril-forming collagen, in the two units during post-natal growth and aberrant loading-induced remodeling. In 3-month-old Col5a1+/– TMJ disc (Fig. 1a,b), reduction of collagen V led to thickened collagen fibrils (Fig. 1c), reduced modulus only at the inferior side, posterior end (Fig. 1d), and no significant changes of cell density or morphology relative to the wild-type (WT) control. In contrast, condylar cartilage exhibited pronounced reduction in both cell proliferation and modulus. When aberrant loading was induced by the unilateral anterior crossbite (UAC) prosthesis procedure, Col5a1+/– mice exhibited increased sGAG staining (black arrowheads), higher Mankin scores and reduced modulus in condylar cartilage relative to WT, but no significant biomechanical changes in the disc (Fig. 1e-g). Collectively, our results demonstrated a more crucial role of collagen V in regulating the biomechanical functions and remodeling of condylar cartilage, but a lesser role in the disc.

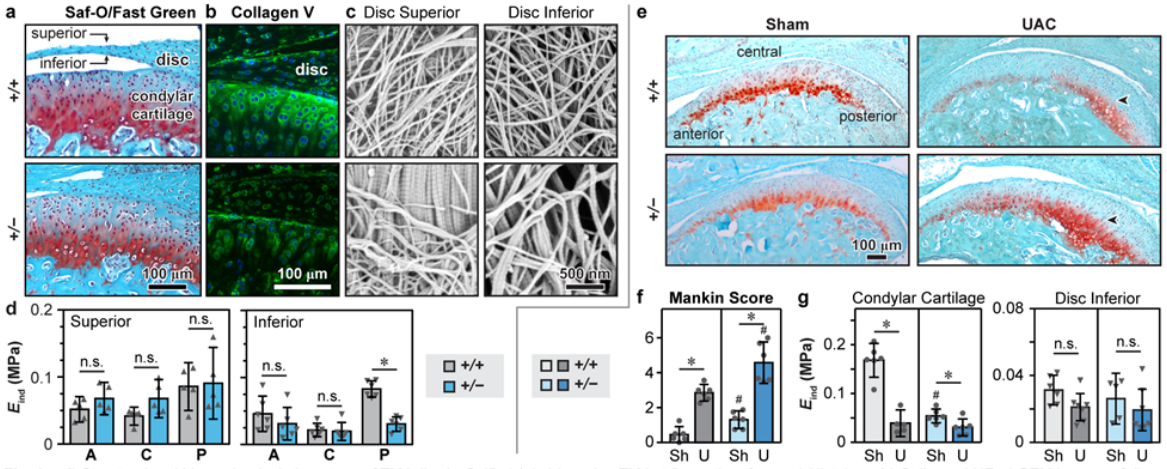

Fig. 1 a-d) Structural and biomechanical phenotype of TMJ disc in *Col5a1*$^{+/-}$ (+/–) murine TMJ at 3 months of age. **a)** Histology, **b)** Collagen V IF, **c)** SEM images of disc surface central region, **d)** AFM modulus across disc anterior (A), central (C) and posterior (P) regions (*: $p < 0.05$, mean ± 95% CI, $n \geq 5$). **e-g)** Remodeling of *Col5a1*$^{+/-}$ (+/–) versus WT (+/+) TMJs at 3 weeks after UAC (U) and Sham (Sh) procedures applied to 3-month-old mice. **e)** Histology, **f)** Mankin scores, and **g)** AFM modulus of condylar cartilage and disc inferior side (*: $p < 0.05$, #: $p < 0.05$ for Sh vs. U, mean ± 95% CI, $n \geq 5$).

1:15 PM Profiling the molecular heterogeneity of mandibular condylar cartilage with single-cell RNA-sequencing

Reed DA,[1] Banks J,[1] Shin J,[1] Drnevich J[2]
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2: University of Illinois Urbana-Champaign, Roy J. Carver Biotechnology Center, Urbana IL

The mandibular condylar cartilage (MCC) is comprised of a heterogeneous population of cells undergoing lineage conversion from a progenitor cell to a fully differentiated hypertrophic chondrocyte. Each sub-population is hypothesized to contain a unique transcriptional profile that regulates the differentiation dynamics responsible for growth at the MCC. We characterize this molecular heterogeneity of the MCC using single-cell RNA-sequencing. MCC cells from ten skeletally mature c57 BL/6 J mice were isolated, processed with the 10x Genomics Chromium Single Cell 3' / Illumina NovaSeq 6000. Cell calls and UMI counts were generated with Cell Ranger (version 6.1.1) resulting in 1,824 cells, with 122,389 mean reads per cell, 317 median genes per cell, and 92.2% of reads mapped to the genome. Quality control, normalization, and clustering were done in the Seurat R Package (version 4.0.6). Clustering analysis identified 6 distinct sub-populations. Genes for each sub-population were analyzed using a gene ontology enrichment analysis (ShinyGO v0.741), identifying cluster-specific biological processes that correspond to the predicted differentiation trajectory of the MCC including mesenchymal cell differentiation, cartilage development, wound healing/skeletal system development, biomineralization, and ion homeostasis. Future studies will define how the transcriptional heterogeneity and differentiation dynamics of the MCC are altered during degenerative arthropathies.

1:30 AM MMP levels in TMJ tissues of surgical patients with TMJ disorders track pain scores more than radiological damage scores

Ita M, Ghimire P, Granquist E, Winkelstein B
Dept. of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania

1:45 PM Exosome-Based Lineage-Specific Differentiation for Tissue Regeneration

Ganesh V[1,2]; Gomez-Contreras P[1]; Fredericks D[1]; Petersen E[1]; Martin J[1,2]; Shin K[3]; Cucco C[4]; Seol D[1,3]
1: Orthopedics and Rehabilitation, University of Iowa, Iowa City, IA, USA
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3: Orthodontics, University of Iowa, Iowa City, IA, USA
4: Endodontics, University of Iowa, Iowa City, IA, USA

Exosome has attracted attention due to their great potential to promote intercellular communication leading to enhanced cell recruitment, differentiation to specific cell lineage, and tissue regeneration. The purpose of this study was to determine the effect of exosomes on in vitro lineage-specific differentiation for tissue regeneration. Exosomes were isolated from rabbit dental pulp stem cells (DPSCs) cultured under growth (Exo-G) or lineage-specific differentiation conditions, angiogenesis (Exo-A) and osteogenesis (Exo-O). The characterization of exosomes was confirmed by nanoparticle tracking analysis and Exo-CheckTM exosome antibody array. Mean size of particles was the biggest in Exo-O (206.2 nm versus approximately 104 nm in Exo-G and Exo-A), and the concentration of Exo-G (11.6x1010 particles/ml) was 2-3 times higher than Exo-A (5.5x1010 particles/ml) and Exo-O (3.1x1010 particles/ml). All exosomes showed positive expression of ANXA5, TSG101, FLOT1, ICAM, ALIX, and CD81. In cytotoxicity test, there was no significant cell death in less than 5x108 exosome, however, the viability was significantly decreased in 5x109 Exo-G and Exo-A. In gene expression analysis, DPSC-Exos enhanced the expression of angiogenic and osteogenic markers including vascular endothelial growth factor (VEGF) and alkaline phosphatase (ALP), respectively. In

conclusion, our exosome-based lineage-specific differentiation strategy has a significant therapeutic potential for TMJ tissue regeneration.

2:00 PM Methodology to quantify collagen subtypes and crosslinks: application in minipig cartilages

Bielajew BJ, Hu JC, Athanasiou KA
University of California, Irvine, Department of Biomedical Engineering

Introduction

This study develops assays to quantify collagen subtypes and crosslinks with liquid chromatography-mass spectrometry (LC-MS) and characterizes the cartilages in the Yucatan minipig.

Methods

For collagen subtyping and bottom-up proteomics, LC-MS analysis was performed on tissues digested in trypsin. For collagen crosslinks, LC-MS analysis was performed on hydrolysates. Ten cartilages (femoral condyle, femoral head, facet joint, floating rib, true rib, auricular cartilage, annulus fibrosus, two meniscus locations, and TMJ disc) were analyzed.

Results

Collagen subtyping quantified collagen types I and II. The collagen crosslink assay quantified mature and immature crosslinks. Elastic cartilage and fibrocartilages had more mature collagen crosslink profiles than hyaline cartilages. Bottom-up proteomics quantified 24 collagen alpha-chains and 12 minor collagen types.

Discussion

The novel assays developed in this work are sensitive, inexpensive, and use a low operator time relative to other collagen analysis methods. Unlike the current collagen assays, these assays quantify collagen subtypes and crosslinks without an antibody-based approach or lengthy chromatography. They apply to any collagenous tissue, with broad applications in tissue characterization and tissue engineering. For example, a spectrum of hyaline and fibrous cartilages was shown, and all collagen subtypes in TMJ disc matrix were quantified.

2:15 PM **CLOSING REMARKS**