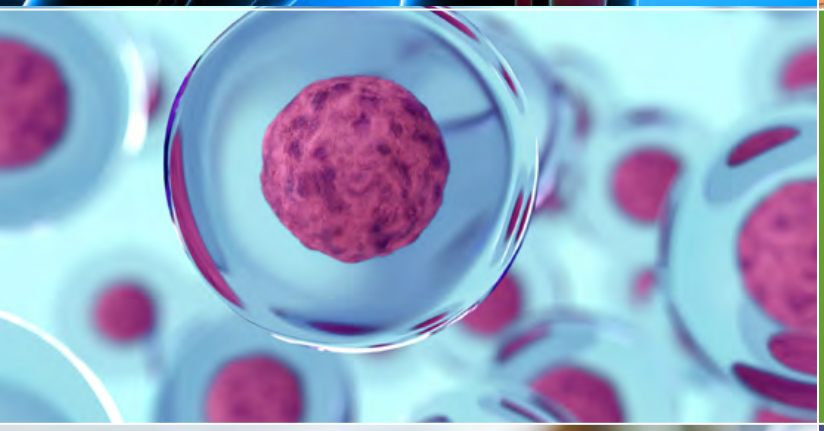
IFATS

International Federation for
Adipose Therapeutics and Science

19TH ANNUAL IFATS CONFERENCE



FORT LAUDERDALE, FL
November 4-6, 2022

THERE'S ONLY ONE IFATS
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Presidential Note

On behalf of the IFATS Board of Directors, I have the pleasure to welcome you to our 19th Annual Meeting in beautiful Ft. Lauderdale, Florida.

In 2002, IFATS was founded by four clinical and scientific pioneers following the historical discovery of mesenchymal stem cells in human subcutaneous adipose tissue. Since then, the annual IFATS Conference has developed into a premier scientific meeting worldwide focusing on adipose tissue *and* purposefully bringing together clinicians and basic researchers. This approach fosters the focused basic research towards translation as well as the implementation of new developments into clinical use.

A broad spectrum of true experts including plastic surgeons, cell biologists, research scientists and physicians in many other fields attend this annual meeting where they both present and learn about cutting edge scientific and clinical research. Also this year, the meeting will provide an exciting exchange of the most current knowledge on basic, translational, and clinical research in the area of adipose tissue and adipose-derived products.

At the 2022 IFATS Conference, we will present a wide range of fascinating Keynotes and Panel discussions. New focus areas include Adipose Tissue Models and Microphysiological Systems, Biofabrication including 3D Bioprinting, and Adipose Tissue in Cancer. Translational and clincial aspects will be covered in Keynotes and Panels on Adipose-derived Cell-based Therapies, Adipose Therapeutics in Musculoskeletal Disorders, Newest Developments in Fat Grafting, and Insight Views into Translation and Regulatory Affairs. The Free Paper Sessions very well reflect those topics and additionally cover other adipose-relevant aspects. A newly implemented Industry Roundtable will present a discussion on the challenges and opportunities in product translation.

The IFATS Annual Meeting continues to provide all our attendees with the opportunity to learn about state-of-the-art research, technology, and clinical practice, view cutting-edge products developed by our exhibiting companies, and interact with the brightest minds in the field. We thank our participating companies for their support and encourage you to meet with them in our exhibit hall during this conference.
Building on our last year's successful experience, this year the conference is again offererd as a hybrid meeting. Those who cannot attend due to schedules or travel restrictions may join us "Live" on-line streaming. The 2022 meeting includes a reception & dinner on the hotel pool deck overlooking beautiful Fort Lauderdale beach during which attendees will enjoy a memorable evening with colleagues and international leaders in the field.

We are very pleased that you join us at the IFATS meeting this year. Take the opportunity to benefit from exciting new ideas and valuable tools for your research and clinical practice, and enjoy your time in Fort Lauderdale!

Torsten Blunk, PhD

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Invited Speakers & Session Moderators

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James J. Hickman, PhD

LaTonya J. Hickson, MD

Chia Chi Kao, MD

Adam J. Katz, MD

Nathan Katz, PhD

Lauren Kokai, PhD

Fred Koelle, MD, PhD

Mikhail Kolonin, PhD

Ramon Llull, MD, PhD

Peter Loskill, PhD

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Elizabeth Martin, PhD

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Suzanne Trott, MD

Kotaro Yoshimura, MD

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Singapore Bioimaging Consortium / Duke-NUS Graduate Medical School

Filip Stillaert, MD
University Hospital Ghent

Dmitry Traktuev, PhD
University of Florida College of Medicine

THURSDAY - NOVEMBER 3

5:30 - 6:30 pm	**IFATS EXCO & BOARD MEETINGS/DINNER**	

FRIDAY - NOVEMBER 4

7:00 - 8:00 am	**Continental Breakfast in Exhibit Hall**	
8:00 - 8:15 am	**Welcome Remarks and Overview** Torsten Blunk, PhD - IFATS President	*Las Olas Ballroom*
8:15 - 8:45 am	**OPENING TALK: Strategies for Engineering Human Tissues: Advancing Towards Translation of Adipose-derived Stem/Stromal Cells-based Applications** Julie Fradette, PhD *Full Professor, Dpt of Surgery, Université Laval* *Researcher, Centre de recherche en organogénèse expérimentale de l'Université Laval* *LOEX Division of Regenerative Medicine, CHU de Québec Research Centre-Université Laval, Québec, Canada*	*Las Olas Ballroom*
8:45 - 10:00 am	**PANEL: Adipose Tissue Models and Microphysiological Systems** Moderators: Rosalyn Abbott, PhD & Lauren Kokai, PhD **Introduction and Overview of the Field** Rosalyn D. Abbott, PhD *Assistant Professor | Carnegie Mellon University* *Biomedical Engineering | Materials Science and Engineering (courtesy)* Lauren Kokai, PhD *Assistant Professor, Plastic Surgery and Bioengineering* *University of Pittsburgh School of Medicine* **Engineering Naturally-derived Human Adipose Tissues for In-vitro and In-vivo Studies** Julie Fradette, PhD *Full Professor, Dept of Surgery, Université Laval* *Researcher, Centre de recherche en organogénèse expérimentale de l'Université Laval / LOEX Division of Regenerative Medicine, CHU de Québec Research Centre-Université Laval, Québec, Canada* **Engineering Human Blood-Derived and Adipose Tissue-Derived Products for Metabolic Research and Regenerative Medicine** Jeffrey M. Gimble, MD, PhD *Chief Medical Officer, Obatala Sciences, Inc.* **Multi-organ Systems to Investigate Metabolic Diseases** James J. Hickman, PhD *University of Central Florida, NanoScience Technology Center, Professor of Nanoscience Technology, Chemistry, Biomolecular Science and Electrical Engineering, and Hesperos, Inc., Chief Scientist* **Autologous Human Immunocompetent White Adipose Tissue-on-Chip Models** Peter Loskill, PhD *Organ-on-Chip-Research, Eberhard Karls University Tübingen* *Natural and Medical Sciences Institute* *Vice Chair European-Organ-on-Chip-Society*	
10:00 - 10:30 am	**Coffee Break in Exhibit Hall**	
10:30 - 11:45 am	**Free Papers 1 - 3D Tissue Models and MPS** Moderators: Susanna Miettinen, PhD & Keith L. March, MD, PhD	
10:30 am	**1** (p.23) - **Human-Derived Hydrogels to Support Stem Cell Derived Organ on a Chip** Presenter: Cecilia Sanchez, PhD (USA) Affiliation: Obatala Sciences Authors: Cecilia Sanchez, Trivia Frazier, Xiying Wu, and Jeffrey Gimble	
10:39 am	**2** (p.23) - **Investigation of Hydrogel Composition for Superior Adipose Stem Cell Spheroid Adipogenesis** Presenter: Charles Amurgis (USA) Affiliation: University of Pittsburgh Medical Center Department of Plastic Surgery Authors: Charles Amurgis, Lindsey Huff, Lauren Kokai, Rosalyn Abbott, Vincent Nerone, Shawn Loder, Elizabeth Johnston	
10:48 am	**3** (p.24) - **Comparison of Different Advanced 3D Adipose Tissue Models Based on Spheroids, Hydrogels Containing ASC, or Mature Adipocytes with a 2D Monolayer and Explanted Lobules** Presenter: Franziska Albrecht, M.Sc. (Germany) Affiliation: Reutlingen University Authors: Franziska Albrecht, Svenja Nellinger, Freia F. Schmidt, Petra J. Kluger	

10:57 am	**Discussion**
11:07 am	**4** (p.25) - **3D Breast Cancer Microtumors to Investigate the Expression of Tumor-Associated Markers Mediated by the Direct Interaction between ASCs/Adipocytes and Breast Cancer Cells** Presenter: Martin Watzling (Germany) Affiliation: University Hospital of Würzburg Authors: Watzling M, Klaus L, Horder H, Blunk T, Bauer-Kreisel P
11:16 am	**5** (p.26) - **Supercritical Carbon Dioxide-foamed Poly(L-lactide-co-ε-caprolactone) Scaffold Embedded with Ascorbic Acid Derivative for Urethral Tissue Engineering: An In-vitro Study** Presenter: Alma Kurki (Finland) Affiliation: Tampere University Author: Alma Kurki
11:25 am	**6** (p.26) - **Biocompatible Hydrogels for Soft Tissue Engineering - Strategies to Promote Vascularization** Presenter: Paul Gatenholm, Kristin Oskarsdotter (Sweden) Affiliation: Sahlgrenska University Hospital, Gothenburg Authors: Paul Gatenholm, Kristin Oskarsdotter
11:34 am	**Discussion**
11:45 - 12:30 pm	**INDUSTRY SHOWCASE** Moderator: Lauren Kokai, PhD **Scaling Engineered MSCs and MSC-EVs to Accelerate Therapeutic Clinical Translation** Joseph Candiello, PhD *Senior Product Manager, RoosterBio, Inc* **Lipogems: Redefining Regenerative Medicine without Disrupting the Adipose Tissue Niche** Camillo Ricordi, MD, FNAI *Stacy Joy Goodman Professor of Surgery and Chief, Division of Cellular Transplantation* *Distinguished Professor of Medicine* *Professor of Biomedical Engineering, Microbiology and Immunology* *Director, Diabetes Research Institute and Cell Transplant Center, University of Miami* *Representing Lipogems* **Orthobiologics 360** Nathan Katz, PhD *Co-founder and CEO, Jointechlabs, Inc.*
12:30 - 1:30 pm	**Lunch & Learn with Allergan an AbbVie company** *Las Olas Ballroom*
1:30 - 2:45 pm	**PANEL: Fat Grafting I - Breast and BBL** Moderator: Fred Koelle, MD, PhD **New Perspectives in Fat Grafting to the Breast** Summer E. Hanson, MD, PhD *Associate Professor of Surgery, Director* *Plastic and Reconstructive Surgery Research Chicago, IL* **Fat Grafting for Reconstruction and Revitalization of the Breast** Kotaro Yoshimura, MD *Professor and Chair Department of Plastic Surgery, Jichi Medical University* *Yakushiji, Shimotsuke, Tochigi, Japan* **Stem Cell Enriched Fat Grafting to the Breast** Fred Koelle, MD, PhD, BC Plastic Surgeon *Head Surgeon of Plastic Surgery, Department of Plastic Surgery, CeriX Private Hospital, Denmark* **Fat Transfer to the Breast in Southern California** Suzanne Trott, MD, BC Plastic Surgeon *Head Surgeon of Plastic Surgery, Silhouette Cosmetic Center Beverly Hills, CA* **Buttock Reshaping with Fat Contour and Volume** Luisa Magalhães Ramos, MD, PhD *Head Surgeon of Plastic Surgery* *LMR Cirurgia Plástica, Portugal*
2:45 - 3:15 pm	**Coffee Break in Exhibit Hall**
3:15 - 4:30 pm	**Free Papers 2 - Fat Grafting I** Moderators: Sherry Collawn, MD, PhD & Ramon Llull, PhD

3:15 pm	**7** (p.27) - **Engineered Fat Graft Enhanced with Adipose-Derived Stromal Vascular Fraction Cells for Breast Augmentation and Reconstruction: Clinical, Histological and Instrumental Evaluation** Presenter: Pietro Gentile (Italy) Affiliation: University of Rome Author: Pietro Gentile
3:24 pm	**8** (p.27) - **Clinical Applications of MEST & ARAT** Presenter: Hasim Eray Copcu (Turkey) Affiliation: Gene, Cell and Tissue Academy Author: Hasim Eray Copcu
3:33 pm	**9** (p.28) - **Cell Enriched Lipotransfer (CELT), A New Technology to Increase Autologous Fat Grafting by Stem Cell Enrichment to Accelerate Tissue Healing and Regeneration in Humans** Presenter: Lukas Prantl (Germany) Affiliation: University Hospital Regensburg Authors: Lukas Prantl, Andreas Eigenberger, Oliver Felthaus
3:42 pm	**Discussion**
3:51 pm	**10** (p.28) - **A Potential Two-Step Mechanism for the Generation of Fat Emboli** Presenter: William Molair (USA) Affiliation: Wake Forest University Authors: Will Molair, Adam Katz, Ramon Llull
4:00 pm	**11** (p.29) - **Designing an Explorative Clinical Trial Investigating Multiple Applications of Adipose-derived Stem/Stromal Cells? Our Clinical Experience and Suggestions** Presenter: Jesper Jensen (Denmark) Affiliation: StemMedical A/S Authors: Frederik Penzien Mamsen, Lea Munthe-Fog, Jesper Dyrendom Svalgaard, Jesper Due Jensen, Thomas Hartvig Lindkær Jensen, Anne Fischer-Nielsen, Mikkel Taudorf, Bo Jønsson, Adam J. Katz, Stig-Frederik Trojahn Kølle
4:09 pm	**12** (p.30) - **Tissue Structure and Retention Rate of Adipose-derived Stem/Stromal Cell-enriched Fat Grafts w/o Collagen: An Explorative, Randomized, Controlled, Triple Blind, Paired, Clinical Trial** Presenter: Frederik Mamsen (Denmark) Affiliation: StemMedical A/S Authors: Frederik Mamsen, Lea Munthe-Fog, Jesper Dyrendom Svalgaard, Jesper Due Jensen, Thomas Hartvig Lindkær Jensen, Anne Fischer-Nielsen, Mikkel Taudorf, Bo Jønsson, Adam J. Katz, Stig-Frederik Trojahn Kølle
4:18 pm	**Discussion**
4:30 - 5:30 pm	**PANEL: Cell-based Therapies** Moderator: LaTonya J. Hickson, MD **Optimizing Kidney Repair through Adipose-Tissue Derived Mesenchymal Stromal Cell Therapy** LaTonya J. Hickson, MD *Chair of Nephrology and Hypertension at Mayo Clinic* **Getting Cells in the Right Mood (and Mode): BonoFill and MesenCure as Examples for Adipose Stromal Cells' Professionalization Enhancing their Therapeutic Utility** Tomer Bronshtein, PhD *VP Business Development, Bonus BioGroup* **Defining New Criteria for Adipose-derived Stromal Vascular Fraction Use: A Multicentric Experience in Marseille and Lugano** Jeremy Magalon, PharmaD, PhD *Medical Biologist in Cell Therapy* *Marseille La Conception University Hospital Aix Marseille University, France*
5:30 - 5:45 pm	**Break - Toast with our Keynote**
5:45 - 6:45 pm	**Keynote Speaker - Kotaro Yoshimura, MD** **Strategic Use of the Regenerative Potential of Adipose Stem Cells** *Professor and Chair Department of Plastic Surgery* *Jichi Medical University* *Yakushiji, Shimotsuke, Tochigi, Japan* Moderator: Fred Koelle, MD, PhD *Las Olas Ballroom*
6:45 pm	**Meeting Adjourns for the Day**

SATURDAY - NOVEMBER 5

7:00 - 8:00 am	**Continental Breakfast in Exhibit Hall**	*Las Olas Foyer*
8:00 - 8:45 am	**Keynote Speaker - Juergen Groll, PhD** **Present and Future of 3D Bioprinting** *Professor and Chair, Department for Functional Materials in Medicine and Dentistry* *Executive Director, Institute for Functional Materials and Biofabrication* *University of Wuerzburg, Germany* *Spokesman, Collaborative Research Center SFB/TRR 225 Biofabrication* Moderator: Torsten Blunk, PhD	*Las Olas Ballroom*

	Las Olas Ballroom 1 - 3		Las Olas Ballroom 4 - 6
8:45 - 10:00 am	**Free Papers 3 - 3D Bioprinting for Soft Tissue Regeneration and Adipose Tissue Models** Moderators: Paul Gatenholm, PhD & Torsten Blunk, PhD	8:45 - 10:00 am	**Free Papers 4 - Adipose Tissue Processing** Moderators: Ricardo Rodriguez, MD & Fred Koelle, MD, PhD
8:45 am	**13** (p.30) - **Gellan Gum as Promising Material for Manually and Additively Manufactured Long-term Stable and Functional 3D Adipose Tissue Models** Presenter: Petra Kluger (Germany) Affiliation: Reutlingen University Authors: Vera Dolderer, Svenja Nellinger, Freia Schmidt, Petra Kluger	8:45 am	**18** (p.33) - **Comparison of Adipose-derived Stem Cells, The Stromal Vascular Fraction and Prepared Adipose Tissue Grafts in Autologous Fat Transfer** Presenter: Summer Hanson (USA) Affiliation: University of Chicago Medicine and Biological Sciences Authors: Summer Hanson , Malke Asaad, Yewen Wu, Cynthia Branch-Brooks, Qixu Zhang, Peiman Hematti
8:54 am	**14** (p.31) - **Machine Learning-based Predictive Model for Printability and Shape Fidelity in Biofabrication** Presenter: Luciano Vidal (France) Affiliation: Centrale Nantes Authors: Vidal, L, Hascoët N, Kavrakova T, Arduengo Garcia J., Chinesta F., Hascoët JY.	8:54 am	**19** (p.34) - **Cell Culture Dynamics of Isolated versus Aggregated Populations of Adipose Derived Stromal Fraction: Stromal Aggregates Remain Viable, Lag Proliferation before Exponential Proliferation** Presenter: Mary Duet (USA) Affliation: Wake Forest Baptist Health Authors: Mary Duet, Hulan Shang, Abigail Peoples, William Molair, Adam Katz, Ramon Lllull
9:03 am	**15** (p.32) - **Developing Next Generation Adipose Tissue Grafts for Soft Tissue Reconstruction** Presenter: Gretel Major (New Zealand) Affiliation: University of Otago Christchurch Authors: Gretel Major, Alessia Longoni, Jeremy Simcock, Tim Woodfield, Khoon Lim	9:03 am	**20** (p.34) - **Mechanical Processing of Lipoaspirate does not Lead to a Significant Manipulation of the White Adipose Tissue or Adipose Derived Stem Cells** Presenter: Andreas Eigenberger (Germany) Affiliation: University Hospital Regensburg Authors: Andreas Eigenberger, Oliver Felthaus, Lukas Prantl
9:12 am	**Discussion**	9:12 am	Discussion
9:21 am	**16** (p.32) - **Long-term in Vivo Survival of 3D-bioprinted Human Lipoaspirate-derived Adipose Tissue: Proteomic Signature and Cellular Content** Presenter: Karin Saljo (Sweden) Affiliation: Department of Plastic Surgery, Sahlgrenska Academy and Sahlgrenska University Hospital Authors: Karin Saljo , Peter Apelgren, Linnea Stridh Orrhult, Susann Li, Matteo Amoroso, Paul Gatenholm, Lars Kölby	9:21 am	**21** (p.35) - **A Simple, Reproducible Estimation of Adipocyte Cell Lysis in Lipoaspirate Samples and their Disaggregated Byproducts** Presenter: Aina Llull Canellas (USA) Affiliation: Wake Forest Baptist Health Authors: Aina Llull Canellas , Gabriela Aguilo-Seara, William Molair, Adam J Katz, Ramon Llull
9:30 am	**17** (p.33) - **Functional and Morphological Studies of In Vivo Vascularization of 3D Bioprinted Human Fat Grafts** Presenter: Peter Apelgren (Sweden) Affiliation: Department of Plastic Surgery, Sahlgrenska University Hospital Authors: Peter Apelgren, Matteo Amoroso, Karin Säljö, Mikael Montelius, Linnea Strid Orrhult, Mona Engström, Paul Gatenholm, Lars Kölby	9:30 am	**22** (p.36) - **Extent of Tissue Washing Can Significantly Alter the Composition and Activity of SVF Preparations: Implications for Clinical Translation** Presenters: Adam Katz, MD (USA) Affiliation: Wake Forest Baptist Health Authors: Adam Katz, Gabriela Aguilo-Seara, William Molair, Hulan Shang, Scott Northrup, Josh Grosser, Ramon Llull

9:39 am	**Discussion**	9:39 am	**23** (p.37) - **Optimal Fat Graft Sizing with the 'Micronizer' System** Presenter: Hilton Becker, MD (USA) Affiliation: beckerMD Author: Hilton Becker
		9:48 am	**Discussion**
10:00 - 10:30 am	**Coffee Break in Exhibit Hall**		*Las Olas Foyer*
	Las Olas Ballroom 1 - 3		**Las Olas Ballroom 4 - 6**
10:30 - 12:00 pm	**Free Papers 5 - Secretory Activity of ASC and SVF** Moderators: Ramon Llull, PhD & Bruce A. Bunnell, PhD	10:30 - 12:00 pm	**Free Papers 6 - Skin, Wound Healing and Radiation Injury** Moderators: Julie Fradette, PhD & Ricardo Rodriguez, MD
10:30 am	**24** (p.37) - **Syngeneic Adipose-Derived Stromal Cells Modulate the Immune Response within Decellularized Adipose Tissue Scaffolds in a Murine Subcutaneous Implant Model** Presenter: John Walker (Canada) Affiliation: Western University Authors: Walker, J., Cooper, Tyler T.; Dunmore-Buyze, Joy; Drangova, Maria; Lajoie, Gilles; Dekaban, Gregory A.; Flynn, Lauren E.	10:30 am	**32** (p.41) -**The Effect of Adipose-derived Stem/Stromal Cell- and Collagen Enriched Fat Grafts on Quality of Wound Healing: An Explorative, Randomized, Controlled, Triple Blind, Paired, Clinical Trial** Presenter: Jesper Jensen (Denmark) Affiliation: StemMedical A/S Authors: Frederik Penzien Mamsen, Lea Munthe-Fog, Jesper Dyrendom Svalgaard, Jesper Due Jensen, Thomas Hartvig Lindkær Jensen, Anne Fischer-Nielsen, Mikkel Taudorf, Bo Jønsson, Adam J. Katz, Stig-Frederik Trojahn Kølle
10:39 am	**25** (p.38) - **Adipose Stromal Cells for Osteoarthritis: Are All Orthobiologics the Same?** Presenter: Nathan Katz (USA) Affiliation: Jointechlabs Authors: N. Katz, A. Yayon, N. Pancholi , A. Hakimian and S. Chubinskaya	10:39 am	**33** (p.42) - **Skin Rejuvenation Using Ex-vivo Expanded Adipose-derived StemStromal Cells and Collagen Enriched Fat** Presenter: Frederik Mamsen (Denmark) Affiliation: StemMedical A/S Authors: Frederik Mamsen, Lea Munthe-Fog, Jesper Dyrendom Svalgaard, Jesper Due Jensen, Thomas Hartvig Lindkær Jensen, Anne Fischer-Nielsen, Mikkel Taudorf, Bo Jønsson, Adam J. Katz, Stig-Frederik Trojahn Kølle
10:48 am	**26** (p.38) - **Human Platelet Lysate as Supplement to Increase the Anti-inflammatory Properties of Adipose-derived Stem Cells for the Treatment of Osteoarthritis** Presenter: Stefania Brambilla (Italy) Affiliation: IRCCS Ospedale Galeazzi - Sant'Ambrogio, Milano, Italy Authors: Stefania Brambilla, Silvia Lopa, Arianna Lovati, Shima Salehi, Matteo Moretti, Silvia Palombella	10:48 am	**34** (p.42) - **Nanofat Subdermal Injections for Treatment of Burn Scars** Presenter: Maria McKenna (USA) Affiliation: Claytor Noone Plastic Surgery Authors: Julie Holes, Richard Claytor, Maria McKenna
10:57 am	**27** (p.39) - **Wharton's Jelly Mesenchymal Stem Cell-derived Small Extracellular Vesicles as Natural Nanoparticles to Attenuate Cartilage Injury via MicroRNA Regulation** Presenter: Penghong Chen (China) Affiliation: Fujian Medical University Union Hospital Authors: Penghong Chen , Xiaosong Chen	10:57 am	**35** (p.43) **Adipose Stem Cell Exosome (ASCE): Next Generation Regenerative Therapeutics & Aesthetics for Skin and Hair** Presenter: Byong Cho (South Korea) Affiliation: ExoCoBio Inc. Author: Byong Cho
11:06 am	**Discussion**	11:06 am	Discussion
11:15 am	**28** (p.39) - **Development of Collagenolytic Cell Inoculants to Address Dupuytren Disease (and other fibrotic/anchylosing processes)** Presenter: Raghav Agarwal (USA) Affiliation: Wake Forest Baptist Health Authors: Raghav Agarwal, Severiano Dos Anjos, William Molair, Adam J. Katz, Ramon Llull	11:15 am	**36** (p.43) - **Addressing Healing of Complex Skin Wounds Using Tissue Engineering Methods Applied to Adipose-derived Stromal Cells** Presenter: Julie Fradette (Canada) Affiliation: LOEX-Universite Lava Authors: Fradette, J., Safoine M., Diaz C., Ruel J.

11:24 am	**29** (p.40) - **Effects of Two- and Three-Dimensional Culture Under Different Microenvironments on Adipose-Derived Stem Cells and Conditioned Medium** Presenter: Yoshihiro Toyohara (Japan) Affliation: Jichi Medical University Authors: Yoshihiro Toyohara, Masanori Mori, Kayo Yoshizumi, Yoshihiro Yamamoto, Zhang Bihang, Takako Shirado, Natsumi Saito, Wu Yunyan, Kotaro Yoshimura	11:24 am	**37** (p.44) - **Adipose Derived Stem Cell Conditioned Media Gives Mixed Results Regarding Irradiated Human Keratinocytes Viability and Migration** Presenter: Celena Sorgel (Germany) Affiliation: Department of Plastic and Hand Surgery University Hospital of Erlangen Author: Celena Sorgel
11:33 am	**30** (p.40) - **Comparative Quality Analysis of Two-dimensional Versus Large Scale Bioreactor Culturing of Human ASCs** Presenter: Lea Munthe-Fog (Denmark) Affiliation: StemMedical A/S Authors: L. Munthe-Fog, MR. Galera, F.Mamsen, A. Woetmann, AL. Fischer-Nielsen, JD Jensen, SF. Kolle and JD.Svalgaard	11:33 am	**38** (p.45) - **Therapeutic Effects of Conditioned Medium from Adipose-derived Stem Cells Cultured in Xeno-free Medium on Impaired Wound Healing in Irradiated Tissue** Presenter: Bihang Zhang (Japan) Affiliation: Jichi Medical University Authors: Bihang Zhang , Kotaro Yoshimura
11:42 am	**31** (p.41) - **Adipose Derived Stromal Cells under Oxidative Stress Exert a Potent Inhibitory Effect in Bystander Cells via a Secretory Phenotype** Presenter: Anuj Jaiwala (USA) Affiliation: Wake Forest Baptist Health Authors: Anuj Jaiwala, Samuel Kogan, William Sanfelippo, Adam J. Katz, Ramon Llull	11:42 am	**Discussion**
11:51 am	**Discussion**		
12:00 - 1:00 pm	**Lunch & Learn with StemMedical, Stem Cells and Development, and IFATS Leaders**		
	Las Olas Ballroom 1 - 3		**Las Olas Ballroom 4 - 6**
1:00 -2:15 pm	**Free Papers 7 - Nerve Regeneration** Moderators: Lauren Kokai, PhD & Paul Kingham, PhD	1:00 -2:15 pm	**Panel: Adipose Tissue in Cancer** Moderator: Bruce A. Bunnell, PhD
1:00 pm	**39** (p.45) - **Secretome from Stimulated Adipose Stem Cells Enhances Neurite Outgrowth and Angiogenesis** Presenter: Maria Brohlin (Sweden) Affiliaton: Umea University Authors: Maria Brohlin, Rosanna Ching, Malin Andersson, Mikael Wiberg, Paul J Kingham		**Adipose Tissue in Cancer and Other Aging-related Diseases** Mikhail Kolonin, PhD *Professor & Director, Center for Metabolic and Degenerative Diseases* *Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research* *University of Texas Health Science Center at Houston* **The Interplay Between Adipose Stem Cells and Breast Cancer** Bruce A. Bunnell, PhD *Professor & Chair Dept. of Microbiology, Immunology, and Genetics* *University of North Texas Health Science Center* **Evaluation of Stromal Age in Breast Cancer** Elizabeth Martin, PhD *Assistant Professor Department of Biological And Agricultural Engineering* *Louisiana State University* **Mechanical Properties of Adipose Tissue and Their Relevance to Cancer** Claudia Fischbach, PhD *Stanley Bryer 1946 Professor of Biomedical Engineering, Director of Cornell's Physical Sciences Oncology Center (PSOC) on the Physics of Cancer Metabolism Cornell University*
1:09 pm	**40** (p.46) - **Human Platelet Lysate Modulates Anti-inflammatory and Regenerative Properties of Stem Cells Derived from the Stromal Vascular Fraction of Adipose Tissue** Presenter: Silvia Palombella (Italy) Affiliation: IRCCS Ospedale Galeazzi Sant'Ambrogio - Milano, Italy Authors: Silvia Palombella, Stefania Brambilla; Silvia Lopa; Giuseppe Talò; Martino Guiotto; Pietro di Summa		

1:18 pm	**41** (p.46) - **Development of a StarPEG Hydrogel System to Promote Controlled and Sustained Delivery of Adipose Tissue Derived Stem Cells Secretome for CNS Regenerative Medicine** Presenter: Antonio Salgado (Portugal) Affiliation: ICVS, School of Medicine, University of Minho Authors: Lucas Schirmer; Tiffany S. Pinho; Passant Atallah; Jorge R. Cibrão; Rui Lima; João Afonso; Sandra B-Antunes; Cláudia R. Marques; João Dourado; Uwe Freudenberg; Rui A. Sousa; Carsten Werner; António J. Salgado
1:27 pm	**Discussion**
1:38 pm	**42** (p.47) - **Optimizing Stem Cell Based Peripheral Nerve Regeneration - Laminin Surface Coating Increases NGF Secretion of Differentiated Adipose Tissue Derived Stem Cells** Presenter: Oliver Felthaus (Germany) Affiliation: University Hospital Regensburg Authors: Oliver Felthaus, Lukas Prantl, Andreas Eigenberger
1:47 pm	**43** (p.48) - **Hyperactivity of Adipose Stem Cell from an Amyotrophic Lateral Sclerosis Donor** Presenter: Lauren Kokai (USA) Affiliation: University of Pittsburgh Authors: Lauren Kokai, Shawn Loder, Bahaa Shaaban, Wayne Vincent Nerone
1:56 pm	**44** (p.49) - **Effectiveness of Cell Therapy Treatment Applied to a Patient with Lumbar Radiculopathy, Following Failed Lumbar Surgery** Presenter: Ramiro Ramirez (Mexico) Affiliation: Medyca Bosques Authors: Ramiro Ramirez, Dra Daniela Flores
2:05 pm	**Discussion**

Fort Lauderdale, Florida

	Las Olas Ballroom 1 - 3		Las Olas Ballroom 4 - 6
2:15 -3:15 pm	**PANEL: Fat Grafting II - Face** Moderator: Fred Koelle, MD, PhD	2:15 -3:15 pm	**Free Papers 8 - Angiogenesis and Vascularization** Moderators: Rosalyn D. Abbott, PhD & Sara Al-Ghadban, PhD
	Face Bio Remodeling Using Stromal Enriched Lipograft Aris Sterodimas, MD, Msc, PhD, BC Plastic Surgeon *Head Surgeon of Plastic Surgery* *Plastic & Reconstructive Department* *Metropolitan General Hospital* *Athens, Greece* **How PRP Can Improve Fat Grafting to the Face** Ali Modarressi, PhD *Professor and Chair* *Department of Plastic Surgery* *University Hospitals of Geneva, Switzerland* **Stem Cell Enriched Fat Grafting to the Face** Fred Koelle, MD, PhD, BC Plastic Surgeon *Head Surgeon of Plastic Surgery* *Department of Plastic Surgery* *CeriX Private Hospital, Denmark* **How to Use SVF, PRP, and PRS in Facial Surgery and 17 Points of Pan-Facial Fat Grafting** Chia Chi Kao, MD, BC Plastic Surgeon *Founder, KAO Regenerative Therapies*	2:15 pm	**45** (p.49) - **Increased Angiogenic Differentiation of HUVECs Treated with Pre-conditioned Media from Lipedema Adipocytes in Vitro** Presenter: Sara Al-Ghadban (USA) Affiliation: University of North Texas Health Science Center Authors: Sara Al-Ghadban, Samantha G. Walczak, Bruce A. Bunnell
		2:24 pm	**46** (p.50) - **Cell Culture Conditions Influence Angiogenic and Adipogenic Properties of Adipose Stem Cells** Presenter: Anne Therese Lauvrud (Sweden) Affiliation: Umeå University Authors: Anne Therese Lauvrud, Maria Brohlin, Rebecca Wiberg, Mikael Wiberg, Paul J Kingham
		2:33 pm	**47** (p.50) - **Transcriptomic Variations of Endothelial Progenitor Cells from Adipose, Bone Marrow, Cord Blood and Peripheral Blood** Presenter: Srinivas Koduru (USA) Affiliation: Penn State, College of Medicine Authors: Srinivas Koduru, Dino J Ravnic
		2:42 pm	**48** (p.51) - **Influences of Oxygen Tension on Human Adipose-Resident Microvascular Endothelial Progenitor Cells** Presenter: Natsumi Saito (Japan) Affiliation: Jichi Medical University Auhtors: Natsumi Saito , Kotaro Yoshimura
		2:51 pm	**Discussion**
3:15 - 3:45 pm	**Coffee Break in Exhibit Hall**		*Las Olas Foyer*

Las Olas Ballroom 1 - 3		Las Olas Ballroom 4 - 6	
3:45 -5:00 pm	**PANEL: Adipose Therapeutics in Musculoskeletal Disorders** Moderator: Marco N. Helder, PhD	3:45 -5:00 pm	**Free Papers 9 - Fat Grafting II** Moderators: Summer E. Hanson, MD, PhD & J. Peter Rubin, MD
	Adipose-derived Cells for Bone Regeneration: Bone (pre) Fabrication and Developmental Engineering Arnaud Scherberich, PhD *Professor, Research Group Leader Bone Regeneration, Department of Biomedicine, University of Basel, Basel, Switzerland* **Orthopedic Clinical Trials for Biologics – Considerations and Issues for Phase I to Pivotal Studies** William W. Cimino, PhD *CEO at GID BIO, Inc., Louisville, Colorado* **Adipose Tissue for Craniofacial Bone Regeneration: Enzymatic Digestion or Microfragmentation?** Marco N. Helder, PhD *Associate Professor, Head Stem Cell & Nanotechnology Lab, Co-head Bone and Stem Cell lab, Department of Oral & Maxillofacial Surgery/ Oral Pathology, AMS-Tissue Function & Regeneration, Amsterdam Bone Center, Amsterdam UMC-location VUMC and ACTA Amsterdam, Amsterdam, The Netherlands* **Adipose Tissue in Bone Marrow: Friend or Foe?** Nathalie Bravenboer, PhD *Associate Professor, Head Bone and Calcium Metabolism Lab, Co-head Bone and Stem Cell lab, Department of Clinical Chemistry, AMS-Tissue Function & Regeneration, Amsterdam Bone Center, Amsterdam UMC-location VUMC, Amsterdam, The Netherlands*	3:45 pm	**49** (p.51) - **The Fat and the Furious: How Different Oil Preparations Alter Local Inflammatory Patterns** Presenter: William Molair (USA) Affiliation: Wake Forest University Authors: William Molair, Adam Katz, Ramon Llull
		3:54 pm	**50** (p.52) - **Oral Vitamin D Improves Fat Graft Survival in a Large Animal Model** Presenter: Bahaa Shaaban (USA) Affiliation: University of Pittsburgh Authors: B Shaaban, SJ Loder, PL Lee, WV Nerone, C Amurgis, R Ricketts, D Ramkumar and LE Kokai
		4:03 pm	**51** (p.53) - **Platelet Rich Plasma Assisted Adipose Grafting for Scars and Aesthetics** Presenter: Sherry Collawn, MD (USA) Affiliation: University of Alabama Authors: Sherry Collawn, Gagandip Singh, Ann Carol Braswell
		4:12 pm	**Discussion**
		4:21 pm	**52** (p.53) - **Evaluation of the Efficacy of Stromal Vascular Tissue and Microfat Injection in Periorbital Improvement of Pigmented Dark Circles, Hollow Ring, and Loss of Palpebral Elasticity** Presenter: Sophie Menkes (Switzerland) Affiliation: Necsens Clinic Author: Sophie Menkes
		4:30 pm	**53** (p.54) - **Clinical Outcomes of Laser Assisted Liposuction for Body Contouring and Facial Fat Graft Under Local Anesthesia** Presenter: Naoko Hitosugi (Japan) Affiliation: Muse City Clinic Authors: Naoko Hitosugi , Mohamed Abdelhakim, Kyoko Dogo
		4:39 pm	**54** (p.54) - **Swiss Regulations on the Transplantation of Adipose Tissue and Stromal Vascular Fraction** Presenter: Carlo Oranges (Switzerland) Affiliation: Geneva University Hospitals Authors: Carlo Oranges, Mathias Tremp, Daniel F. Kalbermatten
		4:48 pm	**Discussion**

Las Olas Ballroom 1 - 3		Las Olas Ballroom 4 - 6	
5:00 - 6:15 pm	**Free Papers 10 - Musculoskeletal Applications** Moderators: Susanna Miettinen, PhD & Marco N. Helder, PhD	5:00 - 6:15 pm	**Industry Roundtable - The Future of Adipose Directed Therapeutics** Moderator: Lauren Kokai, PhD
5:00 pm	**55** (p.55) - **BonoFill from Bench to Bedside: A Novel Tissue-engineered Product Generated from Adipose-derived Mesenchymal Stromal Cells in Line to Replace Bone Autografts for Large Segmental Bone Defect Applications** Presenter: Dror Ben-David (Israel) Affiliation: Bonus BioGroup Authors: Dror Ben-David , Atara Novak, Tomer Bronshtein, Nimrod Rozen, Ephraim Tzur, Vered Kivity, Shai Meretzki		**Joseph Candiello, PhD** *Senior Product Manager, RoosterBio, Inc* RoosterBio® **Marc Fauerby** *Chief Commercial Officer StemMedical* STEMMEDICAL® **Byong Cho** *Chief Executive Officer ExoCoBio, Inc.* ExoCoBio **James J. Hickman, PhD** *University of Central Florida, NanoScience Technology Center, Professor of Nanoscience Technology, Chemistry, Biomolecular Science and Electrical Engineering, and Hesperos, Inc., Chief Scientist* HESPEROS THE HUMAN-ON-A-CHIP COMPANY
5:09 pm	**56** (p.55) - **Clinical Outcomes of Mechanically Isolated Autologous Stromal Vascular Fraction/Cellular Fraction in Patients with Osteoarthritis of Knees** Presenter: Vinod Jain (India) Affiliation: Sahaj Hospital, Indore Authors: Vinod Jain, Raj Sharma		
5:18 pm	**57** (p.56) - **Microfragmented Fat (MFAT) and BCP for Alveolar Cleft Repair: A Prospective Control Clinical Trial** Presenter: Sabrina Natsir Kalla (Indonesia) Affiliation: Hasanuddin University Authors: Sabrina Natsir Kalla, Abul Fauzi, Andi Tajrin, Rifaat Nurrahma, WEG Müller, HC Schröder, XH Wang, Tymour Forouzanfar, Marco N Helder, Muhammad Ruslin		
5:27 pm	**Discussion**		
5:36 pm	**58** (p.56) - **Nanofat Intraarticular Injections Can Decrease Knee Pain and Delay Knee Replacement Surgery** Presenter: Madis Rahu (Estonia) Affiliation: Tart University Hospital Authors: Madis Rahu, Terje Arak, Leho Rips, Mihkel Luik, Tauno Koovit		
5:45 pm	**59** (p.57) - **Role of Adipose Derived Stem Cell for Osteoarthritis of Knee and Hip Joint: Myth or Reality** Presenter: Kumar Ashok, MD (Dubai) Affiliation: My Doc Specialist Medical Centre DMCC Author: Kumar Ashok		
5:54 pm	**60** (p.57) - **A Novel Method of Stromal Vascular Fraction Separation to be Used in the Management of a Knee and its Clinical Outcomes** Presenter: Ramamoorthy Ramaswamy (India) Affiliation: Sri Ramakrishna Hospital Authors: Ramamoorthy Ramaswamy, R.Krishnamoorthy		
6:03 pm	**Discussion**		
6:15 pm	**Meeting Adjourns for the Day**		
7:00 - 9:30 pm	**Conference Reception & Dinner** Westin Hotel Pool Deck		

SUNDAY - NOVEMBER 6

7:30 - 8:30 am	**Continental Breakfast in Exhibit Hall**
8:00 - 9:00 am	**IFATS Members Meeting**
9:00 - 10:50 am	**Plenary Session BEST PAPERS (Award Eligible)** Moderators: Bruce A. Bunnell, PhD & Keith L. March, MD, PhD
9:00 am	**61** (p.58) - **Engineering an Adipose Tissue Organ-on-a-chip Model for Therapeutic Drug Discovery** Presenter: Lindsey Huff (USA) Affiliation: Carnegie Mellon University Authors: Lindsey Huff, Charles Amurgis, Lauren E. Kokai, Rosalyn Abbott
9:09 am	**62** (p.59) - **Neuroprotective Effects of Adipose-derived Stromal Vascular Fraction on Acute Spinal Cord Injuries in Rats** Presenter: Céline Ertlen (France) Affilation: La Timone Hospital, Assistance Publique - Hôpitaux de Marseille Authors: Céline Ertlen, Mostafa Seblani, Maxime Bonnet, Sara Belluco, Jean-Michel Brezun, Tanguy Marqueste, Nicolas Serratrice, Patrick Decherchi
9:18 am	**63** (p.60) - **Cancer Cell Migration Depends on Adjacent Stromal Cells in 3D Bioprinted Breast Cancer Models** Presenter: Hannes Horder (Germany) Affiliation: University of Würzburg Authors: Horder H; Böhringer D; Grummel N; Hildebrand L; Schweinitzer S; Teßmar J; Groll J; Bauer-Kreisel P; Fabry B; Blunk T
9:27 am	**Discussion**
9:36 am	**64** (p.60) - **White and Beige/Brown Human Adipose Tissue-on-Chips at a Platform for Disease Modeling and Drug Discovery** Presenter: Trivia Frazier (USA) Affiliation: Obatala Sciences Authors: Trivia Frazier, Cecilia Sanchez, Katie Hamel
9:45 am	**65** (p.61) - **Development of 3D Human Neuronal and Vascular Microphysiological Models with Human Adipose Stem/Stromal Cells or Bone Marrow Stem/Stromal Cells as Mural Cell Types** Presenter: Susanna Miettinen (Finland) Affiliation: Tampere University Authors: Susanna Miettinen, Lotta Isosaari, Hanna Vuorenpää, Susanna Narkilahti
9:54 am	**66** (p.61) - **Establishment of a 3D-biofabricated Tumor Model Suitable for Analyzing Tumor Progression, Cell-cell-and Cell-matrix-interactions** Presenter:Annika Kengelbach-Weigand (Germany) Affiliation: University Hospital Erlangen Authors: A. Kengelbach-Weigand, R. Schmid, S. K. Schmidt, R. Detsch, H. Horder, T. Blunk, S. Schrüfer, D. W. Schubert, L. Fischer, I. Thievessen, D. Schneidereit, O. Friedrich, A. Grüneboom, Pamela L. Strissel, Reiner Strick, R. E. Horch, A. K. Bosserhoff, A. Arkudas
10:03 am	**Discussion**
10:12 am	**67** (p.62) - **Efficacy of Microfragmented Adipose Tissue for Treatment of Symptomatic Knee Osteoarthritis: A Randomized, Placebo-controlled Study** Presenter: Joshua Harrison (USA) Affiliation: University of New Mexico, School of Medicine Authors: Joshua Harrison, Anil Shetty, Robert Schenck, Dustin Richter
10:21 am	**68** (p.62) - **Understanding the Role of Subcutaneous Adipose Tissue Extracellular Matrix in Wound Healing Complications Following Caesarean Deliveries** Presenter: Louise Croizat-Vallet (New Zealand) Affiliation: University of Otago Authors: Louise Croizat-Vallet, Gabriella Stuart, Michael Stitely, Lyn Wise
10:30 am	**69** (p.63) - **Management of Deep Tunneling Wounds with Novel Allograft Adipose Matrix: A Natural Off-The-Shelf Treatment Modality** Presenter: Chitang Joshi (USA) Affiliation: Northwestern University Authors: Chitang Joshi , Robert D Galiano

10:39 am	**Discussion**	
10:50 - 11:20 am	**Coffee Break in Exhibit Hall**	
11:20 - 12:30 pm	**Free Papers 11 - Adipose Developmental and Functional Biology** Moderators: Petra Bauer-Kreisel, PhD & Dmitry Traktuev, PhD	
11:20 am	**70** (p.63) - **Activin A Mediates Inflammatory Cell-induced Acquisition of Myofibroblast Phenotype by Adipose Mesenchymal/Stromal Cells** Presenter: Dmitry Traktuev (USA) Affiliation: University of Florida College of Medicine Authors: Sahana Manohar-Sindhu, Stephanie Merfeld-Clauss, Keith L. March, Dmitry O. Traktuev	
11:29 am	**71** (p.64) - **Molecular and Functional Characterization of Phosphodiesterase 10A in Human White Adipocytes** Presenter: Mohammed Hankir (Germany) Affiliation: Würzburg University Hospital Author: Mohammed Hankir	
11:38 am	**72** (p.64) - **Adipocyte Differentiation and Collagen Secretion in Nf1-Deficient Breast Cancer** Presenter: Menusha Arumugam (USA) Affiliation: Van Andel Institute Authors: Menusha Arumugam, Elizabeth A. Tovar, Curt J. Essenburg, Ian T. Beddows, Lisa Turner, Corinne Esquibel, Patrick S. Dischinger, Megan E. Callaghan, Eve E. Gardner, Ghassan Mouneimne, Kirk C. Hansen, Carrie R. Graveel, Matthew R. Steensma	
11:47 am	**Discussion**	
11:55 am	**73** (p.65) - **Immunomodulatory Functions of Adipose Mesenchymal Stromal/Stem Cells Derived from Donors with Type2 Diabetes and Obesity on CD4+ T cells** Presenter: Marwa Mahmoud Mohamed El Shahat (Egypt)) Affiliation: National Research Centre Authors: Marwa Mahmoud*, Laura Kummola, Miia Juntunen, Amna Adnan, Ilkka S. Junttila, Minna Kääriäinenj, Tuula Tyrväinen, Abeer Abd El Fattahl, Khalda Amr, Alaa Mohamad El erian, Mimmi Patrikoski, Susanna Miettinen*	
12:04 pm	**74** (p.65) - **Effects of Weight Loss on Adipose Stem/Stromal Cell Immunomodulation and Mitochondrial Respiration Capacity** Presenter: Amna Adnan (Finland) Affiliation: Tampere University Authors: Amna Adnan, Mimmi Patrikoski, Miia Juntunen, Tuula Tyrväinen, Minna Kelloniemi and Susanna Miettinen	
12:13 pm	**75** (p.66) - **Novel Kinase Inhibitor Screening for Inhibitors of Adipogenic Differentiation in Adipose-derived Stem Cells from Lean and Obese Patients** Presenter: Caroline Rinderle (USA) Affiliation: The University of North Texas Health Science Center at Fort Worth Authors: Caroline Rinderle, Bruce Bunnell	
12:22 pm	**Discussion**	
12:30 - 1:00 pm	**Grab and Go Lunch**	
1:00 - 2:00 pm	**Keynote Speaker - Adam J. Katz** **Fat Reflections: Blinded by the Light** *Director, Plastic Surgery Research Laboratory* *Michael J. Morykwas Endowed Professor of Plastic and Reconstructive Surgery* *Professor, Department of Biomedical Engineering* *Professor, Wake Forest Institute of Regenerative Medicine* Moderator: Lauren Kokai, PhD	*Las Olas Ballroom*

2:00 - 3:00 pm	**PANEL: Insight Views into Translation and Regulatory Affairs** Moderator: Adam J. Katz, MD, FACS *Director, Plastic Surgery Research Laboratory* *Michael J. Morykwas Endowed Professor of Plastic and Reconstructive Surgery* *Professor, Department of Biomedical Engineering* *Professor, Wake Forest Institute of Regenerative Medicine* **When Cells, Regulators & Courts Collide: An Update on FDA and FTC Enforcement Efforts** Mary Ann Chirba, J.D., D.Sc., M.P.H. *John C. Ford, S.J., Distinguished Scholar* *Boston College Law School* **Clinical Studies for Cellular Therapies and the FDA: Phase I to Phase III** William W. Cimino, PH.D. *CEO at GID Bio, Inc., Louisville, Colorado* **Translating Novel Technologies within the University: Musings of an Academic-corporate Chimera** Ed Pagani, PhD *Duke University* *Assistant Vice President, External Partnerships* *Executive Director, Office for External Partnerships*	*Las Olas Ballroom*
3:00 - 3:30 pm	**Awards and Closing Remarks** Lauren Kokai, PhD	*Las Olas Ballroom*
3:30 pm	**Meeting Adjourns**	

THE WESTIN FORT LAUDERDALE BEACH RESORT

Synopsis of Conference Panels

Adipose Tissue Models and Microphysiological Systems

Friday, 11/4/22 – 8:45– 10:00 am

Given the important functions of adipose tissue in health and disease, a critical component of studying adipose in dynamic cultures which recapitulate complex cell:cell and cell:matrix interactions is 3-D modeling. Modern innovations in custom culture-ware and microperfusion systems have enabled scientific pioneers to interrogate increasingly complex relationships between the adipocyte and its micro-milieu. The 2022 IFATS panel on Adipose Tissue Models and Microphysiological Systems is designed to provide a high-level overview on the current state of the art of ex vivo adipose modeling and provide a platform to discuss exciting ongoing research by our speakers who utilize new technologies toward diverse clinical goals.

Introduction and Overview of the Field
Rosalyn D. Abbott, PhD
Assistant Professor | Carnegie Mellon University
Biomedical Engineering | Materials Science and Engineering (courtesy)

Lauren Kokai, PhD
Assistant Professor, Plastic Surgery and Bioengineering
University of Pittsburgh School of Medicine

Engineering Naturally-derived Human Adipose Tissues for In-vitro and In-vivo Studies
Julie Fradette, PhD
Full Professor, Dept of Surgery, Université Laval
Researcher, Centre de recherche en organogénèse expérimentale de l'Université Laval / LOEX Division of Regenerative Medicine, CHU de Québec Research Centre-Université Laval, Québec, Canada

Engineering Human Blood-Derived and Adipose Tissue-Derived Products for Metabolic Research and Regenerative Medicine
Jeffrey M. Gimble, MD, PhD
Chief Medical Officer, Obatala Sciences Inc.

Multi-organ Systems to Investigate Metabolic Diseases
James J. Hickman, PhD
University of Central Florida, NanoScience Technology Center, Professor of Nanoscience Technology, Chemistry, Biomolecular Science and Electrical Engineering and Hesperos, Inc., Chief Scientist

Autologous Human Immunocompetent White Adipose Tissue-on-Chip Models
Peter Loskill, PhD
Organ-on-Chip-Research, Eberhard Karls University Tübingen
Natural and Medical Sciences Institute
Vice Chair European-Organ-on-Chip-Society

Fat Grafting I – Breast and BBL

Friday, 11/4/22 - 1:30 - 2:45 pm

Fat grafting to the breast and buttocks, techniques, challenges and results. How does the future look?

Fat Grafting for Reconstruction and Revitalization of the Breast
Kotaro Yoshimura, MD
Professor and Chair
Department of Plastic Surgery
Jichi Medical University
Yakushiji, Shimotsuke, Tochigi, Japan

Stem Cell Enriched Fat Grafting to the Breast
Fred Koelle, MD, PhD, BC Plastic Surgeon
Head Surgeon of Plastic Surgery
Department of Plastic Surgery
CeriX Private Hospital, Denmark

Fat Transfer to the Breast in Southern California
Suzanne Trott, MD, BC Plastic Surgeon
Head Surgeon of Plastic Surgery
Silhouette Cosmetic Center
Beverly Hills, CA

Buttock Reshaping with Fat Contour and Volume
Luisa Magalhães Ramos, MD, PhD
Head Surgeon of Plastic Surgery
LMR Cirurgia Plástica, Portugal

New Perspectives in Fat Grafting to the Breast
Summer E. Hanson, MD, PhD
Associate Professor of Surgery, Director
Plastic and Reconstructive Surgery Research
Chicago, IL

Cell Based Therapies

Friday, 11/4/22 – 4:30 – 5:30 pm

The cell therapy market is growing at a compound rate due to expanded technologies, investment and scholarly advancements. In this panel, we will present new work expanding adipose-therapeutics in novel clinical arenas.

Optimizing Kidney Repair through Adipose-Tissue Derived Mesenchymal Stromal Cell Therapy
LaTonya J. Hickson, MD
Chair of Nephrology and Hypertension at Mayo Clinic

Getting Cells in the Right Mood (and Mode): BonoFill and MesenCure as Examples for Adipose Stromal Cells' Professionalization Enhancing their Therapeutic Utility
Tomer Bronshtein, PhD
VP Business Development
Bonus BioGroup

Defining New Criteria for Adipose-derived Stromal Vascular Fraction Use: A Multicentric Experience in Marseille and Lugano
Jeremy Magalon, PharmaD, PhD
Medical Biologist in Cell Therapy
Marseille La Conception University Hospital
Aix Marseille University, France

Adipose Tissue In Cancer

Saturday, 11/5/22 – 1:00 – 2:15 pm

Adipose tissue is an endocrine organ that produces a large number of hormones and paracrine factors. Obesity results from hyperplasia and hypertrophy within the adipose tissue, as a result of the excessive accumulation of adipose tissue. The functional alterations in the adipose tissue are a confounding contributing factor to many diseases, including cancer. The increased incidence and aggressiveness of several cancers, including colorectal, postmenopausal breast, endometrial, prostate, esophageal, hematological, malignant melanoma, and renal carcinomas, result from obesity as a contributing factor. There is compelling evidence that endocrine and paracrine signals from adipose tissue contribute to cancer progression. This session is devoted to informing the attendees on the interplay between adipose tissue, adipose stem cells or extracellular matrix as factors driving cancer progression.

The Interplay Between Adipose Stem Cells and Breast Cancer
Bruce A Bunnell, PhD
Professor & Chair
Dept. of Microbiology, Immunology, and Genetics
University of North Texas Health Science Center

Adipose Tissue in Cancer and Other Aging-related Diseases
Mikhail Kolonin, PhD
Professor & Director, Center for Metabolic and Degenerative Diseases
Harry E. Bovay, Jr. Distinguished University Chair in Metabolic Disease Research
University of Texas Health Science Center at Houston

Evaluation of Stromal Age in Breast Cancer
Elizabeth Martin, PhD
Assistant Professor
Department of Biological And Agricultural Engineering
Louisiana State University

Mechanical Properties of Adipose Tissue and Their Relevance to Cancer
Claudia Fischbach, PhD
Stanley Bryer 1946 Professor of Biomedical Engineering
Director of Cornell's Physical Sciences Oncology Center (PSOC) on the Physics of Cancer Metabolism, Cornell University

Fat Grafting II – Face

Saturday, 11/5/22 - 2:15 - 3:15 pm

Fat grafting to the face, techniques, challenges and results. How does the future look?

Face Bio Remodeling Using Stromal Enriched Lipograft
Aris Sterodimas, MD, Msc, PhD, BC Plastic Surgeon
Head Surgeon of Plastic Surgery
Plastic & Reconstructive Department
Metropolitan General Hospital
Athens, Greece

How PRP Can Improve Fat Grafting to the Face
Ali Modarressi, PhD
Professor and Chair
Department of Plastic Surgery
University Hospitals of Geneva, Switzerland

Stem cell enriched fat grafting to the face
Fred Koelle, MD, PhD, BC Plastic Surgeon
Head Surgeon of Plastic Surgery
Department of Plastic Surgery
CeriX Private Hospital, Denmark

How to Use SVF, PRP, and PRS in Facial Surgery and 17 Points of Pan-Facial Fat Grafting
Chia Chi Kao, MD, BC Plastic Surgeon
Founder, KAO Regenerative Therapies

Adipose Therapeutics in Musculoskeletal Disorders

Saturday, 11/5/22 – 3:45 – 5:00 pm

Adipose tissue-derived stem/stromal cell preparations for cartilage and bone applications have found their way to the clinic. In this panel, it will be described with expert input from key opinion leaders in the field which challenges, pitfalls, and solutions have been associated with these clinical implementations. Also, it will address new concepts and strategies for adipose tissue-mediated musculoskeletal regeneration, and will feed discussions on the role of adipose tissue in bone marrow, and how this may possibly affect how regulators may look at homologous and non-homologous use.

Adipose-derived Cells for Bone Regeneration: Bone (pre)Fabrication and Developmental Engineering
Arnaud Scherberich, PhD
Professor, Research Group Leader Bone regeneration, Department of Biomedicine, University of Basel, Basel, Switzerland

Orthopedic Clinical Trials for Biologics – Considerations and Issues for Phase I to Pivotal Studies
William W. Cimino, PhD
CEO at GID BIO, Inc., Louisville, Colorado

Adipose Tissue for Craniofacial Bone Regeneration: Enzymatic Digestion or Microfragmentation?
Marco N. Helder, PhD
Associate Professor, Head Stem Cell & Nanotechnology Lab, Co-head Bone and Stem Cell lab, Department of Oral & Maxillofacial Surgery/Oral Pathology, AMS-Tissue Function & Regeneration, Amsterdam Bone Center, Amsterdam UMC-location VUMC and ACTA Amsterdam, Amsterdam, The Netherlands

Adipose Tissue in Bone Marrow: Friend or Foe?
Nathalie Bravenboer, PhD
Assoicate Professor, Head Bone and Calcium Metabolism Lab, Co-head Bone and Stem Cell lab, Department of Clinical Chemistry, AMS-Tissue Function & Regeneration, Amsterdam Bone Center, Amsterdam UMC-location VUMC, Amsterdam, The Netherlands

Industry Roundtable

Saturday, 11/5/22 – 5:00 – 6:15 pm

The market space for commercializing adipose-derived therapeutics is only beginning to blossom and many clinical needs remain unmet. In this panel, we will take a deep dive into the challenges and opportunities in product translation so as to enable potential entrepreneurs to consider paths left untaken. Panelists will discuss their experiences in the field of adipose therapeutics and provide point-of-view toward future opportunities and major adoption barriers.

Joseph Candiello, PhD
Senior Product Manager
RoosterBio, Inc

Marc Fauerby
Chief Commercial Officer, StemMedical

Byong Cho
Chief Executive Officer, ExocoBio Inc

James J. Hickman, PhD
University of Central Florida, NanoScience Technology Center
Professor of Nanoscience Technology, Chemistry, Biomolecular Science and Electrical Engineering
Hesperos, Inc., Chief Scientist-Hesperos

Insight Views into Translation and Regulatory Affairs

Sunday, 11/6/22- 2:00 – 3:00 pm

1. Provide an update on the legal matters pertaining to the US FDA regulation of cell therapies, as well as commentary and discussion related to the implications of such;
2. Provide insights and stimulate discussion regarding the FDA/regulatory experience of an emerging cell therapy company currently in the midst of a Phase 3 pivotal trial; and
3. Provide insight and guidance into the important aspects and considerations of translating novel technologies from the perspective of US Academia as well as Big Pharma;

Adam J. Katz, MD, FACS - *Moderator*
Director, Plastic Surgery Research Laboratory
Michael J. Morykwas Endowed Professor of Plastic and Reconstructive Surgery
Professor, Department of Biomedical Engineering
Professor, Wake Forest Institute of Regenerative Medicine

When Cells, Regulators & Courts Collide: An Update on FDA and FTC Enforcement Efforts
Mary Ann Chirba, J.D., D.Sc., M.P.H.
John C. Ford, S.J., Distinguished Scholar
Boston College Law School

Clinical Studies for Cellular Therapies and the FDA: Phase I to Phase III
William W. Cimino, PhD
CEO at GID Bio, Inc., Louisville, Colorado

Translating Novel Technologies within the University: Musings of an Academic-corporate Chimera
Ed Pagani, PhD
Duke University
Assistant Vice President, External Partnerships
Executive Director, Office for External Partnerships

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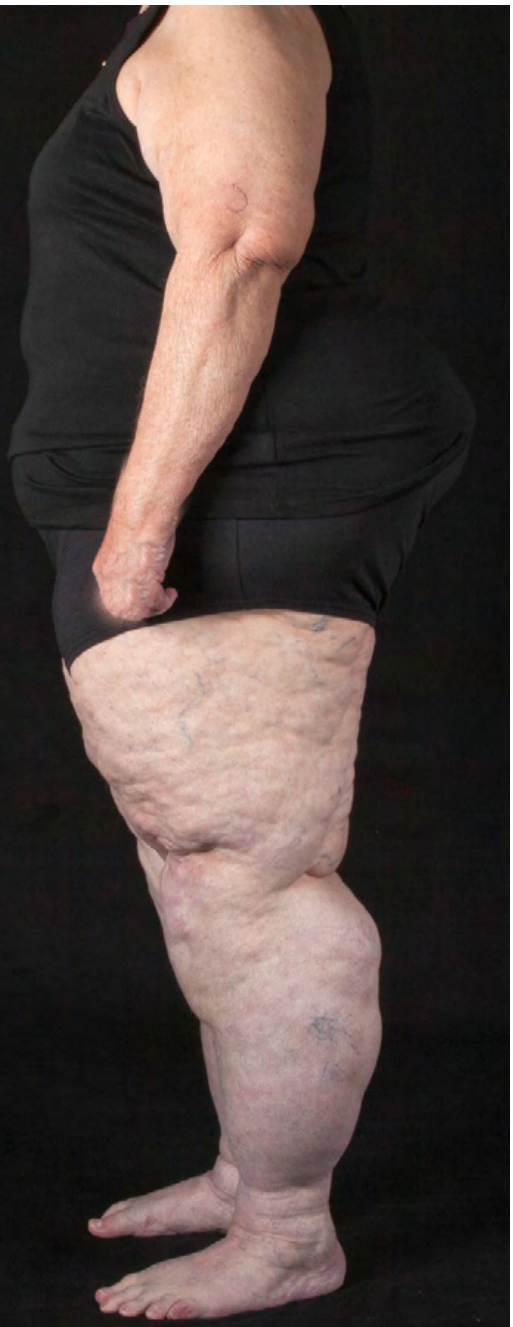

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ABSTRACTS

ABSTRACT 1

Human-Derived Hydrogels to Support Stem Cell Derived Organ on a Chip

Presenter: Cecilia Sanchez, PhD (USA)
Affiliation: Obatala Sciences
Authors: Cecilia Sanchez, Trivia Frazier, Xiying Wu, Jeffrey Gimble

INTRODUCTION: Hydrogels are three-dimensional scaffolds used as alternatives to *in vivo* models for the understanding of human disease. They can also serve as a delivery system for cells and drugs, and as a system for drug screening, reducing the need for animals in preclinical studies. Here we are presenting three human-derived hydrogels – alternatives to current murine-derived and synthetic recombinant hydrogels – with unique physiochemical, biochemical, and biological properties to support stromal vascular fraction and adipocyte differentiation.

METHODS: Three thermoregulated hydrogels were developed from decellularized tissues through a series of processes optimized by Obatala Sciences. The human-derived scaffolds were characterized using rheological studies to determine the viscosity, stiffness, and gelation features. Electron microscopy, proliferation, and differentiation studies were performed on stromal vascular fraction and stromal progenitor cells.

RESULTS: Rheology analyses for stiffness, viscosity, and gelation time demonstrated a potential use of hydrogels as scaffolds for 3D culture. Porosity was studied with scanning electron microscopy (SEM) images (Fig 1), and the biochemical characterization provided differential levels of glycosaminoglycans and protein content. Manufacturing processes were optimized to reduce lot-to-lot variability. Biocompatibility studies demonstrate a significant rate of proliferation and differentiation of the stromal vascular fractions and Adipose Stem Cells (ASC), supporting the role of human-derived hydrogels as a scaffold to promote differentiation into adipose and bone tissue. Moreover, our studies demonstrate the potential of the hydrogel to provide a metabolic responsive environment, providing a platform for drug screening against metabolic disorders.

CONCLUSION: This study provides the characterization of three hydrogels for 3D culture, for drug screening, patient derived xenograft, organ on chip devices, and bioprinting.

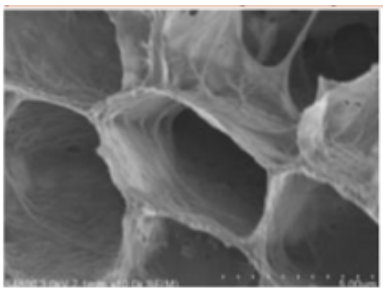


Figure 1

ABSTRACT 2

Investigation of Hydrogel Composition for Superior Adipose Stem Cell Spheroid Adipogenesis

Presenter: Charles Amurgis (USA)
Affiliation: University of Pittsburgh Medical Center Department of Plastic Surgery
Authors: Charles Amurgis, Lindsey Huff, Lauren Kokai, Rosalyn Abbott, Vincent Nerone, Shawn Loder, Elizabeth Johnston

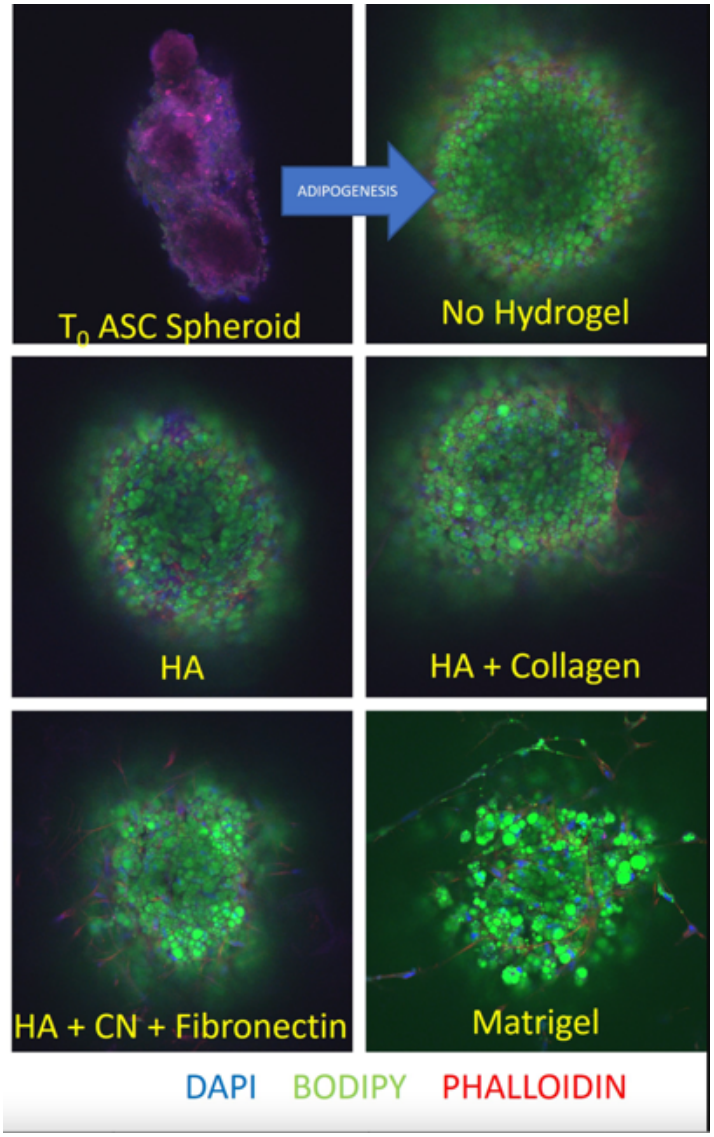
INTRODUCTION: The most used approach for conducting in vitro adipocyte studies employs cell lines or primary human cells differentiated to adipocytes in 2-D. Inherent limitations of this method include lack of complete differentiation into unilocular spheroids and inauthentic matrix and cell-to-cell spatial organization. Further, while 2-D adipogenesis replicates acute phenotypes directed by transcription factors, post-translational modifications that define mature adipocyte gene regulation are lacking. To overcome these challenges while maintaining logistical flexibility of using cryobanked cells, we induced generated spheroids of primary adipose stem cells, however adipogenesis throughout the organoid was incomplete. The goal of this study was to determine if hydrogels containing developmentally appropriate extracellular matrix cues would enhance the architecture of the resulting adipocyte-laden spheroid.

METHODS: Human adipose tissue was obtained from elective body contouring procedures under IRB exemption. The tissue was enzymatically digested and nucleated cells were FACS sorted to isolate CD45-CD31-CD34+ ASCs for culture expansion in EGM media (Lonza). 50,000, passage 2 cells were then suspended in 150 µL in a 96-well low adhesion plate. Spheroids formed after 4 days, at which time they were left free-floating or embedded into the following hydrogels: Hyaluronic Acid (HA), HA-Collagen, HA-CN-Fibronectin, or Matrigel. Adipogenesis occurred over 21 days after which spheroids were fixed and imaged with fluorescent microscopy.

RESULTS: Embedding ASC spheroids in hydrogels prior to inducing adipogenesis increased the complexity and degree of lipid accumulation in resulting adipose organoids. While all adipogenic treated spheroids yielded lipidladen cells, those cultured without supportive hydrogels had numerous multi-locular cells on the perimeter and sparse BODIPY staining within the core. HA hydrogels appeared to repress lipid accumulation, yielding spheroids with higher nuclei-BODIPY ratio compared to controls. Hydrogels with adhesion-related matrix factors such as collagen and fibronectin increased the complexity of spheroids, producing more robust lipid accumulation with dispersed and interwoven elongated stromal elements between adipocytes, resembling primitive fat pads. Finally, Matrigel, which contains high concentrations of laminin, yielded adipocytes with the largest overall lipid vacuoles and complex architecture with interwoven stromal cells.

CONCLUSION: Generating de novo adipose organoids from ASCs with relevant architecture appears feasible. Functional testing is underway.

ABSTRACT 2 (images)



ABSTRACT 3

Comparison of Different Advanced 3D Adipose Tissue Models Based on Spheroids, Hydrogels Containing ASC, or Mature Adipocytes with a 2D Monolayer and Explanted Lobules

Presenter: Franziska Albrecht, M.Sc. (Germany)
Affiliation: Reutlingen University
Authors: Franziska Albrecht, Svenja Nellinger, Freia F. Schmidt and Petra J. Kluger

INTRODUCTION: Various sophisticating adipose tissue models exist in literature, showing the flexibility of suitable cell sources and materials for adipose tissue engineering. Nevertheless, a classification of the respective cell behavior to the in vivo situation is important in order to make predictions regarding the transferability and reliability of the gained results. To start in this direction, we compared three different adipose tissue models with a 2D monolayer and an ex-vivo lobule to evaluate their viability, behavior, and function.

METHODS: Human primary adipose-derived stem cells (ASCs), adipocytes, and lobules were isolated from skin biopsies. ASC were cultured as a monolayer, formed into a spheroid (10.000 cells), or encapsulated in 1% gellan gum (GG) hydrogels, differentiated (two weeks), matured (varying), and finally, for three more weeks maintained. Adipocytes were combined with 0.5% GG to achieve cellular hydrogels, lobules were used unprocessed directly after isolation, and both were maintained for three weeks. Prior to RT-qPCR, RNA was isolated with TRIzol™-phenol precipitation and transcribed into cDNA.

RESULTS: After adipogenic differentiation of ASCs (diffASCs) and during maintenance, cells accumulated lipids. For diffASCs in spheroids and hydrogels, it is visible that multiple cells express an univacuolar morphology while they remain multivacuolar for the monolayer (figure 1). On the other hand, adipocyte hydrogels and lobules showed an univacuolar morphology right at the beginning, which was stable for the maintenance phase. Comparing the different models has demonstrated that the cells exhibited distinct behaviors regarding morphology, size, and function, as also shown in the RT-qPCR.

CONCLUSION: In this work, we succeeded in establishing various tissue models from different primary cells from human adipose tissue. Comparison has shown that, depending on the cell type and model, the functional maximum is at another point in time, and thus, the comparability to the native state varies. This suggests that the models should be chosen according to the research question and the intended purpose.

ABSTRACT 3 (images)

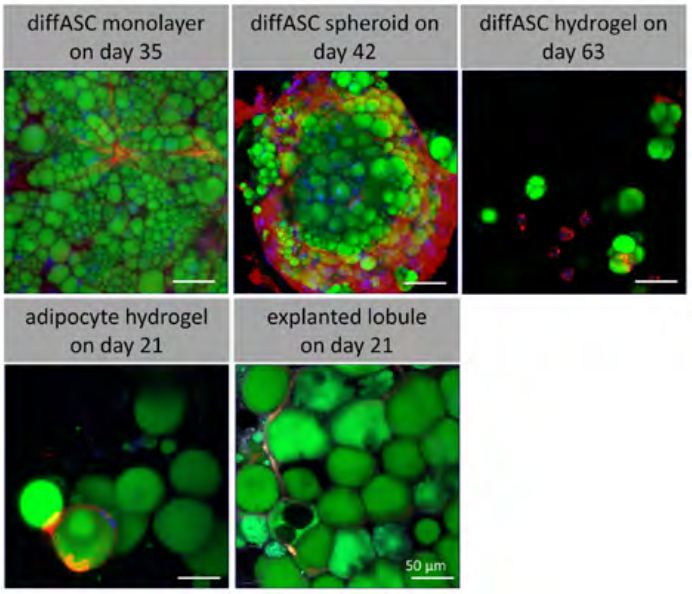


Figure 1: comparison of different adipose tissue models at the latest time point of maintenance. Lipids stained in green by BodiPY, actin stained in red by rhodamine-conjugated phalloidin, and nuclei counterstained with Hoechst in blue. Scale bar 50 µM

ABSTRACT 4

3D Breast Cancer Microtumors to Investigate the Expression of Tumor-Associated Markers Mediated by the Direct Interaction between ASCs/Adipocytes and Breast Cancer Cells

Presenter: Martin Watzling (Germany)
Affiliation: University Hospital of Würzburg
Authors: Watzling, M, Klaus L, Horder H, Blunk T, Bauer-Kreisel P

INTRODUCTION: The tumor microenvironment has recently been recognized as an important factor in tumor development. In breast cancer, adipose-derived stromal cells (ASCs) and adipocytes as integral parts of the mammary fat pad are important players in creating an environment favorable for tumor progression. To adequately mimic cell-cell interactions between tumor and adjacent stromal cells, conventional 2D cultures may prove insufficient. Therefore, in this study, a 3D microtumor model was developed based on spheroids consisting of ASCs or adipocytes and breast cancer cells to investigate the expression of contact-mediated markers, particularly CCL5/RANTES.

METHODS: 3D microtumors consisting of ASCs or adipocytes and breast cancer cells (MDA-MB-231, MCF-7) were generated in agarose-micromolds. Expression of tumor-associated markers was investigated in the co-spheroids and compared to mono-cultures with qRT-PCR, ELISA and immunhistochemistry. Using magnetic-activated cell sorting (MACS), the expression of specific markers in the co-cultures could be assigned to the individual cell type. To study the effects of the co-culture on migration of cancer cells, migration assays were performed. Additionally, a PCR array was performed to identify further contact-mediated markers in the co-culture.

RESULTS: Expression of CCL5/RANTES as a tumor-promoting factor was shown to be specifically dependent on direct cell-cell contact of ASCs or adipocytes and triple-negative breast cancer cells (MDA-MB231) with a specific upregulation in the ASC/adipocyte fraction. Conditioned media from co-cultures distinctly enhanced the migration of tumor cells compared to mono-cultures. Expression of CCR1 as a CCL5 receptor was detected on tumor cells, and the addition of a CCR1 inhibitor decreased the migration of tumor cells indicating that the CCL5/CCR1 axis may increase the migratory ability of triple-negative breast cancer cells in direct vicinity to ASCs/adipocytes.

CONCLUSION: Direct cell-cell interaction between ASC or adipocytes and tumor cells as occuring in the tumor microenvironment of the mammary fat pad led to expression of CCL5/RANTES by ASC and adipocytes. Secreted factors from direct co-culture enhanced migration of triple negative breast cancer cells likely in part via the CCL5/CCR1 axis. Such tumor-specific markers up-regulated upon cell-cell contact with adjacent stromal cells may represent promising targets for theranostic applications in detection and treatment of breast cancer.

ABSTRACT 5

Supercritical Carbon Dioxide -foamed Poly(L-lactide-co-ε-caprolactone) Scaffold Embedded with Ascorbic Acid Derivative for Urethral Tissue Engineering: An In-vitro Study

Presenter: Alma Kurki (Finland)
Affiliation: Tampere University
Authors: Alma Kurki

INTRODUCTION: Surgical repair of urethral defects still lacks optimal repair methods. Autologous genital tissue can be used to a limited extent, whereas larger segments require the use of nongenital tissue grafts. These methods have a high risk for complications and suffer from limited donor site availability. Tissue engineering can provide sustainable approach for urethral repair. Cell-seeded scaffolds have been shown to prevent urethral stricture and scarring during urethral regeneration. Moreover, using stromal cells to regenerate functional urethral smooth muscle and stromal layer has been demonstrated to be effective in preventing complications. This study investigated a novel porous supercritical CO2-foamed poly(L-lactide-co-ε-caprolactone) (PLCL) scaffold with embedded specific ascorbic acid derivative (AAD) to support the regeneration of urethral epithelium and surrounding stromal tissue using in vitro mono- and co-cultures of human urothelial cells (hUCs) and adipose-derived stromal/stem cells (hASCs).

METHODS: Monocultures and co-culture of hUCs and hASCs were cultured on supercritical CO2-foamed PLCL scaffolds with (scPLCLas) and without (scPLCL) embedded AAD. During the 14-day assessment period, the effect of AAD on hUC and hASC growth and viability was assessed with Live/dead viability assay, CyQuant proliferation assay, and SEM- imaging. Effect on collagen production was measured with Sircol total collagen assay. Immunofluorescent staining and qRT-PCR were used to evaluate hUC and hASC phenotype and specific marker expression.

RESULTS: Both hUCs and hASCs remained viable on scPLCLas scaffold and retained their phenotypic characteristics. Urothelial cells on both scaffolds expressed their epithelial cytokeratin profile and maturation markers. The AAD in scPLCLas significantly increased the proliferation and collagen production of hASCs, whereas on scPLCL hASCs formed nonproliferating cell clusters. Moreover, hASCs' expression of early myogenic marker αSMA was increased on scPLCLas. In co-culture, the AAD in scPLCLas supported the viability of hASCs notably better when compared to scPLCL even in suboptimal serum-free co-culture conditions.

CONCLUSION: The scPLCLas scaffold maintains the hUC phenotype and enhances hASC proliferation and collagen production, which makes it potential material for urethral tissue engineering in the future. Future research aims to further investigate hUC maturation and hASC myogenic differentiation on scPLCLas scaffolds.

ABSTRACT 6

Biocompatible Hydrogels for Soft Tissue Engineering - Strategies to Promote Vascularization

Presenter: Paul Gatenholm, Kristin Oskarsdotter (Sweden)
Affiliation: Sahlgrenska University Hospital, Gothenburg
Authors: Paul Gatenholm, Kristin Oskarsdotter

INTRODUCTION: Lipofilling or autologous fat grafting, is an excellent technique to repair soft tissue with patient´s own stem cells. The outcome is however unpredictable. Hydrogels, materials composed of a hydrophilic polymer network capable to hold large amounts of water, yet retaining structural integrity, are attractive biomaterials in that they are biocompatible and can be tuned to deliver cells to repair tissue and organs. In contrast to surgically implanted materials, injectable hydrogels can permit minimally invasive delivery procedures that improve patient outcomes and lower costs for hospitals. We have studied use of injectable hydrogels to deliver stem cell rich micro-fat.

METHODS: Nanocellulose isolated from tunicates (TUNICELL), a novel emerging biomaterial from Ocean Tunicell AS (Norway) and alginate (Pronova SLG100) isolated from brown algae by NovaMatrix (Norway); both medical grade were used in experiments. Micro-fat was prepared using human lipoaspirate and Lipogems device from Lipogems (Italy). In situ gelling was evaluated using crosslinked Calcium alginate particles or Calcium ions generated in situ. Micro-fat, immediately after preparation, was mixed with various ratios of hydrogels. Viscosity, in situ gelling and mechanical properties were studies using TA Rheometer DHR3. Selected compositions were evaluated in in vivo study using nude mice model.

RESULTS: Biocompatible hydrogel formulations were mixed with stem cell-enriched micro-fat isolated from human lipoaspirates. Hydrogel formulations and the ratios between lipoaspirate, TUNICELL and alginates were varied to tune rheological properties affecting injectability. Attention was paid to mechanical properties after crosslinking. Storage moduli was evaluated using a TA rheometer. In situ gelling was tuned by selection of charged TUNICELL, alginate compositions and in situ Calcium ion release. Cell viability was studied in vitro after injection in soft tissue models and in ex vivo soft tissue. Mixing of stem cell enriched micro-fat with biocompatible hydrogels resulted in homogenous and shape formable injectable gels.

CONCLUSION: We have shown that we can tune the injectability and in situ gelling properties of micro-fat by combining it with biocompatible marine biopolymeric hydrogels; tunicate nanocellulose and alginate. This system is promising candidate for soft tissue repair.

ABSTRACT 7

Engineered Fat Graft Enhanced with Adipose-Derived Stromal Vascular Fraction Cells for Breast Augmentation and Reconstruction: Clinical, Histological and Instrumental Evaluation

Presenter: Pietro Gentile (Italy)
Affiliation: University of Rome
Authors: Pietro Gentile

INTRODUCTION: Fat graft enhanced with adipose-derived stem cells (FG-e-ASCs) has been utilized in outcomes of radiotherapy after mastectomy, breast soft tissue defects, ulcers, and loss of substance. The authors present their experience using FG-e-ASCs in breast augmentation and breast reconstruction. The aim of this study was to evaluate the safety and efficacy of a study group (SG) regarding the use of FG-e-ASCs in breast augmentation for aesthetic improvement, comparing the results with a control group (CG) and at the same time the safety and efficacy of a study group (SG-1) regarding the use of FG-e-ASCs in breast reconstruction, comparing the results with a control group (CG-1).

METHODS: A total of 46 patients affected by breast hypoplasia were treated with FG-e-ASCs, comparing results with those of a CG (n = 30) treated with fat graft not enhanced with adipose-derived stem cells (FG-ne-ASCs). The preoperative evaluation included a complete clinical evaluation, a photographic assessment, magnetic resonance imaging (MRI) of the soft tissue, ultrasound (US), and mammography (MG). Postoperative follow-up took place at 1, 3, 7, 12, 24, and 48 weeks, and then annually.
121 patients (SG-1) that were affected by the outcomes of breast oncoplastic surgery were treated with FG-e-ASCs, comparing the results with the CG-1(n = 50) treated with FG-ne-ASCs. The preoperative evaluation included a complete clinical examination, a photographic assessment, biopsy, magnetic resonance (MRI) of the soft tissue, and ultrasound (US). Postoperative follow-up took place at two, seven, 15, 21, 36 weeks, and then annually.

RESULTS: The patients treated, for breast augmentation, with FG-e-ASCs showed 58% maintenance of the contour restoring and of 3-dimensional (3D) volume after 3 years compared with the patients of the CG treated with FG-ne-ASCs, who showed 29% maintenance. In 67.4% (n = 31) of breast augmentation treated with FG-e-ASCs, we observed a restoration of the breast contour and an increase of 10.3 mm in the 3D volume after 36 months, which was observed in only 20.0% (n = 6) of patients in the CG treated with FG-ne-ASCs. Volumetric persistence in the SG was higher than that in the CG (P < .0001 SG vs. CG).
In 72.8% (SG-1 n = 88) of breast reconstruction treated with FG-e-ASCs, we observed a restoration of the breast contour and an increase of 12.8 mm in the three-dimensional volume after 12 weeks, which was only observed in 27,3% (n = 33) of patients in the CG-1 that was treated with FG-ne-ASCs. Transplanted fat tissue reabsorption was analyzed with instrumental MRI and US. Volumetric persistence in the SG-1 was higher (70.8%) than that in the CG- 1 (41.4%) (p < 0.0001 vs. control group).

CONCLUSION: The use of FG-e-ASCs was safe and effective in this series of cases performed.

ABSTRACT 8

Clinical Applications of MEST & ARAT

Presenter: Hasim Eray Copcu (Turkey)
Affiliation: Gene, Cell and Tissue Academy
Authors: Hasim Eray Copcu

INTRODUCTION: Obtaining stromal cells from adipose tissue using sharp blade systems and resizing adipocytes in different diameters in fat graft applications is a revolutionary approach. Mechanical stromal cell transfer using ultra-sharp blades was called MEST, and regenerative transfer of adipocytes by resizing was called ARAT. In this study, the 5-year clinical results of this method were reviewed and presented in the light of the literature.

METHODS: In this study, using ultra-sharp blade systems, ARAT technique, which is both a new paradigm, was used to obtain adipocytes (4000, 2400, 1200i 600, 400 and 100 micron) in different anatomical areas and depths in face, body and breast regions. At the same time, this system was used for regenerative purposes by obtaining stromal cells by mechanical methods defined as MEST. ARAT and MEST procedures were applied to a total of 334 cases. Their early and long-term results are presented in this study.

RESULTS: With the ARAT technique, adipose tissue can be applied to different anatomical areas and depths in 2400, 1200 and 600 micron thicknesses in the face area, 100 micron thickness in hair, 400 micron thickness in penile girth surgery and female beautification, 4000, 2400 and 1200 micron thickness in different depths of the breast and other areas of the body. Different thicknesses of adipose tissue were obtained and used with success. MEST, that is, the last products obtained for regenerative purposes, apart from hair, face and skin regeneration, have been successfully used in the treatment of erectile dysfunction, Peyronie's disease, congenital anomaly, OA, aseptic necrosis, scar treatment, plantar fasciitis, urinary incontinence, RT injury, vocal cord paralysis. used.

CONCLUSION: The use of ultra-sharp blades provides more natural and permanent results in fat graft applications. The main reason for this can be explained both by obtaining the most appropriate adipocyte size according to the appropriate anatomical area and depth, and by liberating the stromal cells, enabling them to transform from quiescence mode to regenerative mode, resulting in greater permanence. Similarly, with different modifications (such as IPs, supercharged) in the MEST method, 5-7 more cells than normal and a much wider variation of stromal cell types can be obtained.

ABSTRACT 9

Cell Enriched Lipotransfer (CELT), A New Technology to Increase Autologous Fat Grafting by Stem Cell Enrichment to Accelerate Tissue Healing and Regeneration in Humans

Presenter: Lukas Prantl (Germany)
Affiliation: University Hospital Regensburg
Authors: Lukas Prantl, Andreas Eigenberger, Oliver Felthaus

INTRODUCTION: Fat grafting has become one of the most frequent procedures in surgery. Different studies have shown the safety and efficacy of lipofilling to treat volume loss in breast and other body regions. A remaining problem is the unpredictable engraftment rate of the transplanted tissue caused by insufficient vascularization and impaired adipocyte viability. Through Cell Enriched Lipotransfer (CELT), a procedure we developed for the enrichment of the Stromal Vascular Fraction and Adipose Derived Stem Cells, we aim to increase the take rate of grafted fat tissue.

METHODS: We developed a method for objective volume measurements in anatomic structures to record volume changes after breast lipofilling reliably in the clinical setting. Furthermore, the wrinkle depth was evaluated after wrinkle treatment. Additionally, CELT-tissue and control tissue was digested enzymatically to evaluate cell enrichment. Furthermore, the cells from CELT-tissue and control tissue were compared regarding cell vitality, proliferation, differentiation potential and gene expression.

RESULTS: Utilizing CELT much higher take rates of up to 90% can be achieved even for an extended period of time. The cells from CELT-treated tissues do not show any impairment in vitality, proliferation, differentiation potential or gene expression.

CONCLUSION: Cell Enriched Lipotransfer is a simple and effective procedure for improvement of take rates in autologous fat grafting, presumably through improved vascularization and the proliferative and immunomodulatory properties of adipose tissue derived stem cells. The method also allows for further applications in osteoarthritis, tendinitis and incontinence.

ABSTRACT 10

A Potential Two-Step Mechanism for the Generation of Fat Emboli

Presenter: William Molair (USA)
Affiliation: Wake Forest University
Authors: William Molair, Adam Katz, Ramon Llull

INTRODUCTION: Fat emboli are a poorly-understood, rare, and devastating complication most commonly associated with liposuction and gluteal fat grafting. To date, no animal models have consistently induced pulmonary fat emboli outside of direct injection of lipids into large vessels. Here, we present a novel model of fat emboli that mirrors clinical manifestations and a potential mechanism behind the generation of fat emboli in both this model and clinical practice.

METHODS: C57BL6 mice were injected with either NS, human oil, emulsified human oil, sunflower oil, or pristane. Mice were monitored daily with weekly paracentesis starting 5 days after initial injection. Any mice that died were autopsied with histological sections taken of their lungs, livers, spleen, and intra-abdominal lymph nodes. Histological sections were stained with either Hematoxylin and Eosin (H&E) or Oil Red O to determine if oil is present.

RESULTS: Of 8 mice injected with emulsified human oil, 3 died within 72 hours of paracentesis. No deaths were observed in either human oil or NS control groups. One mouse died in the sunflower group immediately post-paracentesis. Oil was found within the abdominal lymphatic chain (Figure 1) and within the lungs (Figure 2) of deceased emulsified oil mice.

CONCLUSION: Fat emboli is a rare, but feared complication of procedures involving fat displacement. Based on the findings above we propose a two-stage mechanism of emulsification of liberated oil, followed by generation of a pressure gradient that causes fat uptake into local vessels, including lymphatics. Emulsified human oil not only generated lethal, histologically confirmed fat emboli, but also was found throughout the lymphatic system of the mice post-mortem. In contrast, non-emulsified human oil did not generate lethal fat emboli in C57BL6 mice. We find that the emulsification with a subsequent intra-cavity pressure gradient, a potential consequence of both liposuction and gluteal fat grafting, is a sentinel event for the formation of a clinically relevant pulmonary emboli. Further research should be performed to understand the threshold pressure and concentrations of emulsified lipid that may precipitate lipid absorption into local vasculature.

ABSTRACT 10 (images)

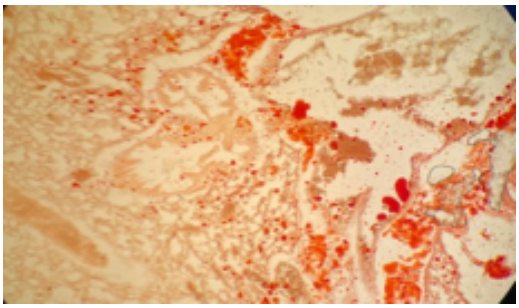


Figure 1. Oil Red O stain of Lung Tissue. Lungs from a mouse that died within 48 hours of paracentesis were harvested, fixed with OCT, and stained using Oil Red O. The lungs of this mouse were showered with oil, likely from the oil emulsification that was placed into its peritoneum.

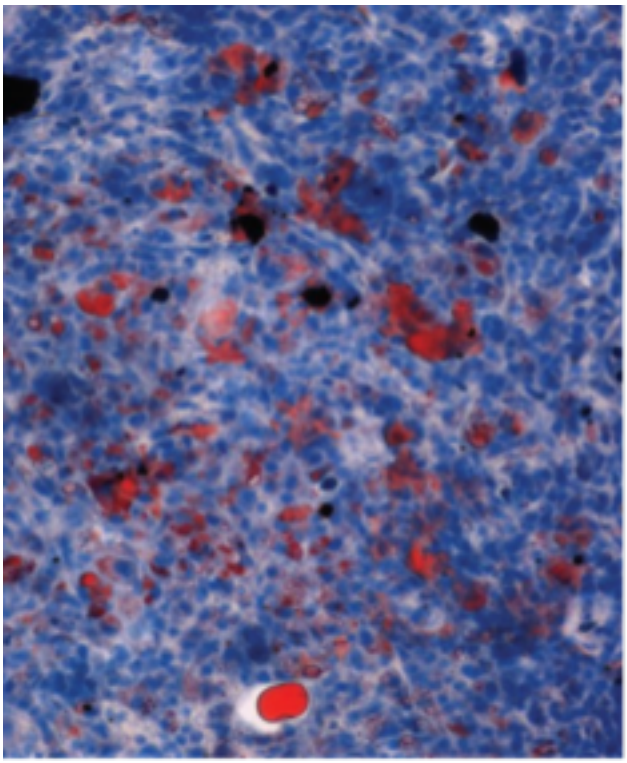


Figure 2. Post-mortem Image of the abdominal lymphatics. After death, emulsified oil mice had lymphatics that were filled with oil. These high concentrations of oil could have contributed to their eventual demise from fat emboli.

ABSTRACT 11

Designing an Explorative Clinical Trial Investigating Multiple Applications of Adipose-derived Stem/Stromal Cells? Our Clinical Experience and Suggestions

Presenter: Jesper Jensen (Denmark)
Affiliation: StemMedical A/S
Authors: Frederik Penzien Mamsen, Lea Munthe-Fog, Jesper Dyrendom Svalgaard, Jesper Due Jensen, Thomas Hartvig Lindkær Jensen, Anne Fischer-Nielsen, Mikkel Taudorf, Bo Jønsson, Adam J. Katz, Stig-Frederik Trojahn Kølle

INTRODUCTION: An explorative clinical trial must be carefully designed to ensure that the results leave more solutions than unanswered questions. It is important to acknowledge that studies are time consuming and expensive. In our opinion traditional study methods may sometimes be inadequate for generating relevant data or even impossible or unethical to perform. This presentation introduces a novel clinical study design that we believe can provide valuable clinical data in a safe and elegant way for indications that are.

METHODS: Five patients fit for an abdominoplasty received eleven unique treatments in the skin and fatty tissue of the abdominal region. Three indications for adipose-derived stem/stromal cell (ADSC)-enriched fat grafts w/o collagen were investigated: soft tissue augmentation, skin rejuvenation and wound healing. The abdominal tissue determined for resection served as a canvas for treatments. This allowed the investigators to tattoo the skin of the patients to precisely locate the treated areas for sampling after the treatment. The tattoos further served as guide for measurement of volume, as nada needles were inserted along the tattoos prior to CT scanning. The high material density of the needles made it easy to assess the volume of tissue underneath the skin of that exact area. Each patient received the same 11 treatments and thus served as their own control. The location of the soft tissue- and dermal treatments respectively were allocated using two separate randomizations to minimize potential bias. Three months after the injections, biopsies were taken to assess the effect of treatments on the skin. The wounds from the biopsies further served as wound bed for healing assessment minimizing the number of biopsies and reducing patient discomfort. The patients carried the interventional treatments for six months whereafter the whole area of treatments was removed by an abdominoplasty.

RESULTS: No patients withdrew from the trial, and all reported the trial to be manageable and worth the outcome. The measured outcomes were all of good quality in terms of volume assessment by CT scans and histological assessments.

CONCLUSION: We believe this study design can be used for various indications, were the investigator wants to assess outcomes of multiple implantable- or injectable solutions pared for creating studies with higher evidence level.

ABSTRACT 12

Tissue Structure and Retention Rate of Adipose-derived Stem/Stromal Cell-enriched Fat Grafts w/o Collagen: An Explorative, Randomized, Controlled, Triple Blind, Paired, Clinical Trial

Presenter: Frederik Mamsen (Denmark)
Affiliation: StemMedical A/S
Authors: Frederik Mamsen, Lea Munthe-Fog, Jesper Dyrendom Svalgaard, Jesper Due Jensen, Thomas Hartvig Lindkær Jensen, Anne Fischer-Nielsen, Mikkel Taudorf, Bo Jønsson, Adam J. Katz, Stig-Frederik Trojahn Kølle

INTRODUCTION: Advancements in fat grafting include the addition of regenerative cells to increase volume retention. Our research team have previously published the results from a clinical study assessing the effect of adding adipose-derived stem/stromal cells (ADSCs) to fat grafts for cosmetic breast augmentation resulting in >80% graft retention This is a significant improvement compared to the reported volume retention of conventional fat grafts. With this study we want to confirm our previous findings using ADSC-enriched fat grafts, and to get a better understanding of the tissue composition in the grafts. Furthermore, we hypothesize that the fat graft retention and composition may be even further improved with the use of a collagen scaffold.

METHODS: This explorative study investigates the histological changes of fat grafts enriched with ex-vivo expanded adipose-derived stem/stromal cells (ADSCs) and collagen as well as the volume retention of the grafts. Five patients underwent liposuctions for the isolation and expansion of ADSCs. The patients received three injections into the subcutaneous tissue of the abdomen comprising of fat, fat enriched with ADSCs, and fat enriched with ADSCs and collagen. Prior to the intervention, the patients were tattooed on the skin above the treated areas to localize the injections at follow-up. The patients received needle marked CT-scans to evaluate the retention rates. At six months the patients had a cosmetic abdominoplasty to remove the treated areas including the tattoos. The excised tissue was histologically analyzed to examine the structure and components of the grafts.

RESULTS: The result from the study is still pending. The initial visual inspection of the fat grafts was performed after the abdominal tissue was resected during the abdominoplasty and after fixation at the department of pathology. A clear height difference was observed between the three treated areas, but due to blinding and randomization it is not possible to conclude which samples were injected into which area. The histological examination has not been analyzed but it will be completed in time for the conference.

CONCLUSION: We expect that the ADSC-enriched fat grafts w/o collagen have a better fat graft retention compared to conventional fat grafts.

ABSTRACT 13

Gellan Gum as Promising Material for Manually and Additively Manufactured Long-term Stable and Functional 3D Adipose Tissue Models

Presenter: Petra Kluger (Germany)
Affiliation: Reutlingen University
Authors: Vera Dolderer, Svenja Nellinger, Freia Schmidt, Petra Kluger

INTRODUCTION: Adipose tissue fulfills important functions besides energy storage, it protects internal organs, and secretes numerous hormones, influencing the whole body's metabolism. Setting up in vitro models with long-term stability is still challenging due to the lack of suitable biomaterials and adequate adipogenic maturation. Due to its biological comparability and easily tunable material properties, we chose the bacterial exopolysaccharide Gellan Gum (GG) to create manual and bioprinted functional and long-term stable human adipose tissue models.

METHODS: Human primary adipose-derived stem cells (ASCs) were isolated from skin biopsies, encapsulated in 1% GG, adipogenically differentiated, and cultured for up to 98 days. Further, bioprinted and non-bioprinted models were compared regarding viability and function.

RESULTS: To achieve a stable and processable bioink with sufficient printing resolution, adding divalent ions into the GG-solution was necessary (figure 1A). Adipocytes, as the characteristic cell of adipose tissue, exhibit a roundish, univacuolar cell morphology and secrete hundreds of adipokines. The cells used for in vitro models should resemble adipocytes as closely as possible. As shown in figure 1, encapsulated and differentiated ASC are viable for 42 days after manual setup and bioprinting. They exhibit a roundish, univacuolar morphology, displaying their similarity to adipocytes. As the amount of lipid-positive and univacuolar cells increase, it can be concluded that the GG hydrogels successfully support adipogenesis. The intact lipid vacuole suggests that the cells are functional. There is no evident difference between printed and manual models, indicating the suitable bioprinting process.

CONCLUSION: Our work showed that GG is a promising material for adipose tissue engineering. Using 1%, GG enabled the setup of non-toxic, non-monocyte activating long-term stable hydrogels. It enabled the culture of viable human primary ASCs for 98 days, successful adipogenic differentiation, and maintenance. The encapsulated cells accumulated significant amounts of intracellular lipids, exhibited an univacuolar morphology, and secreted adipokines, demonstrating the in vivo-close cell state and function. In addition, we established the extrusion-based bioprinting of pre-crosslinked GG, which showed no process-related effects on cell viability or functionality over 42 days compared to manual models.

ABSTRACT 13 (images)

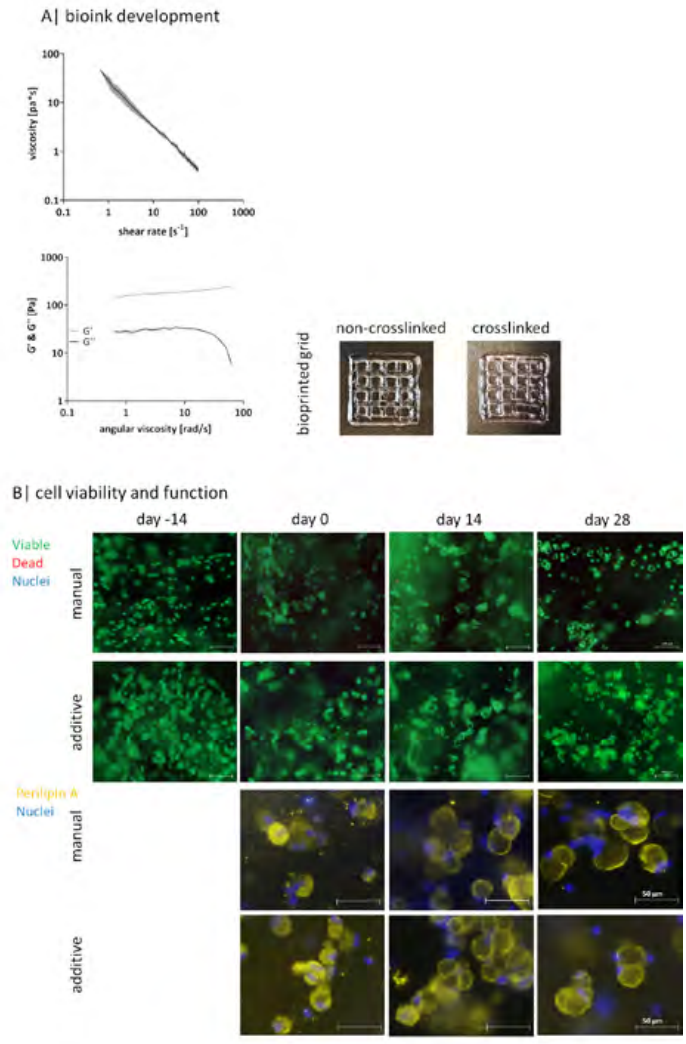


Figure 1: A| viscosity, loss- and storage module of GG bioink and macroscopic images of bioprinted grid structures before and after crosslinking. B| adipogenic differentiated ASCs on days -14, 0, 14, and 28 of manual and additive manufactured models. Live-dead staining, viable cells in green, dead in red and cell nuclei in blue, scale bar 100µm. Anti perilipin A staining on days 0, 14, and 28, perilipin A in yellow, cell nuclei in blue, scale bar 50µm

ABSTRACT 14

Machine Learning-based Predictive Model for Printability and Shape Fidelity in Biofabrication

Presenter: Luciano Vidal (France)
Affiliation: Centrale Nantes
Authors: Vidal, L., Hascoët N, Kavrakova T, Arduengo Garcia J., Chinesta F., Hascoët JY.

INTRODUCTION: Today, hierarchically organized living constructs are developed using additive manufacturing techniques. This emerging technology is named three-dimensional bioprinting.
These biofabricated organized structures are conceived thanks to the use of hydrogels. One of the most critical challenges in bioprinting is the development of bioinks that are suitable for additive manufacturing. These hydrogels are subjected to different physicochemical constraints such as temperature, speed, and pressure during the bioprinting procedure.

METHODS: The bioprinted structure's fidelity can significantly be affected by these constraints. Data was collected through experiments to understand the more significant parameters to support this assumption.. This data was used for conducting a forward machine learning approach. Indeed, an inverse model was developed that allows specifying the machine's operating data according to the dimensions of the desired structure.

RESULTS: A new standard of digital biofabrication is presented to obtain an accurate construct, developing a predictive model using artificial intelligence for microextrusion bioprinting.

CONCLUSION: Considering these previously mentioned physicochemical variables, this model predicts the best parameters to establish in microextrusion bioprinting.

ABSTRACT 15

Developing Next Generation Adipose Tissue Grafts for Soft Tissue Reconstruction

Presenter: Gretel Major (New Zealand)
Affiliation: University of Otago Christchurch
Authors: Gretel Major, Alessia Longoni, Jeremy Simcock, Tim Woodfield and Khoon Lim

INTRODUCTION: Autologous fat grafting has favorable potential as a regenerative strategy to repair large soft-tissue defects. Clinically, there is a limit on the volume of lipoaspirate which can be utilized to repair a soft-tissue defect, as occurrences of graft resorption, tissue calcification and necrosis are proportionate to graft size.

METHODS: Surgical complications are the result of poor graft vascularization and are hindered further by the poor structural fidelity of lipoaspirate as a filling material. This study aims to engineer lipoaspirate-derived adipose tissue grafts with biologically and clinically-admissible structural and functional properties.

Patient-derived lipoaspirate was crosslinked by establishing bonds between extracellular matrix (ECM) proteins within the lipoaspirate material. The degree of crosslinking could be tuned and regulated, with the relative abundance of covalent bonds measured using liquid chromatography-mass spectrometry. Crosslinked lipoaspirate was either extruded for fibre analysis or cast into discs for in vitro culture over 2 weeks. Construct morphology, metabolic activity and tissue function were assessed over the culture period to measure tissue health and survival. To predict patient response, SWATH-MS was used to identify differences in patient ECM and crosslinked grafts were implanted in vivo using a subcutaneous mouse model. Functional vessel formation and resorption were quantified using micro-CT and tissue-remodeling assessed via histology

RESULTS: There was an increase in the relative abundance of covalent bonds present with increasing degree of crosslinking (<10-fold increase). When extruded, crosslinked lipoaspirate had better shape fidelity compared with native lipoaspirate - demonstrated by a 60% reduction in fibre diameter ($p<0.05$). Crosslinked lipoaspirate remained viable and metabolically active over long-term culture at low photoinitiator concentrations. However, high photoinitiator concentrations had a negative effect on the metabolic activity of cells within the constructs after 24 h, with evidence of increased lipolysis. When implanted in vivo crosslinked lipoaspirate demonstrated more predictable resorption profiles (standard deviation reduced by 52%) with functional vessels growing throughout the graft.

CONSLUSIONS: The crosslinking approach described here is tunable and functional across different patient samples. Improving the structural properties of lipoaspirate through minimal manipulation has clinical utility for the delivery of grafts with higher shape-fidelity and therefore increased graft survival when implanted.

ABSTRACT 16

Long-term in Vivo Survival of 3D-bioprinted Human Lipoaspirate-derived Adipose Tissue: Proteomic Signature and Cellular Content

Presenter: Karin Saljo (Sweden)
Affiliation: Department of Plastic Surgery, Sahlgrenska Academy and Sahlgrenska University Hospital
Authors: Karin Saljo , Peter Apelgren, Linnea Stridh Orrhult, Susann Li, Matteo Amoroso, Paul Gatenholm, Lars Kölby

INTRODUCTION: Three-dimensional (3D)-bioprinted lipoaspirate-derived adipose tissue (LAT) is a potential alternative to lipo-injection for correcting soft-tissue defects. This study investigated the long-termin vivo survival of 3D-bioprinted LAT and its proteomic signature and cellular composition

METHODS: We performed proteomic and multicolour flow cytometric analyses on the lipoaspirate and 3D-bioprinted LAT constructs were transplanted into nude mice, followed by explantation after up to 150 days. The constructs were investigated histologically and with immunohistochemistry.

RESULTS: LAT contained adipose-tissue-derived stem cells (ASCs), pericytes, endothelial progenitor cells (EPCs) and endothelial cells. Proteomic analysis identified 6,067 proteins, including pericyte markers, adipokines, ASC secretome proteins, proangiogenic proteins and proteins involved in adipocyte differentiation and developmental morphogenic signaling, as well as proteins not previously described in human subcutaneous fat. 3D-bioprinted LAT survived for 150 days in vivo with preservation of the construct shape and size. Furthermore, we identified human blood vessels (Ku-80 and CD31 positive cells) after 30 and 150 days in vivo, indicating angiogenesis from pre-existing capillaries in the LAT that have survived the 3D-bioprinting process.

CONCLUSION: LAT has a favourable proteomic signature, contains ASCs, EPCs and blood vessels that survive 3D bioprinting and can potentially facilitate angiogenesis and successful autologous fat grafting in soft-tissue reconstruction.

ABSTRACT 17

Functional and Morphological Studies of In Vivo Vascularization of 3D Bioprinted Human Fat Grafts

Presenter: Peter Apelgren (Sweden)
Affiliation: Department of Plastic Surgery, Sahlgrenska University Hospital
Authors: Peter Apelgren, Matteo Amoroso, Karin Säljö, Mikael Montelius, Linnea Strid Orrhult, Mona Engström, Paul Gatenholm, Lars Kölby

INTRODUCTION: 3D bioprinting offers a new possibility to design and biofabricate 3D structures based on autologous fat. Lack of vascularization of larger 3D bioprinted constructs is however one of the limiting factors thereby hampering translation of this technology from bench to bedside. By 3D bioprinting with microfractured fat mixed with nanocellulose-alginate hydrogel, vascularization by the connections of fragments of vessels included in the fat can contribute to vascularization. Aim of the present work was to determine the perfusion and diffusion characteristics in 3D bioprinted fat constructs by MRI and to correlate the perfusion to angiogenesis within the printed constructs.

METHODS: Microfractured human fat from liposuction was printed with tunicate nanocellulose/alginate hydrogel. The constructs (10 x 10 x 3 mm in size) were transplanted in nude mice that underwent longitudinal MRI investigations up to 99 days in vivo.

RESULTS: Before implantation, the constructs contained abundant fat tissue and fragments of human blood vessels (CD31+ and KU80+). In vivo MRI showed that, in general, the perfusion was low in the constructs and they survived mainly by diffusion. The diffusion coefficient was high, around 2 x 10-3 mm2/s, and was preserved throughout the observation time. After explantation, the constructs had a preserved histology. There was a mix of human. (Ku80+) and murine (Ku80-) erythrocyte-containing vessels in the constructs.

CONCLUSION: MRI, histology and immunohistochemistry confirmed vascularization of 3D bioprinted fat constructs. Fragments of blood vessels in the microfactured human fat had interconnected by angiogenesis and formed a vascular network that integrated with the host circulation.

ABSTRACT 18

Comparison of Adipose-derived Stem Cells, The Stromal Vascular Fraction and Prepared Adipose Tissue Grafts in Autologous Fat Transfer

Presenter: Summer Hanson (USA)
Affiliation: University of Chicago Medicine and Biological Sciences
Authors: Summer Hanson , Malke Asaad, Yewen Wu, Cynthia Branch-Brooks, Qixu Zhang, Peiman Hematti

INTRODUCTION: There are multiple methods to prepare lipoaspirate for autologous fat transfer; however, graft retention remains unpredictable. The objective of this study was to compare the cell and protein content of adipose grafts and the stromal vascular fraction (SVF) resulting from three common means to prepare adipose grafts.

METHODS: Adipose grafts were harvested from healthy female donors and processed via three commonly used techniques: centrifugation, a single-filter device (SF, RevolveTM) or a double filtration system (DF, PuregraftTM 250). A portion of each resulting graft was analyzed or further processed to isolate the SVF. Cell viability and surface markers between the graft and SVF were compared. Cytokine and growth factor analysis was performed and included adipose derived stem cells (ASCs) for comparison.

RESULTS: Overall, we found variations across the three processing techniques and among the graft components (ASCs, SVF, fat). Cell viability within the grafts were similar (88.5-90%); however, there was a greater percentage of ASCs in the SVF from SF versus DF or centrifugation (10.1%, 4.3% and 2.8% respectively). Adipogenic markers were similar among all three grafts. VEGF and angiogenin values were higher in the grafts processed by SF compared to DF or centrifugation. C-reactive protein was not expressed in the ASCs but found in similar concentrations in the SVF and grafts across all three techniques while IL-8 had an opposite trend. CD14 was negligible in pure ASCs but comparable in the filtered SVF and grafts; however, there was nearly two-fold higher concentration in the centrifuged grafts with minimal expression in the SVF. Fibroblast growth factor was comparable among all samples and all techniques. Hepatocyte growth factor was similar in the SVF and grafts resulting from filtration devices (SF and DF) but negligible in the SVF and grafts from centrifugation. MMP9 was highest in the SVF from all processing techniques compared to ASCs or fat.

CONCLUSION: While there were many similarities, we identified differences in cytokine expression both in the graft and the associated SVF, particularly in inflammation and wound healing. These secretomes may impact graft retention and fat necrosis in the clinical setting or have implications in cell-assisted lipotransfer.

ABSTRACT 19

Cell Culture Dynamics of Isolated versus Aggregated Populations of Adipose Derived Stromal Fraction: Stromal Aggregates Remain Viable, Lag Proliferation before Exponential Proliferation

Presenter: Mary Duet (USA)
Affiliation: Wake Forest Baptist Health
Authors: Mary Duet, Hulan Shang, Abigail Peoples, William Molair, Adam Katz, Ramon Lllull

INTRODUCTION: Enzymatic and mechanical disaggregation of adipose tissue separates the parenchyma from its stromal cell fraction, rendering an isolated stromal cell population, enzymatic or mechanical stromal vascular fraction (eSVF or mSVF), and aggregated cell populations: the enzymatic (eTR), or mechanical tissue residue (mTR). Contrary to the expected, the tissue residues (TRs) contain high cell densities. We seek to confirm the viability of TR aggregated cells and further understand how different processing methods of adipose tissue maintain a viable cell population and affect the stromal cell aggregates and their regenerative nature to potentially improve clinical regenerative therapies.

METHODS: Human abdominal fat was harvested from the operating room, centrifuged, washed, and subjected to mechanical or enzymatic disaggregation. Fragments were centrifuged to pellet the isolated cell subset and separate the supernatant aggregated cells in the TR. Cultures were seeded at equivalent cell density (500k/cm2). Cell number, viability, cell cycle and imaging were determined at 7 day intervals up to 3 weeks (n=7, ran in triplicate wells).

RESULTS: Cell Procurement (Table One): Cell number and viability was severely compromised in mSVF. eSVF yielded reduced cell fractions when compared to TRs. Viability quantification was difficult in the TRs due to their three-dimensional nature but shared a similar range.

Lag Phase: eSVF cultures initiated expansion at day 4. TR cultures displayed a protracted lag phase (14 days) compared to their isolated counterparts. No differences in mTR vs eTR were found. Isolated cells remained quiescent with only 3-8 % in G2 phase.

Log Phase: Once eSVF cultures initiated sub-confluent expansion, G2 fraction increased to 12% and reached confluence at day 14 (density 4.0x103cells/cm2) with stabilization at 8%. TRs remained in log phase up day 14. mTR cells initiated a log phase with G2 fractions twice as fast as eTR cells. However, cell densities at confluence were 40 and 120, respectively.

CONCLUSION: Tissue residues generated by enzymatic or mechanical disaggregation of adipose tissue contain the majority of cell biomass present in the stromal fraction. TR cells remain viable yet initially contact inhibited, explaining a protracted lag phase. However, their log phase reaches 3 fold the monolayer density compared to their controls.

Table 1. Cell Procurement

	Cell Number (cells)	Cell Viability	
mSVF	50000	59%	**mSVF**
eSVF	$8.0x10^5$	85%	**eSVF**
mTR	$3.5x10^7$	60-72%	**mTR**
eTR	$7.2x10^7$	60-72%	**eTR**

ABSTRACT 20

Mechanical Processing of Lipoaspirate does not Lead to a Significant Manipulation of the White Adipose Tissue or Adipose Derived Stem Cells

Presenter: Andreas Eigenberger (Germany)
Affiliation: University Hospital Regensburg
Authors: Andreas Eigenberger, Oliver Felthaus, Lukas Prantl

Introduction: Autologous lipotransfer is a promising method for tissue regeneration. To improve the outcome, adipose tissue can be washed and processed before application. Even if there is a need to increase the number of adipose derived stem cells in the graft, their proliferation and secretion properties must not be altered. We developed a procedure for processing lipoaspirate by shear force only. In order to assess the safety of the procedure, we investigated the effects of mechanical processing on adipose derived stem cells and the surrounding tissue.

Methods: Lipoaspirate was harvested from patients using water jet-assisted liposuction. Subsequently, it was processed using sedimentation, first time centrifugation, homogenization, and second time centrifugation. Average adipocyte size, stromal vascular cell count, and adipocyte-depot size were examined histologically for every processing step. Adipose derived stem cells were isolated and counted. Furthermore, their secretome was analyzed using mass spectrometric and seeded stem cells were differentiated adipogenically and osteogenically.

Results: Homogenization causes a disruption of large adipocyte accumulations, however, the shape and size of the remaining adipocytes is not changed. Mechanical processing leads to a cell enrichment attributable to volume reduction without reducing the viability of the stem cells. Neither their proliferation rate nor their differentiation potential was altered significantly. Protein composition of the secretome did not change after shear force processing.

Conclusion: Shear force assisted enrichment of lipoaspirate constitutes no substantial manipulation of the stem cells secretome or differentiation potential. The changes due to processing correspond more to a filtration of mechanically fewer stable components than to a manipulation of the tissue. Therefore, it appears mechanical processed lipoaspirate is promising to repair even mechanically stressed tissue.

ABSTRACT 21

A Simple, Reproducible Estimation of Adipocyte Cell Lysis in Lipoaspirate Samples and their Disaggregated Byproducts

Presenter: Aina Llull Canellas (USA)
Affiliation: Wake Forest Baptist Health
Authors: Aina Llull Canellas , Gabriela Aguilo-Seara, William Molair, Adam J Katz, Ramon Llull

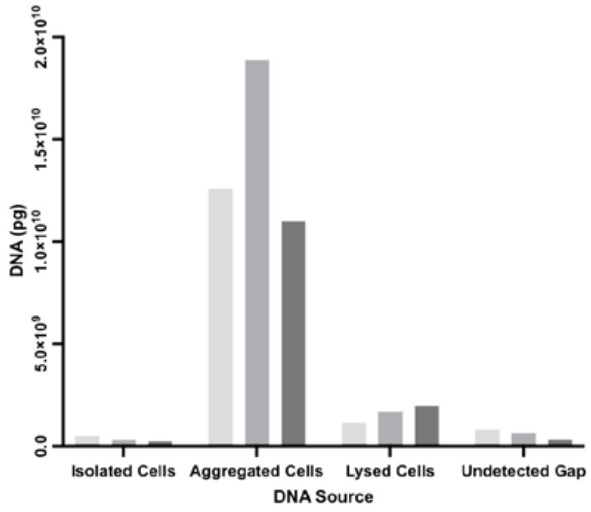
INTRODUCTION: Lipoaspiration is the preferred procurement method of adipose tissue and it consistently involves a degree of mature adipocyte lysis. Additionally, lipoaspirate disaggregation methods, enzymatic or mechanical, are profusely utilized to separate the volume occupying adipocytes and thus concentrate the stromal component of adipose tissue. We hereby describe a method to estimate the number of adipocyte cell lysis resulting from adipose tissue processing.

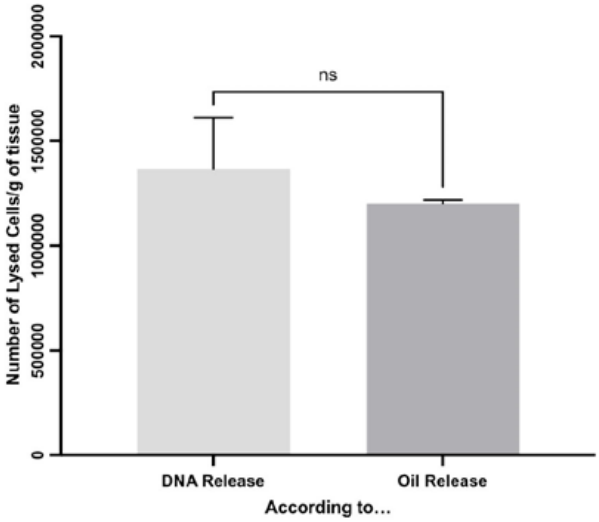
METHODS: Human lipoaspirate samples were collected under standard conditions (n=3) and subjected to enzymatic or mechanical disaggregation. Oil, tissue, water and sediment (pellet) phase volumes were recorded. DNA quantification was performed for all phases. Tissue Residue DNA, Isolated cell DNA, and dissolved DNA in water were then correlated with the oil volume considering relatively constant factors for these representative samples: fat fraction in adipose tissue is 86%, adipocyte mean radius 50 µm, proliferation fraction lower than 3%. DNA per human cell 6.7 pg/cell.

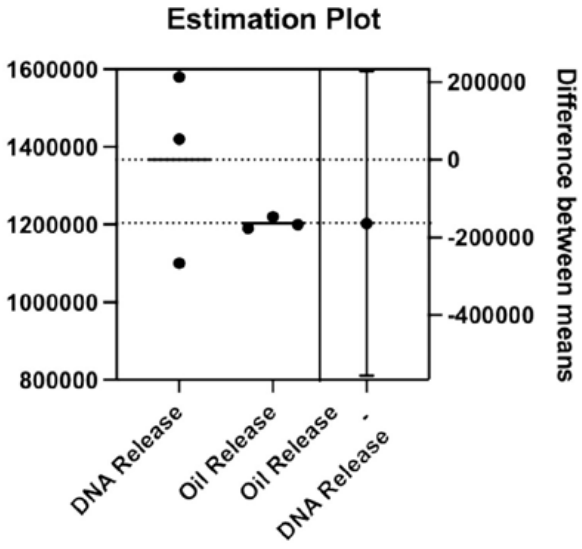
RESULTS: DNA elution was constant (78%), rendering a reliable regression line (R^2 0.9) from which cell number was interpolated. DNA from Isolated, Aggregated and Lysed cells accounted for nearly the entire biomass present in adipose tissue before procurement or disaggregation (Figure 1). DNA in the water phase was found to correlate with the oil phase volume: oil phase volume, when divided by the single adipocyte volume (5x10^5µm^3) and corrected by the fat fraction, renders a number of adipocytes which approximates to the quantity of DNA released into the water phase (Figure 2A and B)

CONCLUSION: Following adipose tissue procurement or disaggregation, DNA is absent in the oil phase, yet it distributes in the tissue residue, water and pellet phases proportional to the partial biomass present in each phase. Summation of the different phase DNA contents correlates with the total biomass present in the original sample. The undetected gap present is likely due to the contribution of non-adipocytic cell component, further supporting the low fraction of post-processing stromal. The number of post-processing lysed adipocytes appear to shed proportional triglyceride volume and DNA to the oil and water phase respectively, thus confirming the intuitive reasoning that the more the oil volume the more the adipocyte lysis.

ABSTRACT 21 (images)







ABSTRACT 22

Extent of Tissue Washing Can Significantly Alter the Composition and Activity of SVF Preparations: Implications for Clinical Translation

Presenters: Adam Katz, MD (USA)
Affiliation: Wake Forest Baptist Health
Authors: Adam Katz, Gabriela Aguilo-Seara, William Molair, Hulan Shang, Scott Northrup, Josh Grosser, Ramon Llull

INTRODUCTION: Autologous, point-of-care cell therapies using adipose-derived stromal vascular fraction (SVF) cells have advanced to late-stage clinical trials for a growing number of indications.The objectives of this paper were to evaluate the effects of t tissue washing and RBC lysis - separately and together on SVF cell composition. Our central hypothesis is that the extent of initial washing of lipo-aspirated adipose tissue can significantly impact the final composition of SVF preparations and that blood derived cells can confer detectable and modifiable effects on adipose-derived stromal cell activity.

METHODS: Using various cell culture assays, flow cytometry and ELISA analysis of human-derived SVF preparations we examined the effect of tissue washing and hypotonic RBC lysis on blood-derived cell (BDC) concentrations within SVF, and whether BDCs can confer detectable and modifiable effects on adipose-derived cell activity.

RESULTS: Our findings demonstrate that thorough washing of adipose tissue prior to enzymatic dissociation effectively removes RBCs from SVF preparations as well as standard lysis methods and significantly alters the type and relative quantities of WBCs. In addition, these studies demonstrate that potentially toxic RBC components are detectable for up to 1 week in cultures containing RBC lysate, but not those with intact RBCs; and, that SVF cells and culture-expanded ASCs proliferate significantly more in the presence of intact RBCs.

CONCLUSION: Broadly, these data exemplify how different, seemingly mundane tissue processing steps can significantly influence SVF identity/composition, purity and potency. Based on the findings of this work, we propose that translational efforts in the field would benefit by a better understanding of the impact of RBCs, WBCs and non-viable cells on the in-vivo therapeutic activity of SVF therapies.

ABSTRACT 22 (images)

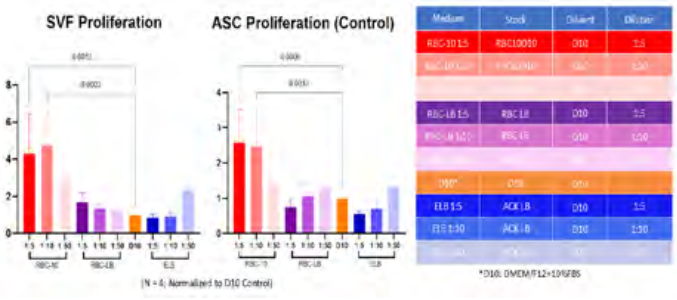


Figure 1: Proliferation of Adipose-derived Cells in Media with Intact RBCs or RBC Lysate. Fresh SVF cells and ASCs were cultured in a variety of conditioned media as summarized above. Presto Blue Assay was used to quantify cell numbers. Human SVF cells and culture-expanded ASCs proliferate significantly more in the presence of intact RBCs compared to standard D10 medium whereas cells cultured in RBC lysate media do not.

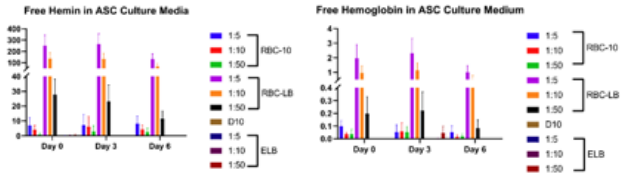


Figure 2: Hemin and Hemoglobin Concentrations in Culture Media Over Time. Both Hemin and Hemoglobin were significantly ($p < 0.05$) higher in lysed RBC media (RBC-LB) than in either control (D10) or intact RBC media (RBC-10) after 6 days of culture. Values were determined using ELISA for each specific compound. Based on these results, potentially toxic RBC components are detectable for up to 1 week in cultures containing RBC lysate, but not in those culture with intact RBCs.

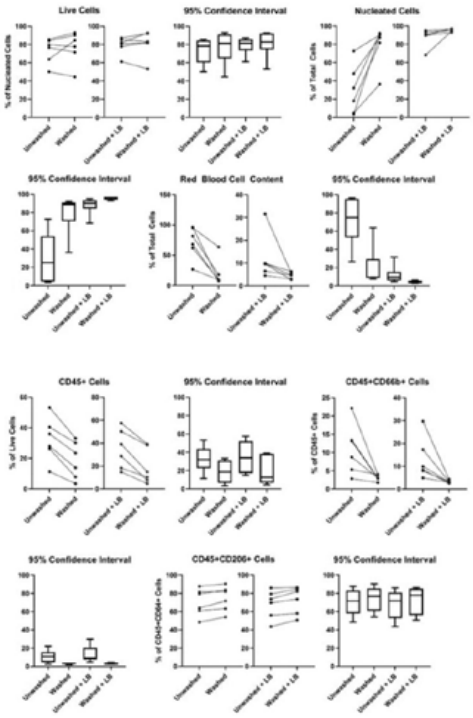


Figure 3: Thorough Adipose Tissue Washing Significantly Reduces the Number and Types of Blood-derived Cells in SVF Preparations. Flow cytometry was used to determine changes in cell populations depending on the method of purification (n=6). Thorough tissue washing is as effective as ELB at removing RBCs from SVF preparations, and provides advantages of time savings and diminished risk of potentially toxic substances. Thorough washing also significantly alters the type and relative quantities of WBCs in SVF preparations, significantly reducing neutrophils while increasing the percentage of T2 macrophages.

ABSTRACT 23

Optimal Fat Graft Sizing with the 'Micronizer' System

Presenter: Hilton Becker, MD (USA)
Affiliation: beckerMD
Author: Hilton Becker

INTRODUCTION: It has been shown that to ensure fat graft survival, it is important that fat cells do not exceed 1mm in diameter, but at the same time contain a high concentration of stromal vascular fraction. A method of harvesting large sized fat grafts and reducing them in size to less than 1mm is described.

METHODS: The Micronizer system
Fat is harvested with a 4mm cannula

1. The lipo-blade attaches to the canister.
It has a 4mm inner diameter containing a blade that cuts the fat to 2mm particles .

2. The 2.4mm micronizer cuts 2 mm particles to 1mm particles (Microfat) .
Microfat can be injected atraumatically through a 1mm cannula.
3. The 1.4 micronizer cuts 1mm particles to 0.5 mm Fat particles, which can then be injected atraumatically through a 0.7 mm cannula. The fat can be further reduced in size by passing the fat back and forth several times. (Ultra micro fat)
4. To obtain so called nano fat , the fat is passed through multiple times and then strained. Depending on the strainer size, the fluid can be injected through a 27g or 30 g needle.

RESULTS: Using the micronizer system, it is possible to harvest fat with a 4-5 mm cannula and by means of the micronizer system reducing the size of the fat grafts to 2mm, 1mm and 0.5mm and even smaller if required. As the grafts are cut from 4-5 mm particles they contain a higher SVF content than fat harvested with a 1mm cannula. The micronizer is the only system having a single ultra sharp blade that actually cuts the fat particles with minimal trauma. Furthermore the fat particles can be cut to the specific size that will passthrough the selected cannula or needle atraumatically.

CONCLUSION: 4mm fat particles containing a higher concentration of SVF than 1mm particles ,can be reduced in size to less than 1mm using the Micronizer system.

ABSTRACT 24

Syngeneic Adipose-Derived Stromal Cells Modulate the Immune Response within Decellularized Adipose Tissue Scaffolds in a Murine Subcutaneous Implant Model

Presenter: John Walker (Canada)
Affiliation: Western University
Authors: Walker, J., Cooper, Tyler T.; Dunmore-Buyze, Joy; Drangova, Maria; Lajoie, Gilles; Dekaban, Gregory A.; Flynn, Lauren E.

INTRODUCTION: Despite a remarkable capacity to expand and regress throughout mammalian life, adipose tissue typically fails to regenerate following reconstructive surgeries or tissue injury and is often replaced by scar tissue. Biomaterials, including decellularized adipose tissue (DAT), have shown promise for stimulating adipose regeneration in pre-clinical models, with an enhanced response often reported when the scaffolds are seeded with adipose-derived stromal cells (ASCs). While ASC-based therapies have shown promise for a range of applications, the extent and mechanisms through which ASCs support tissue regeneration in vivo are not well characterized.

METHODS: An established decellularized adipose tissue (DAT) delivery platform was employed to investigate the pro-angiogenic and immunomodulatory effects of transgenic mouse syngeneic DsRED+ donor ASCs within a subcutaneous implant model in immunocompetent C57BL/6 mice, with endpoints up to 8 weeks post-implantation. It was hypothesized that ASC seeding would promote a more regenerative macrophage phenotype and enhance localized vascular perfusion, resulting in improved scaffold integration compared to unseeded scaffolds. Flow cytometry was used to assess macrophage phenotype and track ASCs, and fluorescence-activated cell sorting enabled isolation of these populations for proteomics assessment via biological mass spectrometry. The integration of scaffolds with surrounding tissue was assessed histologically, and vascular perfusion was measured using a μCT- based angiography approach.

RESULTS: Initial proteomics data indicate that ASCs modulate cell surface receptor and extracellular matrix protein expression over time; however, few ASCs persisted in the tissue beyond 2 weeks post- implantation. Histological assessment showed qualitatively similar tissue integration between seeded and unseeded scaffolds over time. While vascular perfusion was not affected by ASC seeding, a more regenerative macrophage phenotype was noted in the seeded group, but only up to 2 weeks post- implantation while ASCs were most abundant. Interestingly, DsRED fluorescence was detected in a small subset of macrophages, indicative of a population that had phagocytosed ASC-derived protein. Notably, these macrophages displayed a more regenerative phenotype than their DsRED- counterparts.

CONCLUSION: ASCs modulated macrophage phenotype within the DAT scaffolds at early time points, potentially mediated in part through their phagocytosis. Investigation of strategies to increase the localized persistence of ASCs in vivo may help to augment cell-mediated regeneration.

ABSTRACT 25

Adipose Stromal Cells for Osteoarthritis: Are All Orthobiologics the Same?

Presenter: Nathan Katz (USA)
Affiliation: Jointechlabs
Authors: N. Katz, A. Yayon, N. Pancholi , A. Hakimian and S. Chubinskaya

INTRODUCTION: Adipose stromal cells (ASC) were reported as effective orthobiologics for OA. However, there are very few studies on the mechanisms of action and possible differences between available treatments.
We have studied the effect of different doses of ASC on the cartilage in vitro and compared our results to those reported for other orthobiologics.

METHODS: Secretory activity of ASC was assessed in the OA model in vitro. The effect on the cartilage was evaluated by PG synthesis, histology and PCR of the cadaver cartilage simulated for OA inflammatory environment.
Literature search and comparative analysis was performed for Bone marrow and PRP research for joint OA.

RESULTS: ASC actively respond to inflammatory environment by secreting complex panel of pro- and anti-inflammatory factors. The secretory activity of ASC is dynamic and changes over the period of time.
Embodiment of ASC into scaffolding carrier Regenogel (ProcoreBio) supports metabolic activity of the cells and promotes anti-inflammatory and structural support of ASC effect on cartilage.

CONCLUSION: ASC survive and function for prolonged time upon application in OA model in vitro. Regenogel supports ASC metabolism and secretory activity, posing promising delivery modality for therapeutic applications.
ASC have accumulative and increasing over time anti-inflammatory and structural effect on cartilage. While both PRP and BMAC demonstrate anti-inflammatory pain mitigating effect in OA, ASC exhibit unique structural effect on cartilage, reversing the deterioration of the cartilage at OA.
Further animal and human studies are required to determine the disease modifying mechanisms of orthobiologics and corresponding choice of treatments at different phases of OA.

ABSTRACT 26

Human Platelet Lysate as Supplement to Increase the Anti-inflammatory Properties of Adipose-derived Stem Cells for the Treatment of Osteoarthritis

Presenter: Stefania Brambilla (Italy)
Affiliation: S Ospedale Galeazzi - Sant'Ambrogio, Milano, Italy
Authors: Stefania Brambilla, Silvia Lopa, Arianna Lovati, Shima Salehi, Matteo Moretti, Silvia Palombella

INTRODUCTION: Adipose-derived stem cells (ASCs) have a high potential for regeneration. ASCs are capable of localizing on damaged tissue sites and secrete trophic factors, restoring the biological environment. Moreover, human platelet lysate (HPL) has become an alternative supplement for ASCs culture: it can be easily obtained without ethical limits and without safety issues for recipient patients. HPL can be used allogeneically and increases cell metabolism and secretory activity, since it contains a higher concentration of growth factors than FBS. Considering their anti-inflammatory properties, ASCs can be used as treatment to target osteoarthritis (OA) in which inflammation plays a pivotal role and several soluble inflammatory factors cause the progressive degeneration of cartilage. Therefore, our aim was to investigate the effect of HPL-cultured ASCs on OA inflammation.

METHODS: We analyzed the morphology and metabolism of ASCs cultured in FBS (FBS-ASCs) and HPL (HPL-ASCs). Furthermore, we measured the levels of the inflammatory-related mediators IL-6 and IL-8 secreted by ASCs. Then, FBS-ASCs and HPL-ASCs were co-cultured with chondrocytes stimulated with TNFα and IFNγ to simulate inflammation. After five days of co-culture, morphology, cell viability, and proliferation of both ASCs and chondrocytes were assessed.

RESULTS: Our results indicated that HPL-ASCs maintained their classic morphology, whereas FBS-ASCs were larger and less spindle-shaped. In addition, the viability and proliferation of HPL-ASCs were superior to those of FBS-ASCs. The level of IL-6 secreted by HPL-ASCs was twice as high as FBS-ASCs (FBS-ASCs: 285 pg/mL, HPL-ASCs: 600 pg/mL). Conversely, IL-8 was detected in FBS-ASCs, but not in the HPL-ASCs conditioned medium (FBS-ASCs: 202 pg/mL, HPL-ASCs: 0 pg/mL). As expected, chondrocyte proliferation was stimulated during co-culture with HPL-ASCs compared to FBS-ASCs. Furthermore, the metabolism of chondrocytes was similar when grown in co-culture with FBS-ASCs or HPL-ASCs.

CONCLUSION: Our data suggest that HPL has the potential to improve the anti-inflammatory properties of ASCs. The effects exerted by HPL-ASCs on inflamed chondrocytes support their potential use in the treatment of OA. In a future phase, we will analyze the levels of inflammatory-related mediators and oxidative stress in chondrocytes co-cultured with HPL-ASCs.

ABSTRACT 27

Wharton's Jelly Mesenchymal Stem Cell-derived Small Extracellular Vesicles as Natural Nanoparticles to Attenuate Cartilage Injury via MicroRNA Regulation

Presenter: Penghong Chen (China)
Affiliation: Fujian Medical University Union Hospital
Authors: Penghong Chen, Xiaosong Chen

INTRODUCTION: The main strategy of tissue repair and regeneration focuses on the application of mesenchymal stem cells and cell-based nanoparticles, but there are still multiple challenges that may have negative impacts on human safety and therapeutic efficacy. Cell-free nanotechnology can effectively overcome these obstacles and limitations. Mesenchymal stem cell (MSC)-derived natural small extracellular vesicles (sEVs) represent ideal nanotherapeutics due to their low immunogenicity and lack of tumorigenicity.

METHODS: sEVs harvested from Wharton's jelly mesenchymal stem cells (WJMSC) were identified. In vitro experiments, including CCK-8, transwell assay, macrophage polarization assay were performed. Then, the potential therapeutic effects of WJMSC-sEVs and WJMSCs in rat OA models were compared by macroscopic evaluation, immunohistochemical staining and micro-CT. Furthermore, small RNA sequencing were utilized to decode the underlying mechanism.

RESULTS: In vitro results showed that WJMSC-sEVs efficiently entered chondrocytes in the osteoarthritis (OA) model, further promoted chondrocyte migration and proliferation and modulated immune reactivity. In vivo, WJMSC-sEVs notably promoted chondrogenesis, which was consistent with the effect of WJMSCs. RNA sequencing results revealed that sEV-microRNA-regulated biocircuits can significantly contribute to the treatment of OA, such as by promoting the activation of the calcium signaling pathway, ECM-receptor interaction pathway and NOTCH signaling pathway. In particular, let-7e-5p, which is found in WJMSC-sEVs, was shown to be a potential core molecule for promoting cartilage regeneration by regulating the levels of STAT3 and IGF1R.

CONCLUSIONS: Our findings suggest that WJMSC-sEV-induced chondrogenesis is a promising innovative and feasible cell-free nanotherapy for OA treatment.

ABSTRACT 28

Development of Collagenolytic Cell Inoculants to Address Dupuytren Disease (and other fibrotic/anchylosing processes)

Presenter: Raghav Agarwal (USA)
Affiliation: Wake Forest Baptist Health
Authors: Raghav Agarwal, Severiano Dos Anjos, William Molair, Adam J. Katz, and Ramon Llull

INTRODUCTION: Intralesional delivery of Collagenase (Xiapex®) is a treatment for Dupuytren Disease limited in its indications, facing post-injection uncomfortable forceful manipulation, inflammatory convalescence and tendon rupture complications. Since collagen degradation occurs naturally by cell released collagenases, the administration of a cell inoculant overexpressing collagenase activity could result into a controlled clinically relevant collagen lysis. As stromal vascular fraction cells (SVF) express potent collagenolytic activity during acute infectious process, we hypothesized that Lipopolysaccharide (LPS) activated Stromal Vascular fraction could potentially exert a quantifiable collagenolytic activity.

METHODS: Human stromal cells from adipose tissue (150 mL) where harvested (n=7) and their Methyl-Metalloprotease MMP expression was activated by exposing them to LPS in vitro. Subsequently washed to eliminate LPS traces. LPS-SVF or their soluble supernatant (putatively containing soluble MMP) were further cultured in presence of fluorescent gelatin. As gelatin (collagen) is digested, fluorescence release to the media is proportional to the collagenase catalytic activity, where its luminescence can be quantified.

Xiapex® was also exposed to fluorescent gelatin to determine the enzymatic functional concentration of its clinical formulation (0,25 mL containing 0,58 mg). Collagenolytic activities from serially concentrated LPS SVF were then compared to the nominal collagenolytic effect exerted by a Xiapex dose.

RESULTS: LPS activated SVF were able to release fluorescein to the supernatant, and that was extinguished upon addition of TIMP-1 (tissue inhibitor of MMP), thus proving that they LPS SVF were able to reproducibly cleave collagen (14 fold the activity of quiescent not activated SVF). LPS activated and control supernatant failed to display catalytic activity of soluble collagenase. A minimum of 5x10(7) LPS-SVF are able to support membrane bound, selective collagenolysis equivalent to one Xiapex dose.

CONCLUSION: Cell mediated collagenolysis requires stimulation, is cell membrane bound and not due to MMPs in its soluble form. A collagenolytic activity equivalent to that of a FDA approved treatment can be obtained with a minimally invasive lipoaspirate harvest (150mL).

Further research might vindicate that, if successfully engrafted, a safe cell suspension generated from an expendable tissue source with minimal morbidity can possibly exert a sustainable site-specific collagenolytic activity, and possibly a clinically relevant fibrolysis.

ABSTRACT 29

Effects of Two- and Three-Dimensional Culture under Different Microenvironments on Adipose-Derived Stem Cells and Conditioned Medium

Presenter: Yoshihiro Toyohara (Japan)
Affiliation: Jichi Medical University
Authors: Yoshihiro Toyohara, Masanori Mori, Kayo Yoshizumi, Yoshihiro Yamamoto, Zhang Bihang, Takako Shirado, Natsumi Saito, Wu Yunyan, Kotaro Yoshimura

INTRODUCTION: Conditioned medium (CM) collected from adipose-derived stem cells (ASC) contains a large number of growth factors, and various effects such as tissue repair and angiogenesis have been reported. CM has already been clinically applied in some medical institutions, but the type and amount of growth factors contained are affected by the culture environments, there are issues regarding the uniformity of quality and stability of effects. In this study, we investigated the effects of various culture environments on ASC and CM and examined the optimal culture conditions for CM rich in growth factors.

METHODS: Human ASC, isolated from adipose tissue collected by liposuction, cultured for 28 days in two-dimensional using dishes coated by extracellular matrix protein (collagen I, fibronectin, or laminin) or three-dimensional using carrier beads under normoxic (21% O2) or hypoxic (6% O2 or 1% O2) conditions. The number of cells and the concentrations of growth factors (HGF, KGF, and VEGF) were measured every 7 days.

RESULTS: Three-dimensional culture with carrier beads showed the highest levels in both the number of cells and the concentrations of growth factors and maintained the high levels for 28 days. In two-dimensional culture with dishes coated by extracellular matrix protein, HGF and VEGF levels gradually decreased, but KGF levels continued to increase. In comparison with various oxygen concentrations, HGF levels was high in 6% O2 and VEGF levels was high in 1% O2.

CONCLUSION: Three-dimensional culture increases proliferation of ASC and secretion of growth factors. The extracellular matrix protein and oxygen concentrations in cell culture affect the type and amount of growth factors. We will continue further studies to establish optimal culture conditions.

ABSTRACT 30

Comparative Quality Analysis of Two-dimensional Versus Large Scale Bioreactor Culturing of Human ASCS

Presenter: Lea Munthe-Fog (Denmark)
Affiliation: StemMedical A/S
Authors: L. Munthe-Fog, MR. Galera, F. Mamsen, A. Woetmann, AL. Fischer-Nielsen, JD. Jensen, SF. Kolle and JD.Svalgaard

INTRODUCTION: Two-dimensional cell culture is still the standard method used for research and cell therapy. Cell culture on flat plastic surfaces is a well-established technique in which cell proliferation is easy to observe. However, although being well described in the literature, the culture dynamic, e.g., effects of media gradients, passage regime, of 2D cell cultures is less known, leading to unrecognized subtle differences in the manufacturing of cells. This may contribute to the discrepancies found in the effectiveness of ASC in clinical trials.

As new tightly controlled cell culture platforms are emerging e.g., bioreactors, in response to an increasing demand of large-scale cell manufacturing, the static 2D cell culture platform e.g., in flasks, is to be transferred (translated) into bioreactors, while maintaining the clinical effectiveness of the produced ASCs. In this work we describe the transition of our current clinically proven handheld 2D cell culturing platform into a scalable 3D-like bioreactor platform, thereby reducing the workload and culture inconsistencies while maintaining the quality and clinical effectiveness of our ASCs.

METHODS: Isolated stromal vascular fraction (SVF) will be cultured in well-established 2D platform (cell culture flasks) and in parallel in our newly developed 3D-like platform. We aim to compare (among other parameters) tri-lineage differentiation, surface marker analysis, secretory profiling, and gene expression, to address the quality and effectiveness of the culture expanded ASCs.

RESULTS: The results from this study are still pending. The results will be presented at the conference.

CONCLUSION: The conclusion will be interpreted based on the results.

ABSTRACT 31

Adipose Derived Stromal Cells under Oxidative Stress Exert a Potent Inhibitory Effect in Bystander Cells via a Secretory Phenotype

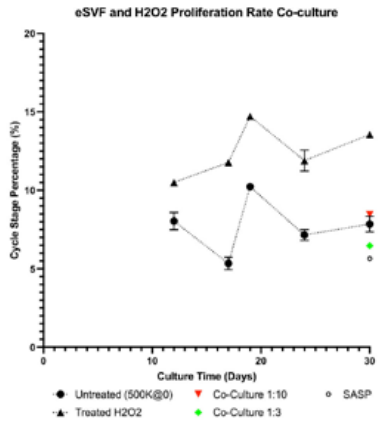
Presenter: Anuj Jaiwala (USA)
Affiliation: Wake Forest Baptist Health
Authors: Anuj Jaiwala, Samuel Kogan, William Sanfelippo, Adam J. Katz, Ramon Llull

INTRODUCTION: Multiple pathological and senescence processes are associated to the release of Oxygen Free Radicals which induce oxidative stress to participating cells (Oxidative stressed cells, OSC). Upon stress, OSC activate synthesis cyclin inhibitors (i.e. p16, p21) which reprogram cells to release a Senescent Associated Secretory Phenotype (SASP) profiles in soluble tumor suppressor factors. Based on that, It is hereby hypothesized that Adipose Derived Stromal Vascular fraction (SVF) cells, when exposed to Oxygen Free Radical media shall undergo oxidative stress. Furthermore, SVF-OSCs shall exert a paracrine inhibitory effect on bystander subsets.

METHODS: Human SVF cells (n=3 different donor samples), enzymatically dissociated, were cultured at sub-confluent density (5x10^5 cells/cm^2) in triplicate. Following their Lag phase, Oxygen Free Radical containing media was fed to a separate identical batch to stress cultures between day 5 and 9. 10 days post stress, SVF-OSCs (ratios 1:10 and 1:3) or their putative SASP (Supernatant from SVF-OSC cultures) were added in co-culture to sub-confluent normal SVF cultures. Cell density (δ), cell cycle (G1,S,G2), doubling times (dT), morphology and immunohistochemistry were recorded.

RESULTS: Generation of OSCs: In vivo, constitutive presence of cyclin inhibited cells (p16) were found in fresh samples of enzymatically dissociated from adipose tissue (3-12%). In vitro, Immediately following OS, cell morphology was consistent with OS changes, cell density was severely and consistently arrested in OSC cultures (10 vs 24x10^3 cells/cm^2, $P < 0.001$), δ displayed comparable percentages of cells in G0/G1 yet statistically significant accumulation of cells in G2 (5-9 vs 11-15%). This was associated to decaying, extended dT.
OSCs Co-culture or addition of OSC's SASP, resulted in uniform δ decrement and the return of G2% to control levels (P NS).

CONCLUSION: SVF exposed to OS results in cells with senescent-like morphological changes, with decaying cell populations, despite G2% increment, suggesting a slow dividing population arrested in the S to G2 (likely p21 inhibitor) and not at the G1-S check point (p16). When normal bystander cells are exposed to OSCs even at their low, physiologically equivalent percentage (10%) or their SASP, the cell system undergoes proliferative arrest that is not dependent upon cyclin inhibitors (nuclear), but rather dependent on cytoplasmic regulators.



ABSTRACT 32

The Effect of Adipose-derived Stem/Stromal Cell- and Collagen Enriched Fat Grafts on Quality of Wound Healing: An Explorative, Randomized, Controlled, Triple Blind, Paired, Clinical Trial

Presenter: Jesper Jensen (Denmark)
Affiliation: StemMedical A/S
Authors: Frederik Penzien Mamsen, Lea Munthe-Fog, Jesper Dyrendom Svalgaard, Jesper Due Jensen, Thomas Hartvig Lindkær Jensen, Anne Fischer-Nielsen, Mikkel Taudorf, Bo Jønsson, Adam J. Katz, Stig-Frederik Trojahn Kølle

INTRODUCTION: The process of wound healing is complicated and involve the function of various cell types at difference stages of healing. Furthermore, patient-depended factors such as cardiovascular status, diabetes, hygiene, infections, medicines etc. can impair healing. In recent years, the application of regenerative cells in wound healing has shown promising potential to improve the environment of the wound bed, likely by secreting proangiogenic growth factors, activating nearby fibroblasts, recruiting endothelial cells and macrophages, thus facilitating the formation of the granulation tissue.

METHODS: This explorative study investigates the effect of adipose-derived stem/stromal cells, collagen, and fat on wound quality. Five patients were carefully injected with various combinations of the three components in the dermis of the skin, in eight separate squares marked by tattoos on the abdomen. Above four of the squares, a CO2 laser was applied to initiate tissue damage. Three months after the injections, circular 28.3 mm2 defects were made in the eight treated skin areas and sutured to leave a central defect of 3.14 mm2 for conservative healing. Three months after, samples were taken from the scar tissue and analyzed by immunochemistry and histology. The treated areas including the tattoos, were removed by an abdominoplasty at the end of the trial.

RESULTS: The results are pending but will be presented at the conference.

CONCLUSION: We expect that the wounds created in the ADSC and collagen-enriched tissue to have less scarring and have a more regular skin architecture.

ABSTRACT 33

Skin Rejuvenation Using Ex-vivo Expanded Adipose-derived StemStromal Cells and Collagen Enriched Fat

Presenter: Frederik Mamsen (Denmark)
Affiliation: StemMedical A/S
Authors: Frederik Mamsen, Lea Munthe-Fog, Jesper Dyrendom Svalgaard, Jesper Due Jensen, Thomas Hartvig Lindkær Jensen, Anne Fischer-Nielsen, Mikkel Taudorf, Bo Jønsson, Adam J. Katz, Stig-Frederik Trojahn Kølle

INTRODUCTION: Skin aging is eminent; however, researchers investigate ways to slow down or even reverse skin aging by enriching the skin with regenerative cells. Adipose-derived stem/stromal cells (ADSCs) hold regenerative properties as they modulate the immune system, secrete cytokines, chemokines, and growth factors important for maintenance, differentiation, and repair of cells. Measuring the effect of dermal facial injections can be difficult as the assessment of treatments often rely on camera software which is unable to capture cellular changes in the aging skin. This explorative study investigates the effect of injecting fat, ADSC-enriched fat, and ADSC-enriched fat with collagen into the dermis of the abdominal skin to safely and ethically evaluate the effect of treatments relevant for facial skin applications.

METHODS: The study used the abdominal skin of five patients as a surrogate for facial skin treatments due to the ethical concerns regarding skin biopsies of the face. To mark the treatment area, each patient got eight 2x2 cm squares tattooed on the skin of their abdomen. Four different solutions containing the three graft components separately or in combination, were carefully injected into the dermis of each square. Each solution was injected in duplicates and the skin surface of one of the duplicates was treated with CO2 laser to induce an initial cellular reaction. Biopsies from the treated area were collected at three- and six months after the intervention. At the end of the treatment period, the patients underwent an abdominoplasty completely removing the treated area of the abdomen.

RESULTS: The results are pending but will be presented at the conference.

CONCLUSION: We expect the ADSC-enriched fat grafts to improve skin thickness and rejuvenate the structure of the skin towards a more youthful structural composition.

ABSTRACT 34

Nanofat Subdermal Injections for Treatment of Burn Scars

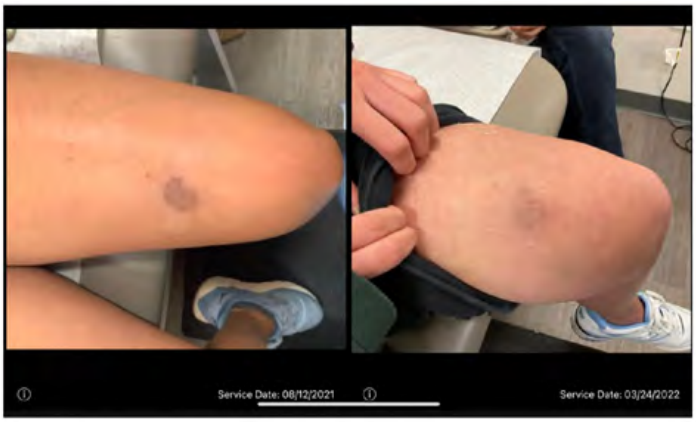
Presenter: Maria McKenna (USA)
Affiliation: Claytor Noone Plastic Surgery
Authors: Julie Holesh, Richard Claytor, Maria McKenna

INTRODUCTION: Burn scars which are full thickness result in thinning of dermis and poor aesthetic results. Full scar excision is often the only treatment. We hypothsize that subdermal injections of nanofat will induce regenerative properties to thicken dermis and improve skin color.

METHODS: A 19 year old patient with deep second degree burn to her thigh induced by a curling iron desired aesthetic improvement of the unsightly scar. 3 treatments were performed with nanofat harvested from her abdomen and immediately processed after fat removal. Treatments were 2 months apart and were performed under local anesthesia. Fat was harvested and processed in standard nanofat mechanical preparation for as described by Dr Verpaele and Tonnard. Multiple passes with small aliquots injected through #27 gauge blunt needles wtih a total of 3 cc injected.

RESULTS: Clinical end points and patient satisfaction were used to determine that the scar showed dramatic improvement in color and thickness. No infection or wound breakdown were evidenced in the patients recovery. The patient was very pleased with her aesthetic results.

CONCLUSIONS: Established chronic burn scars can safely and effectively be managed with subdermal injections of nanofat to improve aesthetics and function. Minimal downtime and local procedures can improve patient compliance and satisfaction. Additional studies including tissue biopsy can further quantify the clincial findings.



ABSTRACT 35

Adipose Stem Cell Exosome (ASCE): Next Generation Regenerative Therapeutics & Aesthetics for Skin and Hair

Presenter: Byong Cho (South Korea)
Affiliation: ExoCoBio, Inc
Author: Byong Cho

INTRODUCTION: Exosomes, nano-sized extracellular vesicles, are the most important mediator for intercellular communication. The dual function of skin regeneration and anti-inflammation mediated ASCE has been well known from a number of research. ASCEs have been developed as next generation regenerative therapeutics and aesthetics as well. From our various studies, ASCE have been demonstrated as an innovative biomaterial for the treatment of atopic dermatitis, acne scar, facial redness, & scalp rejuvenation/hair loss.

METHODS: Human adipose mesenchymal stem cell-derived exosomes (ASCE) and a specific formulation including ASCE were applied or treated for a variety of in vitro, in vivo, & clinical studies.

RESULTS:

1. ASCE could attenuate severe inflammation in skin in atopic dermatitis model. The AD score was significantly improved and proinflammatory cytokines including IL-4, IL-13, TLSP, & others we re down-regulated.
2. ASCE could promote the de novo synthesis of ceramide, key molecules in skin barrier formation, with the significant improvement of atopic dermatitis model.
3. ASCE showed strong anti-particulate matter effect by 1) promoting the synthesis of filaggrin and loricrin, 2) reversing the level of proinflammatory IL-6 and IL-1b in a co-culture model of 3 types of cells of fibroblasts, keratinocytes, and mast cells.
4. In combination of CO2 fractional laser procedure, ASCE could synergistically show the improve ment of acne scar, which was the world's first double blinded clinical study in exosome field.
5. From 2 patient case study, the topical application of ASCE by electroporation could successfully improve refractory DFR.
6. When compared to PRP, ASCE and a specific formulation could successfully improve scalp reju venation and hair loss.

CONCLUSION: ASCE may serve as next generation technology with competitive advantages over conventional regenerative aesthetics or therapeutics, in terms of regeneration and anti-inflammation.

ABSTRACT 36

Addressing Healing of Complex Skin Wounds Using Tissue Engineering Methods Applied to Adipose-derived Stromal Cells

Presenter: Julie Fradette
Affiliation: LOEX-Universite Laval (Canada)
Authors: Fradette J., Safoine M., Diaz C., Ruel J.

INTRODUCTION: Mesenchymal stem cell-based therapies offer a promising approach to potentially cure complex skin wounds. Cell delivery methods (isolated cells vs 3D formulation) can impact therapeutic potential. There is also a need for the development of tissue engineering methods suited for clinical applications. Thus, we have engineered manipulatable biological dressings from human adipose-derived stem/stromal cells (ASC). Using the self-assembly approach of tissue engineering, we hypothesized that all important parameters of tissue reconstruction can be maintained in a system devoid of FBS from cell extraction to tissue reconstruction.

METHODS: We studied a commercially available serum-free (SFM) medium for its impact on tissue reconstruction using human ASC in comparison to serum-containing medium. Topical application of the ASC dressings provided evidence of improved healing in murine models of complex wounds, namely for full-thickness excisional wounds on skin of diabetic animals (NONcNZO/LtJ mice) or irradiated skin (4 weeks post radiation, 45 Gray dose).

RESULTS: A detailed characterization of tissues produced under SFM conditions showed a substantial 50% reduction of production time without compromising key tissue features such as thickness, mechanical resistance and pro-angiogenic secretory capacities (plasminogen activator inhibitor 1, hepatocyte growth factor, vascular endothelial growth factor, angiopoietin-1) when compared to tissues produced in the control FBS-containing medium. For the diabetic mice model, global wound closure kinetics determined from macroscopic images showed that ASC-based dressings accelerated wound closure by 83% at day 8, 57% at day 12 and 35% at day 16 compared to untreated wounds which consistently had a 1-week delay in healing. Neovascularization (CD31+ labeling) was 1.3-fold higher in the treated wounds. For the post-radiation model, global wound closure evaluation revealed that wounds in irradiated tissues featured delayed healing (58% vs 90% healed surface area, P=0.007). ASC dressings promoted wound closure in these irradiated tissues (86% vs 58% healed surface area, P=0.01). While irradiation initially decreased tissue vascularity (1.3-fold fewer CD31+ vessels), this was increased 2.5-fold after treatment with ASC dressings.

CONCLUSION: In summary, these natural tissue-engineered dressings made of ASC and endogenous extracellular matrix represent promising candidates for cutaneous healing in vivo, by stimulating, among others, granulation tissue formation and neovascularization.

ABSTRACT 37

Adipose Derived Stem Cell Conditioned Media Gives Mixed Results Regarding Irradiated Human Keratinocytes Viability and Migration

Presenter: Celena Sorgel (Germany)
Affiliation: Department of Plastic and Hand Surgery University Hospital of Erlangen
Author: Celena Sorgel

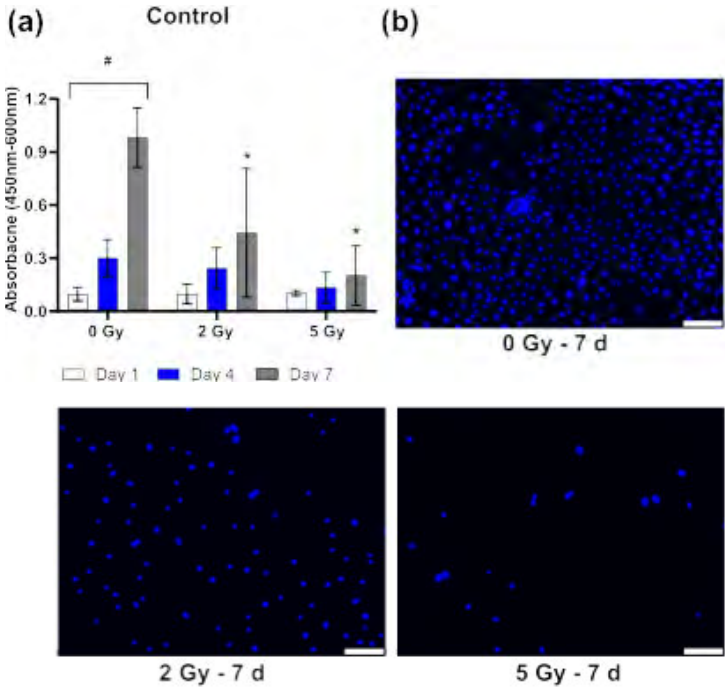
INTRODUCTION: Ionizing radiation has become an integral part of modern cancer therapy regimens. Patients undergoing radiation therapy for the treatment of various types of cancer often experience side effects such as radiation dermatitis. It can affect patients in acute and chronic forms and decrease therapy compliance significantly. A gold standard for therapy and prevention of radiation dermatitis is still lacking.
The objective of the presented study was to find novel therapeutic strategies for the regeneration and repair of damaged skin areas after irradiation.

METHODS: Primary keratinocytes were irradiated in a 2-dimensional (2D) culture as well as on a 3-dimensional (3D) collagen- elastin matrix with doses of 2 and 5 Gy. The effect of different concentrations of IGF-I, KGF, platelet lysate (PL), high and low molecular weight hyaluronic acid (H-HA, L-HA) and adipose-derived stem cell (ADSC) conditioned medium (CM) was analyzed in respect to cell viability (WST-8), wound closure (migration) and gene expression (quantitative real time PCR) of 2D cultures. The 3D culture was evaluated by WST-8.

RESULTS: Hyaluronic acid and IGF-I effectively reduced irradiation damage of primary keratinocytes by stimulating viability and migration and simultaneously reducing cell apoptosis and necrosis. On day 7, cell viability was significantly lower in the 2 Gy and 5 Gy irradiated group compared to the non-irradiated control. Interestingly, this effect could be significantly reversed when keratinocytes were treated with 1 mg/ml H-HA/L-HA, 0.15 mg/ml L-HA, 0.15 mg/ml H-HA, 1 mg/ml H-HA or 100 ng/ml IGF-I after irradiation with 5 Gy. Cell viability experiments revealed a rather inhibiting effect of 3-fold concentrated ADSC-CM. Stimulating effects of H-HA/L-HA and IGF-I were able to be confirmed in 3D culture. A positive influence on cell viability, migration and gene expression was achieved after the treatment with H-L-HA and IGF-I.

CONCLUSION: These results open the possibility of a novel therapeutic method for both prevention and treatment of radiation dermatitis. They indicate that the negative effects of irradiation on keratinocytes located in the patient's skin can be decreased or even counterbalanced with hyaluronic acid and IGF-I treatment. Both HA/L-HA and IGF-I may offer a promising therapeutic option for the prevention and treatment of irradiation dermatitis.

ABSTRACT 37 (images)



Keratinocyte viability after irradiation of the control groups without growth factor treatment; (a) Y-axis shows absorbance on 1 d, 4 d and 7 d after 0, 2, 5 Gy irradiation; (b) Representative images of irradiated keratinocytes without growth factor supplementation at time point 7 d stained with DAPI (blue = living cells). * $P \leq 0.05$, significances were correlated to the non-irradiated respective control. * =significant compared to the control without growth factors in the respective irradiation dose at the respective time point. # = significances were correlated to the control on day 1. Scale bar 100 μm.

ABSTRACT 38

Therapeutic Effects of Conditioned Medium from Adipose-derived Stem Cells Cultured in Xeno-free Medium on Impaired Wound Healing in Irradiated Tissue

Presenter: Bihang Zhang (Japan)
Affiliation: Jichi Medical University
Authors: Bihang Zhang , Kotaro Yoshimura

INTRODUCTION: Radiotherapy is widely used in clinical applications while it can lead to devitalized tissue and impaired wound healing. Conditioned medium (CM) from adipose-derived stem cells (ASCs) may have therapeutic effects on it.

METHODS: A xeno-free medium was adopted to culture ASCs and get its CM. The ASC-CM's components of cytokines and growth factors were quantified and fibroblast proliferation in this CM was investigated. Fractional localized radiations of 40 Gy were delivered to the dorsal skin of nude mice and waited for 6 months. Then cutaneous wounds were created at the radiated skin and locally injected with DMEM, ASC-CM, ASCs, while another group of healthy control was injected with DMEM. Skin samples harvested at day 28 were analyzed with histological examinations.

RESULTS: The ASC-CM contained high concentrations of growth factors and cytokines including HGF, KGF and VEGF, and improved fibroblast proliferation in vitro. The CM significantly enhanced the impaired wound healing in radiated tissue most, followed by the ASC-treated irradiated group and the healthy control group, compared to the irradiated-control group. And the CM group showed highest CD34 positive area compared to other groups of irradiated tissue, with no significant difference with the healthy tissue.

CONCLUSION: The ASC-CM obtained from ASCs cultured in xeno-free medium has therapeutic effects on impaired wound healing in irradiated tissue, which may be a promising tool for wound healing and tissue regeneration, especially in stem cell-depleted pathological tissues.

ABSTRACT 39

Secretome from Stimulated Adipose Stem Cells Enhances Neurite Outgrowth and Angiogenesis

Presenter: Maria Brohlin (Sweden)
Affiliaton: Umea University
Authors: Maria Brohlin, Rosanna Ching, Malin Andersson, Mikael Wiberg, Paul J Kingham

INTRODUCTION: Adipose stem cells (ASC) secrete a wide range of growth factors which have the potential to boost axon regeneration and ameliorate muscle atrophy after nerve injury. In this study we have investigated the regenerative potential of ASC grown under xeno- and serum-free conditions with a focus on the extracellular vesicle component of the secretome.

METHODS: Human ASC were isolated from lipoaspirate samples (n=5 female donors) and expanded for 1-2 passages in a xeno- and serum-free medium (PRIME-XV Expansion XSFM; FujiFilm Irvine Scientific). Medium was collected from untreated cells or cultures treated with a stimulating cocktail (basic fibroblast growth factor, platelet derived growth factor-AA, neuregulin and forskolin). Extracellular vesicles were isolated from the conditioned medium using polymer based precipitation and the particle size was analysed by nanoparticle tracking analyses (NTA). Angiogenic and neurotrophic growth factor expression was analysed by qRT-PCR, and protein secretion by ELISA and antibody arrays. In vitro angiogenic and neurite outgrowth assays were used to measure biological activity.

RESULTS: NTA showed similar size profiles for untreated and stimulated cells with a major peak at approximately 150nm and a smaller peak at 50-70nm. IGF1 gene was markedly up-regulated (6 fold) by the stimulation protocol. In the whole secretome, urokinase-type plasminogen activator protein was consistently up-regulated by the stimulation protocol whereas hepatocyte growth factor protein was detected at high levels in the extracellular vesicles prepared from the stimulated cells. Conditioned medium from stimulated ASC enhanced neurite outgrowth of SHSY-5Y cells (this was significantly higher when compared with medium from untreated cells). Extracellular vesicles from both untreated ASC and stimulated ASC enhanced endothelial cell capillary-like network formation in vitro. The latter group showed the greatest effects.

CONCLUSION: An *in vitro* stimulation of ASC may be a useful conditioning step to boost the neuroregenerative and angiogenic properties of the cells. The relative role of extracellular vesicles versus traditional paracrine factor secretion remains to be determined and this will inform future treatment strategies which might overcome the need for direct cell transplantation.

ABSTRACT 40

Human Platelet Lysate Modulates Anti-inflammatory and Regenerative Properties of Stem Cells Derived from the Stromal Vascular Fraction of Adipose Tissue

Presenter: Silvia Palombella (Italy)
Affiliation: IRCCS Ospedale Galeazzi Sant'Ambrogio - Milano, Italy
Authors: Silvia Palombella, Stefania Brambilla; Silvia Lopa; Giuseppe Talò; Martino Guiotto; Pietro di Summa

INTRODUCTION: Regenerative therapies based on stem cells derived from the stromal vascular fraction of adipose tissue (ASC) have great promises for musculoskeletal disorders. ASC are able to home on injury sites and secrete bioactive factors with anti-inflammatory and trophic effects on tissue-specific resident stem cells that in turn induce tissue repair and regeneration. The ASC efficacy can be incremented with the use of biological adjuvants, such as human platelet lysate (HPL) that can be easily obtained in large quantities from expired buffy coats and used allogenically. Moreover, the use of HPL surpass ethical and safety problems related to animal derived reagents. Our main aim was to investigate the influence of HPL on the anti-inflammatory and regenerative properties of ASC using two different in vitro models.

METHODS: ASC anti-inflammatory properties were evaluated with an osteoarthrosis in vitro model based on cartilage explants inflamed with TNFα and IFNγ. Explants were co-cultured with ASC in the presence of FBS (FBS- ASC) or HPL (HPL-ASC) and metabolism was detected using Alamar blue assay. Moreover, dorsal root ganglia (DRG) were used as a functional assay to assess ASC regenerative properties. FBS-ASC were pre-differentiated into Schwann cells (SC-ASC) and Nestin level was determined with FACS for both SC-ASC and HPL-ASC. Then, SC-ASC and HPL-ASC were co-cultured with DRG and the length of DRG extensions was measured after the immunodetection of βIII-tubuline.

RESULTS: As expected, without inflammation, the presence of HPL-ASC increased the cartilage metabolism compared to FBS-ASC. Surprisingly, the co-culture with HPL-ASC reduced the metabolism of inflamed cartilage compared to FBS-ASC. On the other hand, significantly higher levels of Nestin were detected in HPL-ASC than in SC- ASC. Moreover, DRG co-cultured with HPL-ASC showed longer extensions than DRG in the presence of SC-ASC, indicating that the regenerative ability of ASC was increased by HPL.

CONCLUSION: Our results suggest that the appealing properties of ASC can be deeply influenced by the culture conditions, such as the presence of classic FBS or the human derivate HPL. Our future plans include the proteomic analysis of FBS-ASC and HPL-ASC to assess the changes induced by HPL on anti-inflammatory and regenerative ASC properties.

ABSTRACT 41

Development of a StarPEG Hydrogel System to Promote Controlled and Sustained Delivery of Adipose Tissue Derived Stem Cells Secretome for CNS Regenerative Medicine

Presenter: Antonio Salgado (Portugal)
Affiliation: ICVS, School of Medicine, University of Minho
Authors: Lucas Schirmer; Tiffany S. Pinho; Passant Atallah; Jorge R. Cibrão; Rui Lima; João Afonso; Sandra B-Antunes; Cláudia R. Marques; João Dourado; Uwe Freudenberg; Rui A. Sousa; Carsten Werner; António J. Salgado

INTRODUCTION: Spinal Cord Injuries (SCI) affect both motor and sensorial functions of the body. One of the most promising approaches to tackle tissue regeneration lies on the development of drug delivery systems that could increase the availability of the therapeutic agent at the lesion site, while protecting it from degradation. Herein we propose the delivery of Adipose-derived stem cells (ASCs) secretome in star-shaped poly (ethylene glycol) (starPEG) in situ forming hydrogels, that when crosslinked with heparin (Hep) increase the affinity to cytokines, chemokines and growth factors promoting prolonged release.

METHODS: ASCs secretome was loaded in a 1:1 ratio (10ul secretome / 10ul starPEG-Hep) and the release profile evaluated by fluorescence intensity over 21 days as well as analytes detection by multiplex assay for 10 days. Moreover, mechanical properties of hydrogels were accessed using rheology, mesh size and swelling ratio. Additionally, bioactivity effects of the released secretome was evaluated by the capacity to promote differentiation of Neural Progenitor Cells (NPCs), as well through the assessment on axonal growth on organotypical spinal cord slices. Finally, an in vivo proof of concept study was performed in a full T8 transection model of SCI in rats.

RESULTS: Multiplex analysis allowed to identify, among others, pro-regenerative signaling mediators such as IL-4, IL-6, BDNF, GDNF, and β-NGF (Figure 1) . Regarding mechanical properties, hydrogels could assume rheological values in a range of 500Pa-3000Pa, swelling ratio 1-2 and mesh size 10-20 nm, which are within the range that is adequate to allow axonal growth. In vitro assays with NPCs show an increase in the percentage of differentiated cells in secretome-loaded hydrogels. A similar trend was also observed for axonal growth. Finally, in a complete transection SCI rat model, the secretome-loaded hydrogel significantly improved motor function by reducing the percentage of ameboid microglia and anti-inflammatory cytokines (Figure 2).

CONCLUSIONS: Under experimental conditions, these data indicate that starPEG hydrogels are a suitable system to promote controlled and prolonged release of secretome, as their characteristics could be modulated in order to improve it implantation *in vivo* to promote SCI regeneration.

ABSTRACT 41 (images)

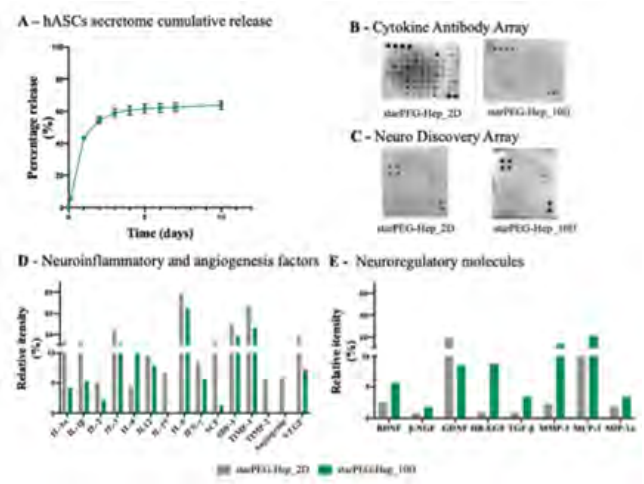


Figure 1. Characterization of hASCs secretome release from starPEG-Hep hydrogels. A- FITC-labeled secretome was loaded to starPEG-Hep hydrogels and samples were collected at 0, 3 hours, 1, 2, 3, 4, 5, 6, 7 and 10 days. Secretome was detected by fluorescence and the cumulative release was calculated over time. Values are plotted as mean + SEM from two independent experiments with seven replicates. To decipher which molecules were being released from starPEG-Hep hydrogels, a membrane based-protein array was performed of samples collected at two and ten days. B and C, show the membranes of Cytokines Antibody arrays and Neuro Discovery arrays, respectively. D and E- Relative intensity of factors involved in neuroinflammatory, angiogenesis and regeneration Values are presented as relative intensity, in percentage, for the positive control in each membrane.

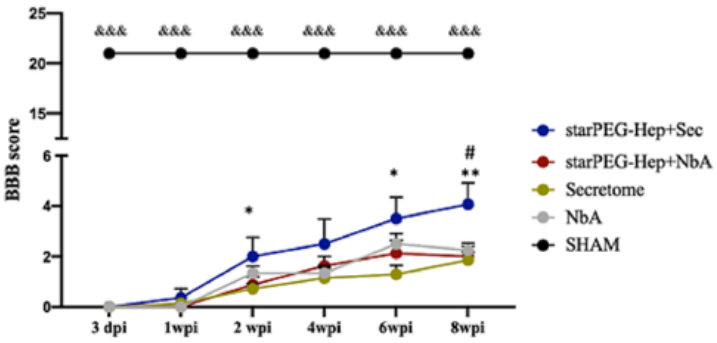


Figure 2. Evaluation of motor performance in SCI rats by BBB test for eight weeks post-injury. Animals treated with secretome presented improved motor recovery eight weeks after treatment. Mixed ANOVA; (*) represented differences between starPEG-Hep+sec and Secretome, (#) differences between starPEG- Hep+sec vs starPEG-Hep+NbA and NbA, and (&) differences between SHAM group and all other; * and #p <0.05; **p <0.001 and &&& p <0.0001. Error bars represent mean ‡ SEM.

ABSTRACT 42

Optimizing Stem Cell Based Peripheral Nerve Regeneration - Laminin Surface Coating Increases NGF Secretion of Differentiated Adipose Tissue Derived Stem Cells

Presenter: Oliver Felthaus (Germany)
Affiliation: University Hospital Regensburg
Authors: Oliver Felthaus, Lukas Prantl, Andreas Eigenberger

INTRODUCTION: Schwann cells (SCs) have been identified as the major regenerative source of the peripheral nervous system. During physiological nerve regeneration, SCs dedifferentiate in response to injury into a state which has been termed activated SC phenotype. Adipose tissue derived stem cells (ASCs) can be harvested in great numbers with minimally invasive methods. ASCs differentiated into SC-like cells can yield significant improvement of functional restitution. Although SC-like ASCs were applied for the purpose of peripheral nerve regeneration in several studies, precise phenotypes of SC-like ASCs have found little attention. In this study, various culture conditions were investigated for their ability to efficiently direct ASCs into specialized SC phenotypes. Furthermore, the capability of differentiated ASCs to promote axonal regeneration in vitro was assessed. Moreover, with regard to culture surface conditions this differentiation may be reproducible on nerve conduit materials.

METHODS: ASCs were treated with three different neural/glial differentiation protocols and on surfaces coated with different matrix proteins. Differentiation into various glial phenotypes was assessed using Real Time-RT-PCRs, Western Blots and ELISA. The influence of differentiated ASCs on neuroblastoma cell line NG108-15 was evaluated using PCRs and neurite outgrowth assay following indirect co-culture. Additionally, neurite outgrowth assays were performed after supplementation with nerve growth factor NGF or a NGF-inhibitor.

RESULTS: Treatment of ASCs with different differentiation protocols leads to the upregulation of marker genes of different glial cell phenotypes. One phenotype mimics the activated Schwann cell phenotype regarding neurotrophine secretion. Indirect Co-culture with these ASCs had the highest impact on NG108-15 neuroblastoma cell line in neurite outgrowth assays. Tretment with NGF had a similar effect on NG108-15 cells as the indirect Co-culture. Supplementation with a NGF inhibitor reversed this effect.

CONCLUSION: ASCs hold great therapeutic promise in the field of nerve regeneration. ASCs comprise the capability to mimic various functional capacities of SCs. NGF is a key to axonal regeneration. Laminin seems to increase NGF release of differentiated ASCs, and hence might be most suitable for coating nerve guidance conduits.

ABSTRACT 43

Hyperactivity of Adipose Stem Cell from an Amyotrophic Lateral Sclerosis Donor

Presenter: Lauren Kokai (USA)
Affiliation: University of Pittsburgh
Authors: Lauren Kokai, Shawn Loder, Bahaa Shaaban, Wayne Vincent Nerone

INTRODUCTION: ALS is a devastating neurodegenerative disease in which motor neurons die-back, resulting in muscle paralysis. The underlying disease pathology includes upregulated stress responses in neural support cells, activated innate immunity, fibrosis of the spinal tracts and systemic hypermetabolism. Conditioned media from human adipose regenerative cells contains an abundance of anti-inflammatory and immune-modulating molecules and has shown utility in mouse ALS models, but the effects were short-lived. Therefore, we hypothesized that direct use of ASCs might provide more durable effects. To test feasibility, we isolated ASCs from a patient with ALS and compared in vitro regenerative capacity to age and sex matched controls.

METHODS: Approximately 80cc of lipoaspirate was obtained from a 71-yo male with ALS under an IRB approved protocol. Matched controls were obtained as discarded tissues from elective body contouring procedures under IRB exemption (N = 4 unique donors). After tissues were enzymatically digested, nucleated cell quantity and viability were measured. Cell proliferation rate of ASCs was measured over several passages. At passage 2-3, ASC metabolism, mitochondrial stress, amino acid toxicity and adaptive response to oxidative insults were measured. Finally, an unbiased metabolomic analysis was performed with Bruker LC-timsTOF Pro PASEF.

RESULTS: ASCs from an ALS donor had significantly increased cell proliferation rate compared with age and sex matched controls. Overall, cell metabolism in the ALS-ASCs was also significantly increased, with elevated levels of peroxides and nitric oxide in cell culture media and increased oxygen consumption rate measured with a Seahorse Bioanalyzer. ALS-ASCs further had significantly increased cell stress including lipid accumulation, elevated production of extracellular ATP and increased ceramides. Analysis of amino acid toxicity revealed ALS-ASCs had sensitivity to lysine and glutamate, a neurotransmitter involved in ALS pathology.

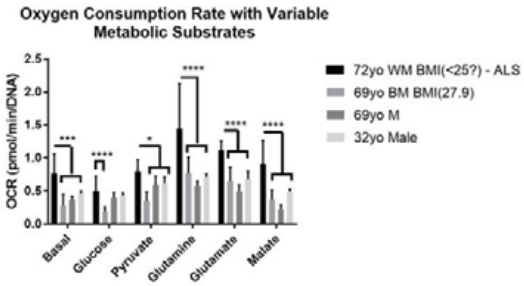
CONCLUSION: Our data from a single ALS donor suggests that ASCs obtained from non-neural tissue, i.e. adipose, maintained elements of underlying disease and had elevated levels of cellular stress in culture. Therefore, further analysis of ASCs from additional ALS donors is warranted to determine if ASCs maintain accumulated stress phenotypes after ex vivo expansion, an indication that autologous cell therapy should be avoided in this population.

ABSTRACT 43 (images)

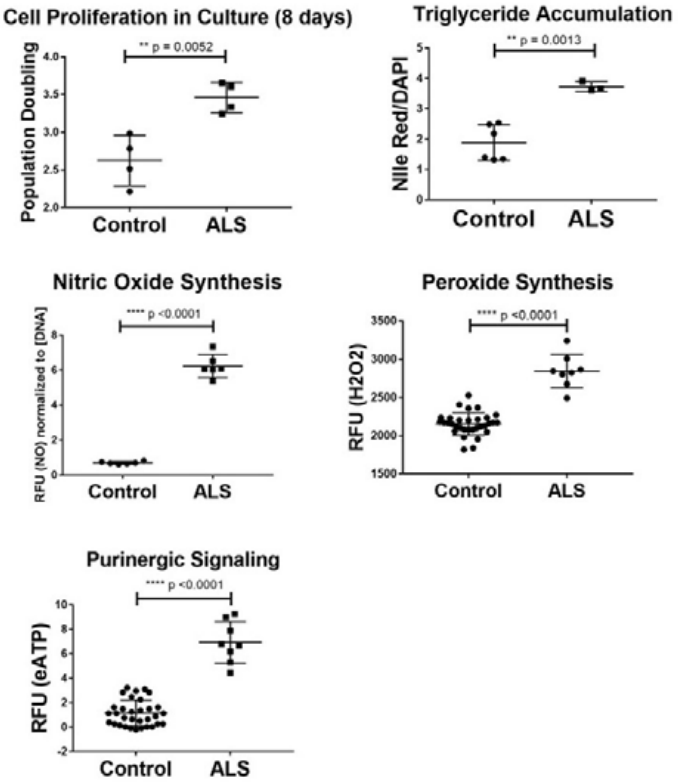
A.

	Demographics	
	Age	Sex
ALS	71	M
Controls	49 - 84	M

B.



C. Cell "Danger" Response



ABSTRACT 44

Effectiveness of Cell Therapy Treatment Applied to a Patient with Lumbar Radiculopathy, Following Failed Lumbar Surgery

Presenter: Ramiro Ramirez (Mexico)
Affiliation: Medyca Bosques
Authors: Ramiro Ramirez, Dra Daniela Flores

INTRODUCTION: Lower back pain is a very common symptomatology, occuring in up to 90% of the population, 70% of these cases resolve themselves on their own in 3 weeks, and 90% resolve themselves in 3 months, without the need of invasive intervention. However, a small percentage of cases do not improve with conservative treatment and require surgery, that can be anything from a simple microdiscectomy to a spinal fusion.
It is a common occurrence that the lumbar surgery fails, causing an economic burden on both the patient and their families. In the USA, 200,000 discectomies are performed annually, 60% of these surgeries result in a complete cure, 40% of patients remain with residual pain, and 15% end up as a failed surgery, resulting in up to 30,000 failed surgeries a year. Mesenchymal cell therapy has demonstrated its great analgesic and anti-inflammatory effects in the spine, especially in spinal fusions.

METHODS: Case report: Effectiveness of placental mesenchymal cells, activated for nerve tissue cells and bilateral transforaminal L5 autologous Nanofat infiltration, in a patient with persistent sciatic pain (Not responsive to surgical treatment, rehabilitation, and post-operative infiltrations).

RESULTS: The results will be measured using the Oswestry low back pain disability scale and visual analogue scale. A monthly report will be given to evaluate the changes in these scales.

CONCLUSION: It is essential to explore new treatment alternatives for our patients using advanced minimally invasive technology methods, with the least amount of side effects. For this we give ourselves the task of evaluating the response of these patients to the treatments by carrying out a constant report and monitoring, recording results and thus obtaining evidence of the effectiveness of cell therapy.

ABSTRACT 45

Increased Angiogenic Differentiation of HUVECs Treated with Pre-conditioned Media from Lipedema Adipocytes in Vitro

Presenter: Sara Al-Ghadban (USA)
Affiliation: University of North Texas Health Science Center
Authors: Sara Al-Ghadban, Samantha G. Walczak, Bruce A. Bunnell

INTRODUCTION: Lipedema is a connective tissue disorder characterized by an increased number of dilated blood vessels (angiogenesis), fibrosis, inflammation and accumulation of interstitial fluid in the subcutaneous adipose tissue (SAT). This project aims to gain insights into the lipedema angiogenesis processes using human umbilical vein endothelial cells (HUVECs) as an in vitro model of angiogenesis. The overall goal of this study is to determine the effect of cellular interaction between HUVECs and adipocytes from healthy and lipedema adipose-derived stem cells (ASCs) on promoting angiogenesis a co-culture system that mimics SAT.

METHODS: HUVECs were cultured in conditioned media (CM) collected from healthy and lipedema adipocytes. The effects on the expression of the endothelial and angiogenic markers [CD31, von Willebrand Factor (vWF), Angiopoietin 2 (Ang2), Hepatocyte Growth Factor (HGF), Vascular Endothelial Growth Factor (VEGF), Matrix Metalloproteinase (MMPs), NOTCH and its ligands] in HUVECs were investigated in 2D monolayer and 3D cultures. Real-time polymerase chain reaction (RT-PCR), flow cytometry and Western blot assays were used to assess the expression of endothelial and angiogenic markers in HUVECs. In addition, the ability of HUVECs to form capillary-like tubes when seeded on Matrigel in 2D was determined by phase-contrast microscopy and the number of tubes formed (number of nodes and branches) was quantified using NIH ImageJ software.

RESULTS: Increased expression of CD31 and Ang2 were observed at both the gene and protein level in HUVECs treated with CM from lipedema adipocytes in 2D monolayer and 3D cultures compared to untreated cells. The number of detected tubes were increased in treated HUVECs cultured in a 2D monolayer. No changes in the gene expression of vWF, VEGF, MMPs, NOTCH was detected; however, Delta 4 and Notch 4 protein expression was increased in HUVECs treated with lipedema CM.

CONCLUSION: These data indicate that lipedema adipocyte-CM promotes the vascular tube formation of endothelial cells through paracrine mechanisms. In addition, the expression of multiple factors that induce angiogenesis was upregulated after exposure to conditioned media from lipedema adipocytes. The definition of the pathways that enhance angiogenesis in lipedema tissues will help researchers develop new therapeutic approaches to treat this disease.

ABSTRACT 46

Cell Culture Conditions Influence Angiogenic and Adipogenic Properties of Adipose Stem Cells

Presenter: Anne Therese Lauvrud (Sweden)
Affiliation: Umeå University
Authors: Anne Therese Lauvrud, Maria Brohlin, Rebecca Wiberg, Mikael Wiberg, Paul J Kingham

INTRODUCTION: Fat grafting is widely used in reconstructive surgery but graft retention is highly variable and enrichment with adipose stem cells (ASC) has been suggested as a way to improve angiogenesis and adipogenesis. In this study we have investigated how these properties are influenced by in vitro culture conditions and how this correlates with expression of CD146+ ASC sub-populations.

METHODS: Human lipoaspirate samples (n=7 female donors) were digested with collagenase NB4 to yield the stromal vascular fraction cells which were then seeded in A) Minimal Essential Medium-α (MEM-α) containing 10% (v/v) foetal bovine serum, B) MEM-α containing 2% human platelet lysate (PLT) or C) PRIME-XV MSC Expansion XSFM (a xeno- and serum-free medium; FujiFilm Irvine Scientific). Groups A and B were cultured on polystyrene flasks (Nunc™ EasYFlask™) and group C on Corning® CellBIND® flasks. Flow cytometry for ASC markers CD73, CD90 and CD105 together with CD146 was performed at passages 1 and 7. Growth rates were monitored by regular passage and re-plating prior to cultures reaching confluence. Angiogenic gene expression was analysed at early and late passage. Expanded ASC were treated with an adipogenic cocktail and analysed for adipokine secretion and stained with Oil Red O.

RESULTS: ASC under all culture conditions were expanded through 90 days in vitro at which point growth arrested. Cells in FBS and PLT grew at similar rates whereas the cells cultured in PRIME-XV proliferated significantly faster with 4-5 population doublings every 4 days. All cultures were >98% positive for CD73, CD90 and CD105 whereas CD146 expression was significantly different between groups (FBS 19±5%, PLT 4±2% and PRIME-XV 28±4% at passage 1). Angiogenic gene expression was variably influenced by the culture conditions. For example, HGF showed 90 fold higher expression in PRIME-XV versus FBS and IGF1 30 fold higher expression in PLT versus FBS. Compared with FBS, the PLT expanded cells showed inferior adipogenic differentiation and the PRIME-XV expanded cells were superior.

CONCLUSION: Culture under xeno- and serum-free conditions supports rapid cell expansion and maintains cells with high adipogenic potential. Consistent with our previous findings, CD146 expression levels correlate with the adipogenic potential of ASC.

ABSTRACT 47

Transcriptomic Variations of Endothelial Progenitor Cells from Adipose, Bone Marrow, Cord Blood and Peripheral Blood

Presenter: Srinivas Koduru (USA)
Affiliation: Penn State, College of Medicine
Authors: Srinivas Koduru, Dino J Ravnic

INTRODUCTION: Endothelial progenitor cells (EPCs) are known to be highly specific and important regulatory cells for blood vessel development. As they can easily differentiate into endothelial cells (ECs) and form vascular networks both in vitro and in vivo, EPCs hold immense promise to regenerative medicine. Although there has been some disagreement over their true definition, it now appears that specific phenotypes do exist and that harvest is possible from various anatomic sites, including adipose tissue (AT), bone marrow (BM), cord blood (CB) and peripheral blood (PB). Our study aimed to determine the transcriptomic differences of human EPCs (CD34+) for data mining.

METHODS: Raw RNA sequencing data downloaded from the NIH BioProject and files were converted to fastq files using to SRA tools. Fastq files were align to hg38 using STAR v2.7.8a aligner and annotated using ensembl v106 with stringent statistical methods applied (FDR <0.05). Data was mined by PartekFlow v10 on linux based HPC and also MetaCore software used for in-depth pathway analysis i.e., Pathway Maps, Go Processes, Process Networks, diseases by biomarkers and Relevant networks individual and comparative analysis between four groups.

RESULTS: AT-EPCs vs BM-EPCs showed most were downregulated in AT-EPCs. Specifically, 20,127 genes were differentially expressed with 12,956 mRNAs downregulated in AT-EPCs and the remaining 7,172 mRNAs upregulated. When AT-EPCs were compared to cord blood EPCs, vast variability in gene expression levels were similarly noted with 17,188 genes with 10,268 were downregulated and 6,920 upregulated in AT-EPCs. Comparing AT-EPCs to PB-EPCs showed a similar gene expression (20,676 mRNAs with 15,902 mRNAs downregulated and 4,774 upregulated). Interestingly, biological pathway analysis revealed that the AT-EPC GEP leaned towards the VEGF/VEFGR signaling network (25.1 % of genes).

CONCLUSION: EPCs are immensely valuable for tissue engineering and regenerative medicine. However, their harvest can be cumbersome (e.g., BM) and low-yield (e.g., PB). AT represents an abundant and easily accessible that mitigates these concerns. While our data analysis showed that the gene expression of AT-EPCs is markedly different and skewed towards vascular signaling. This suggests that AT-EPCs may be suitable for targeted vascular repair both in vitro and in vivo.

ABSTRACT 48

Influences of Oxygen Tension on Human Adipose-Resident Microvascular Endothelial Progenitor Cells

Presenter: Natsumi Saito (Japan)
Affiliation: Jichi Medical University
Authors: Natsumi Saito, Kotaro Yoshimura

INTRODUCTION: Vascular endothelial progenitor cells (EPCs) are one of important cell populations to play pivotal roles in angiogenesis and wound healing. We reported purification of microvascular EPCs from human lipoaspirates (adipose-resident microvascular EPCs; AEPCs) (Saito N., et al., Sci Rep, 12:1775, 2022). Since endothelial cells are generally an oxygen sensitive cell population, we cultured AEPCs at different oxygen concentrations and evaluated the cellular profiles such as stemness, cellular senescence and endothelial marker expressions.

METHODS: Stromal vascular fraction (SVF) was extracted from lipoaspirates through regular enzymatic digestion. AEPCs were isolated from SVF with combination of magnetic-activated cell sorting (MACS) using CD31/CD45 microbeads and adhesive cell culture. The AEPCs were cultured in three different oxygen concentration, 1) humid air, 2) 6%-O2, and 3) 1%-O2, with 5% CO2. We performed characterization of these AEPCs; proliferation, morphology, colony forming unit (CFU) assay, and expression of senescence and endothelial markers.

RESULTS: Stem cellularity was maintained up to 9 passages (P9) under all oxygen conditions. Colony size remained small under 1%-O2 culture. On the other hand, under conditions both air and 6%-O2, colony size tended to increase as the number of generations progressed. The expression of a senescence marker indicates the cellular senescence of AEPCs (P9) of air condition. AEPCs stronger expressed an endothelial cell marker, von Willebrand factor, and binding capacity of isolectin-B4 at 1%-O2 culture than air and 6%-O2.

CONCLUSION: The AEPCs appeared to be better cultured in hypoxic conditions for therapeutics.

ABSTRACT 49

The Fat and the Furious: How Different Oil Preparations Alter Local Inflammatory Patterns

Presenter: William Molair (USA)
Affiliation: Wake Forest University
Authors: William Molair, Adam Katz, Ramon Llull

INTRODUCTION: Free lipids and fatty acids (FAs) have appeared in recent literature as pro and anti-inflammatory, as well as angiogenic. Understanding the nature of these compounds is critical for plastic surgeons, as their presence in fat grafts, especially micro and nano fat grafts, is a near certainty. While adipose progenitor cells are usually implicated as the key effectors of fat grafts, it is essential to understand the biological activity of oils within the graft. To further investigate the effects of adipose-derived oil on tissues, we present a novel quantitative model of inflammatory states within the abdomen of mice that have been exposed to various oil preparations.

METHODS: C57BL6 mice were intraperitoneally injected with 0.5mLs of normal saline (NS), cell-free adipose-derived human oil, emulsified human oil, sunflower oil, or pristane. They were given weekly injections of up to 3mL of NS with subsequent paracentesis to remove any ascitic fluid and NS. Cells were isolated from the ascitic fluid and phenotypically characterized via flow cytometry. Samples were collected weekly and used to develop an 'inflammatory' profile and time course for different oil preparations and controls.

RESULTS: Mice injected with saline served as a negative control group and those injected with pristane served as a positive control group. Saline injected mice showed moderate increases in monocytes from week one to four. Pristane injected mice displayed enrichment of neutrophils in their ascitic fluid from week 2 onwards. Mice injected with sunflower oil and emulsified human oil displayed components of chronic inflammation, primarily enriching monocytes and B Cells. Emulsified oil mice displayed a stronger immune response than their non-emulsified counterparts, showing a more pronounced enrichment of monocytes.

CONCLUSION: Our results demonstrate that different lipids stimulate the proliferation and/or homing of different inflammatory cell types, but also that emulsified lipid is potentially more inflammatory than non-emulsified human lipid. This may be related to the presence of emulsified micelles. These results support the bioactivity of lipids and free fatty acids and may implicate their role in a variety of adipose-derived therapies. Further research will elucidate the mechanism by which these lipids are capable of selecting for specific immune-competent cell types.

ABSTRACT 49 (images)

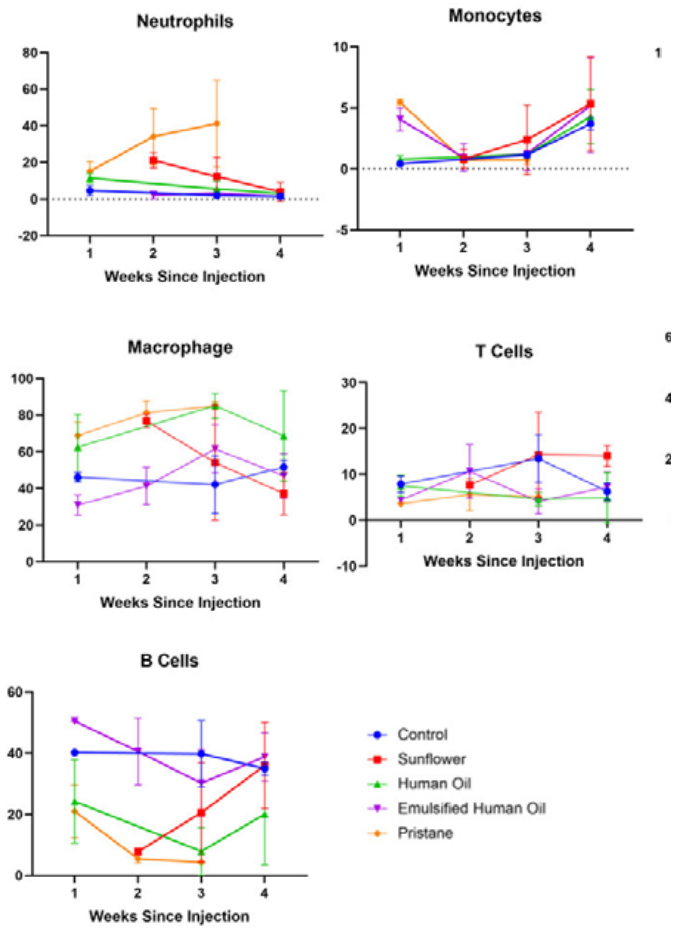


Figure 1. Flow Cytometry of Ascitic Fluid. Ascitic fluid and recovered normal saline were centrifuged to remove any potential immune cells. These cells were then stained and characterized via flow cytometry in order to create a longitudinal record of the inflammatory pattern generated by the injection of different oil species into the peritoneal cavity of mice.

ABSTRACT 50

Oral Vitamin D Improves Fat Graft Survival in a Large Animal Model

Presenter: Bahaa Shaaban (USA)
Affiliation: University of Pittsburgh
Authors: B Shaaban, SJ Loder, PL Lee, WV Nerone, C Amurgis, R Ricketts, D Ramkumar and LE Kokai

INTRODUCTION: A major limitation of fat grafting is unpredictable retention. We previously demonstrated that bioactive Vitamin D (VD3), calcitriol, improves human fat graft retention in a xenograft mouse model, however, use of high dose calcitriol incurs safety risks as key metabolic controls are bypassed. The overall goal of this study was to verify mouse model data that inactive Vitamin D3 (VD3), cholcalciferol, similarly protects adipose stromal cell viability during vascular deficiency post-grafting and improves long term retention outcomes with a porcine model. Our outcomes aim to define a clinical protocol for direct translation of VD3 into practice.

METHODS: Human adipose was collected as lipoaspirate from discarded surgical samples under IRB exemption and cultured ex vivo for 7-days with or without VD3. The residual tissue was enzymatically dissociated to release mature adipocytes and the stromal vascular fraction (SVF), which was evaluated for viability, identity, proliferation, and mitochondrial stress with Seahorse Bioanalyzer. An immunocompromised xenograft mouse model was used to determine survival, retention, and histologic characteristics of VD3 treated vs. untreated fat. Finally, oral VD3 was assessed in a porcine model of engraftment after 3 months. Graft volume and retention were serially assessed by ultrasound and confirmed by gas pycnometry.

RESULTS: ex vivo, adipose tissue-cultures demonstrated greater viability when treated with VD3; cytometric analysis demonstrated significant enrichment of endothelial and pericyte populations. Further, SVF treated with VD3 demonstrated enhanced spare mitochondrial respiratory capacity and survival under acute hypoxic conditions. Xenografted lipoaspirate demonstrated enhanced survival of human cells, reduced fibrosis, increased host-derived vascularity, and increased host-derived adipocyte replacement at 12 weeks with intraperitoneal VD3. Pigs which received oral VD3 therapy further demonstrated significantly enhanced retention of allografted fresh adipose throughout the 3-month study period.

CONCLUSION: Inactive Vitamin D3 significantly increases viability of the vascular and perivascular components of hypoxic adipose tissue. In vivo, VD3 significantly improved graft retention in both xenograft-murine and porcine models, likely through increased recipient bed revascularization and adipocyte replacement. These findings support use of VD3 to minimize graft resorption and improve clinical outcomes.

ABSTRACT 51

Platelet Rich Plasma Assisted Adipose Grafting for Scars and Aesthetics

Presenter: Sherry Collawn, MD (USA)
Affiliation: University of Alabama
Authors: Sherry Collawn, Gagandip Singh, Ann Carol Braswell

INTRODUCTION: The combination of adipose tissue supplemented with platelet rich plasma (PRP) is used for volume and skin improvement. For volume replacement patients have had successful results. Burn scars and burn scar hypertrophy have been improved.

METHODS: This retrospective chart review covers a total of 41 fat graft/ PRP procedures performed from October 2019 through July 2022 (Figure 1). Of these, 11 facelifts were grafted with fat/PRP. Two more cases were for fat/PRP grafting nose. Three cosmetic buttock fat grafting cases were performed. Five breast procedures were for removal of implants with fat grafting/PRP breasts. One was for HIV facial atrophy cheeks. Seven procedures were for burn scars face, neck, and breast. Eight procedures were for hypertrophic scars in various areas of the body, including the face, back, neck, and upper extremities. Four were for keloid excision sites. For hypertrophic scars and keloids injections of five fluorouracil and kenalog were also injected during the same session. Fat was generally harvested from the abdomen, thighs, or flanks using an enclosed power-assisted system or toomey syringes with 3.7 or 3.0mm cannulas, then washed and emulsified. Injection in the face, neck, and some scars was with the 0.9mm Tulip single port injection cannula and/or 18 gauge needle for thick hypertrophic scars at a ratio of 0.8 fat/0.2 PRP.

RESULTS: Of the face grafts, the average amount of fat grafted was 20 ml. The patient in Figure 2 shows the excellent improvement in periorbital fat/PRP grafting 4 months after facelift with injection of 25 ml fat/PRP injected in the face and less than 1 ml nose. Scleral show dramatically decreased with fat grafting and nasal contour improved.

CONCLUSION: Patients have had successful results with volume improvement and reduction of scar deformity. There was a complication of cellulitis in one fat graft breast patient. There were no cases of embolization. In conclusion fat grafting combined with PRP is a safe and reliable method for volumization and scar improvement.

Figure 1

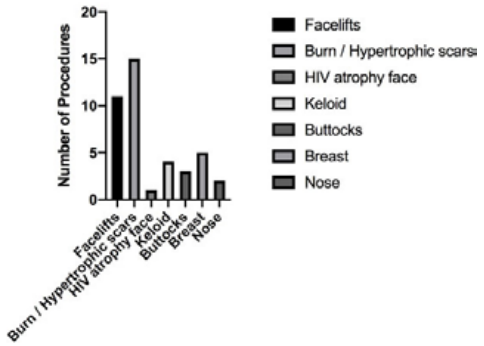


Figure 2

ABSTRACT 52

Evaluation of the Efficacy of Stromal Vascular Tissue and Microfat injection in Periorbital Improvement of Pigmented Dark Circles, Hollow Ring, and Loss of Palpebral Elasticity

Presenter: Sophie Menkes (Switzerland)
Affiliation: Necsens Clinic
Authors: Sophie Menkes

INTRODUCTION: Currently, treatments for pigmented eyelids are not very effective. The treatment of the hollow ring is treated by fillers but involve contraindications and a significant rate of complications in the periorbital region. Microfat and Stromal vascular tissue are very promising alternatives in these indications. We want to evaluate the effectiveness of this technique specifically in the periorbital region in the indications of aesthetic rejuvenation: hollow rings, pigmented eyelids, loss of palpebral elasticity.

METHODS: Prospective multicenter clinical study of 10 patients with pigmented rings, loss of elasticity and hollow rings The harvesting technique consists of infiltration of a mixture of Nacl and xylocaine adrenaline into the adipose tissue and then harvesting in a closed system the adipose tissue. The sample is then rinsed to obtain a volumizing fat of Microfat necessary for filling This fat is then emulsified and filtered with a Tulip kit to obtain an emulsion with regenerative capacities.
These two samples are then reinjected into the periorbital region to fill in the eyelids on the one hand and treat the loss of elasticity and coloring on the other. Evaluation by regular follow-up on photos and self-evaluation questionnaire over 6 months Evaluation of the cell viability and the number of stem cells in the samples

RESULTS: This technique seems to be very promising for the treatment of pigmented eyelids and loss of skin elasticity. It can be applied to the entire face. It allows in the same operating time to fill the periorbital region by Microfat by increasing the viability of adipocytes.

CONCUSION: The treatment of the periorbital region by Microfat and Stromal vascular tissue is a simple and painless technique, in a closed system, without centrifugation, avoiding any risk of contamination.

ABSTRACT 53

Clinical Outcomes of Laser Assisted Liposuction for Body Contouring and Facial Fat Graft Under Local Anesthesia

Presenter: Naoko Hitosugi (Japan)
Affiliation: Muse City Clinic
Authors: Naoko Hitosugi , Mohamed Abdelhakim, Kyoko Dogo

INTRODUCTION: Laser-assisted liposuction emits light energy preferentially absorbed by water resulting in a rapid and localized contouring and skin tightening effect, with minimal scarring. When collected under appropriate conditions, extracted fat samples can be utilized as autologous filling material in fat sculpturing procedures.

METHODS: Our objective is to assess the 1-month contouring efficacy of the laser-assisted liposuction (LAL) carried out under local anesthesia and the volumetric enhancement effect of the harvested tissue in facial fat grafting. 10 female subjects aged between 40 to 50 years old, of body mass index 25-30 underwent liposuction (BeautiFill, Alma Lasers, Inc.) of the lower abdomen. The harvested samples were processed using FILTRON® 250cc and grafted into facial region. Volumetric calculations were then performed using a 3D image analysis application (3D LifeViz Infinity) to circumscribe areas, orient dimensions, and calculate volumes of the malar fat pad (MFP). Treatment safety, body weight, blinded evaluator-assessed aesthetic improvements, and subject-rated satisfaction were also monitored.

RESULTS: One-month post-treatment, most subjects ranked improvements good/excellent and skin tightening satisfactory/very satisfactory (80%). Blinded evaluators noted improved/very much improved aesthetic appearance (87%). Harvested tissue injected as a facial filler led to increase in facial fat thickness. These data revealed significant difference in the MFP volume changes before and after fat injection ($p < 0.05$).
Post filling follow-up showed that the majority of subjects (80%) were satisfied with the outcome. All procedures were well-tolerated.

CONCLUSION: A single laser-assisted abdominal liposuction session under local anesthesia provided an effective and durable reduction of adipose tissue, with skin tightening and aesthetic improvements. The gentle lipofilling method yielded viable filler material, which was well-retained in the injected facial regions. Adipose derived stem cells provides rejuvenate effect in the injected facial area yet further studies are needed to elucidate the mechanism.

ABSTRACT 54

Swiss Regulations on the Transplantation of Adipose Tissue and Stromal Vascular Fraction

Presenter: Carlo Oranges (Switzerland)
Affiliation: Geneva University Hospitals
Authors: Carlo Oranges, Mathias Tremp, Daniel F. Kalbermatten

INTRODUCTION: Different legislations regulate the use of fat grafting among Countries. The Swiss Society of Plastic Surgery has recently submitted a scientific report allowing the competent authorities to elaborate the legal basis on fat transplantation. This paper aims at presenting the Swiss regulatory system, at verifying how it reflects the current scientific knowledge and providing elements for an international comparison.

METHODS: We analyzed the document of the Swiss Agency for Therapeutic Products (Swissmedic) entitled "Adipose tissue and stromal vascular fraction for autologous transplantation," which provides a comprehensive legal context on the use of fat grafting. The most recent authoritative literature on the subject has been identified to confirm the validity of the regulations.

RESULTS: Swissmedic classifies adipose tissue and stromal vascular fraction (SVF) transplantation in two legal groups, further divided into two groups: 1. "autologous transplants", in case of minimal manipulation and homologous use, and "transplant products" in case of functional change or non-homologous use. Adipose tissue/SVF prepared with mechanical methods, including filtration, centrifugation or decanting, are considered as autologous transplants, unless the physiological properties or the function of the tissue or cells are altered. In reverse, transplant products include enzymatically prepared adipose tissue/SVF. Autologous transplants are regulated by the Transplantation Act, implying the obligation to notify Swissmedic for tissue preparation and storage, but only in case of "later use", while the intraoperative use is excluded. Companies and institutions manufacturing or dealing with transplant products need establishment licenses from Swissmedic. The use of transplant products in patients or their standardizable manufacturing process must be authorized by Swissmedic, or their use is only allowed in the context of controlled clinical trial approved by Swissmedic.

CONCLUSION: According to the Swiss regulations, adipose tissue and SVF mechanically prepared and used intraoperatively for homologous use and without functional change are authorized with no need for notification or approval. In reverse, adipose tissue or SVF enzymatically prepared and/or used for delayed transplantation, with functional change, must be notified and authorized. This reflects current knowledge and recommendations from authoritative scientific sources.

ABSTRACT 55

BonoFill from Bench to Bedside: A Novel Tissue-engineered Product Generated from Adipose-derived Mesenchymal Stromal Cells in Line to Replace Bone Autografts for Large Segmental Bone Defect Applications

Presenter: Dror Ben-Davi (Israel)
Affiliation: Bonus BioGroup
Authors: Dror Ben-David , Atara Novak, Tomer Bronshtein, Nimrod Rozen, Ephraim Tzur, Vered Kivity, Shai Meretzki

INTRODUCTION: Significant losses of bone tissue, too large to heal, occur in ≥10% of all open fractures and present an urgent clinical challenge associated with grave morbidities and substantial healthcare costs. Bone autografting, the therapeutic standard in such cases, has a 50% complication rate, with persistent infection or non-union in 18% of the patients and further surgery required in 36%. Bone tissue engineering technologies have been widely investigated as an alternative to autografts. Such technologies were tested in segmental animal bone defects, with success reported mostly for defects under 2.5 cm. Considering the larger defects encountered in the clinic, with a mean of 5.5 cm, Bonus BioGroup developed BonoFill, an autologous tissue-engineered bone graft based on adipose-derived mesenchymal stromal cells (MSC) for the treatment of defects above 2.5 cm.

METHODS: Bonus BioGroup utilizes proprietary technology to isolate MSCs from patients' lipoaspirates, seed them on mineral scaffold particles, and culture them in a specially designed bioreactor. The expansion of the scaffold-seeded MSCs was shown to increase their osteo-inducibility, allowing for a brief osteoinduction while retaining additional regenerative functions. This is exemplified by the higher levels of genes related to osteogenesis, angiogenesis, and ECM remodeling expressed by 3D-osteoinduced MSCs, compared to the 2D ones. The osteo-induced cells seeded on mineral particles are formulated into the final product delivered to the patient fresh but with sufficient shelf-life to support global supply.

RESULTS: BonoFill applied to a 3.2 cm defect in a sheep tibia, led to a full recovery in 12 weeks (N=7). A phase II clinical trial is currently underway testing BonoFill in patients with bone defects ≥2.5 cm. Patients with defects up to 8.5 cm long and history of 2-7 failed prior interventions were treated thus far with encouraging safety and efficacy outcomes. BonoFill is also tested in Phase II trial in patients requiring a bone transplant in the upper maxilla with near complete success reported to date.

CONCLUSION: Overall, the solid underlying science and innovative technologies developed by Bonus BioGroup position BonoFill as an exciting alternative to bone autografting.

ABSTRACT 56

Clinical Outcomes of Mechanically Isolated Autologous Stromal Vascular Fraction/Cellular Fraction in Patients with Osteoarthritis of Knees

Presenter: Vinod Jain (India)
Affiliation: Sahaj Hospital, Indore
Authors: Vinod Jain, Raj Sharma

INTRODUCTION: Osteoarthritis of the knee joint is a debilitating condition in which there is degeneration of articular cartilage. Total knee replacement is the last option after failure of conservative treatment. But recently, regenerative therapy has shown very promising results in the treatment of osteoarthritis of the knee with Kellgren-Lawrence Grade I to III. We had undertaken the study with an aim to evaluate the safety, efficacy, functional and clinical outcomes of intra-articular implantation of autologous adipose-derived stromal vascular fraction or cellular fraction isolated using Australian Patent technology (mechanical method).

METHODS: The present prospective observational study was conducted over a period of 3 years. 58 patients (115 knees) underwent intra-articular implantation of autologous stromal vascular fraction or cellular fraction. Knee Injury and Osteoarthritis Outcome Score (KOOS) was used to assess short and long-term consequences of knee injury. Visual Analogue Scale (VAS) used for pain assessment. The adipose-tissue was harvested from the abdominal area using tumescent anesthesia. The harvested tissue was processed using Australian Patented Technology to isolate stromal vascular fraction or cellular fraction. Comparison of mean KOOS and VAS at different follow-ups was done using Paired 't' test. A p value of <0.05 was considered as significant.

RESULTS: 84.5% were in grade 3 osteoarthritis. Female preponderance was seen (62.1%). Half of the population was overweight. Hypertension was the commonest comorbidity seen followed by diabetes mellitus type-2 and hypothyroidism. There was a significant improvement in KOOS and VAS scores from preoperative level at all the follow-ups ($p<0.05$). All the subscales of KOOS also showed significant improvement ($p<0.05$). No adverse effects were seen in any of the patients.

CONCLUSION: For individuals with degenerative osteoarthritis, autologous SVF or cellular fraction grafting in a same surgical procedure is an innovative and promising therapy option. Even after 3 years, this treatment still has shown a good clinical and functional outcome.

ABSTRACT 57

Microfragmented Fat (MFAT) and BCP for Alveolar Cleft Repair: A Prospective Control Clinical Trial

Presenter: Sabrina Natsir Kalla (Indonesia)
Affiliation: Hasanuddin University
Authors: Sabrina Natsir Kalla, Abul Fauzi, Andi Tajrin, Rifaat Nurrahma, WEG Müller, HC Schröder, XH Wang, Tymour Forouzanfar, Marco N Helder, Muhammad Ruslin

INTRODUCTION: Inorganic bone substitutes such as biphasic calcium phosphate (BCP) are alternative or adjunct to autologous bone grafting in alveolar cleft reconstructions. We hypothesize that BCP can be made osteoinductive by addition of mesenchymal stem cells (MSCs) that are present in adipose tissue.

METHODS: In this study, mechanical fractionation of adipose tissue is used namely microfragmented fat (MFAT), thus avoiding any enzymatic processing. MFAT has shown to have high regenerative capacity due to high pericyte and MSC content and a preserved perivascular cell compartment in micro vessels. The aim of this study was to evaluate the feasibility and safety of the BCP-MFAT combination. This prospective non-blinded first-in-man clinical pilot study enrolled 8 patients (mean age 22.13: range 17-32 years) with residual alveolar bone cleft receiving combination of BCP and MFAT. Patient follow-up was six months. Outcome parameters included safety parameters and close monitoring of possible adverse effects using radiographic imaging, regular blood tests, and physical examinations. Osteoinductivity evaluation using histomorphometric analysis of biopsies was not possible due to COVID-19 restrictions.

RESULTS: All of the patients received BCP and MFAT. Six out of eight patients were assessed up to day 90, two out of eight patients missed the follow up day 90 due to Covid-19 restrictions. Four out of eight patients were able to continue with the final assessment day (day 180). Two out of eight were unable to reach the hospital for follow up day 180 due to Covid-19 restrictions. Two patients decided not to continue with the study. There was no allergic reaction, or remarkable local or systemic side effects noticed in all of the patients. The Bergland scale score of radiographic assessment of all patients ranging from I to III.

CONCLUSION: Therefore, our results indicate that BCP and MFAT graft appear to be safe and feasible graft materials for alveolar cleft reconstruction.

ABSTRACT 58

Nanofat Intraarticular Injections Can Decrease Knee Pain and Delay Knee Replacement Surgery

Presenter: Madis Rahu (Estonia)
Affiliation: Tart University Hospital
Authors: Madis Rahu, Terje Arak, Leho Rips, Mihkel Luik, Tauno Koovit

INTRODUCTION: The hypothesis of the present study was that standardized injection of nanofat close to the Hoffas fat pad relieves pain and decreases functional disability and might provide an opportunity to delay knee arthroplasty in patients with knee OA.

METHODS: 10 patients (5 male, 5 female); mean age 48.9 years (range 30 - 61); 4 right, 6 left knees were included in the study.
The inclusion criterias for patients were Kellegren gr 2 - 3 knee arthrosis, who not responded effective to other intraarticular injections like PRP, hyaluronic acid and to physiotherapy program during six months and they are on the waiting list for total knee arthroplasty. All nanofat intraarticular injections were done under endoscopic visualization close to the Hoffa fat pad. Knee was fixed after nanofat injection with compression bandage in straight position for 3 days.
Clinical evaluation was performed using VAS, Oxford and KOOS scoring systems at inclusion and at 12 months after nanofat injection.

RESULTS: In 5 cases Kellegren and Lawrence grade 3 and in 5 cases grade 2 arthrosis was found at inclusion on MRI, Outerbrigde grade 4 changes were found in 8 cases and grade 3 changes in 2 cases. At 12 months significant impovment in VAS (p- 0.040) was found, All other variables did not show any significant improvement. Total knee arthroplatsy was still delayed in 8 patients one year after nanofat injection.

CONCLUSIONS: At the 12 month follow-up, nanofat intraarticular injection close to the Hoffa's fat pad appears to decrease pain measured as VAS. Furthermore, it appears to have the possibility to delay total knee arthoplasty.

ABSTRACT 59

Role of Adipose Derived Stem Cell for Osteoarthritis of Knee and Hip Joint: Myth or Reality

Presenter: Kumar Ashok, MD (Dubai)
Affiliation: My Doc Specialist Medical Centre DMCC
Authors: Kumar Ashok

INTRODUCTION: Mesenchymal Stem Cell (MSC) therapy in osteoarthritis has been hailed as a promising treatment for osteoarthritis due to their unlimited potential of healing and regeneration. Existing literature regarding their proper name, optimal sources, mechanisms of action, dosage, and route of administration, efficacy, and safety is debatable.

METHODS: Ten patients with 12 joints having symptomatic grade 2-3 (KL) osteoarthritis of hip and knee were included in the study. All the patients received single intra-articular injection of adipose derived culture expanded stem cell (20× 106) under ultrasound guidance.

RESULTS: There were 4 female, 8 male; average age was 43 yrs; minimum follow-up was 12 months. All the patient showed improvement in VAS pain and WOMAC score, and quality of life. No serious adverse events were observed.

CONCLUSION: The author believes that Maintenance Stem Cells (MSC) may be a more suitable term than mesenchymal stem cell or medicinal signaling cells as their origin might not be limited to mesodermal tissue. Also, they have been shown capable of self-renewal, differentiation, and maintaining a cascade of healing & possibly regeneration at the implanted site. Only a small percentage of implanted MSC survive and rest undergo apoptosis after releasing growth factors, cytokines, and extracellular vesicles. These surviving MSC become active due to conformational changes induced by anti-environment stimuli and undergo limited self-renewal, proliferation, and differentiation, but only a few of them might incorporate into the host tissues. These cells generate & maintain a momentum of series of regenerative activities to improve the function of joint, stabilize or possibly enhance the cartilage quality.

Presentation based on clinical study and published paper "Kumar Ashok, Ghosh Kadamb A, Ghosh Kadamb K. Mesenchymal or Maintenance Stem Cell & Understanding Their Role in Osteoarthritis of the Knee Joint: A Review Article. Arch Bone Jt Surg. 2020 Sep;8(5):560-569. doi: 10.22038/abjs.2020.42536.2155. PMID: 33088856; PMCID: PMC7547168."

ABSTRACT 60

A Novel Method of Stromal Vascular Fraction Separation to be Used in the Management of a Knee and its Clinical Outcomes

Presenter: Ramamoorthy Ramaswamy (India)
Affiliation: Sri Ramakrishna Hospital
Authors: Ramamoorthy Ramaswamy, R. Krishnamoorthy

INTRODUCTION: Stromal vascular fraction (SVF) is among the proven regenerative types of interventions used in the management of OA. Here we present a novel augmented mechanical method used in the separation of SVF from the adipose tissue.

METHODS: A commonly used method for isolation of adipose stromal vascular fraction (SVF) requires the use of a collagenase enzyme that has some detrimental effects such as lysis on the cells and longer processing times. Alternatively, nonenzymatic isolation methods using physical techniques have been used to separate cells from the adipose tissue. The main aim of this study is to highlight physical separation using the sonication method along with cymatics low-frequency vibration techniques in the order of 75hz to separate SVF from adipose tissue. Between June 2021 and December 2021, 75 patients presented to our pain management clinic who received an intra-articular injection of SVF. Radiographic evidence and the WOMAC scoring scale were assessed for their severity. Data were collected prior to the procedure and six months post intra-articular injection. Well-informed consent was obtained from all the patients prior to the procedure.

RESULTS: There was a statistically significant improvement in WOMAC scoring in most of the patients followed up to 6 months ($p<0.05$). No complications were observed. The following were observed

1. High cell yield in the range of 20 to 30 million.[7]
2. The minimum adipose tissue required was 20 to 25 cc.
3. Low processing time of 10 to 12 minutes.

CONCLUSISONS: The prevalence of age-related degenerative diseases such as Osteoarthritis of the knee is becoming more cumbersome and involves huge cost factors that are seen in both developing and developed nations. Therefore, Cost-effective and minimally manipulative treatment strategies such as SVF are necessary to benefit in long-term management of degenerative joint diseases. The extraction process using cymatics along with sonication was found to be a minimally manipulative and cost-effective technique which is a promising novel method for the separation of SVF from Adipose tissue with good clinical outcomes, in the management of OA Knee.

ABSTRACT 60 (images)

CellDrop Cell Count Report DeNovix

Trypan Blue

Sample Name:	Rajakkannu	Created Date/Time:	2021-11-30 12:08:22
Account:	General	Passage:	
Software Version:	v1.9.0	Firmware Version:	6.59
Instrument SN:	s-06878	Record:	20211130120822
Description:			

Results

Last Processed Date/Time:	2021-11-30 12:08:22	Gating Min Diameter (um):	6
Gating Max Diameter (um):	30	Live Cell Count:	353
Dead Cell Count:	458	Total Cell Count:	811
% Viability:	43.5265	Mean Diameter (um):	14.32
Live Cell/mL:	2.28e+07	Dead Cell/mL:	2.96e+07
Total Cell/mL:	5.24e+07		

Dilution

Target Cells/mL:	0.00e+00	Target numbers of Cells:	N/A
Target Volume (mL):	0	Original Volume (mL):	0
Dilution Guidance:			

Protocol

Protocol:	Rkh	DirectPipette (or Slide):	Pipette
Chamber Height:	50	Dilution Factor:	15
Protocol Min Diameter (um):	6	Protocol Max Diameter (um):	30
Live Roundness:	30	Dead Roundness:	25
Stained Threshold:	35		
Small Cell Mode:	True		
Irregular Cell Mode:	True		

Fig1. Processed Stromal Vascular Fraction

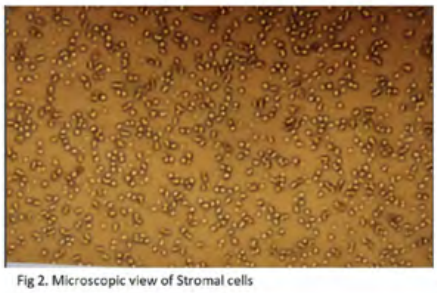


Fig 2. Microscopic view of Stromal cells

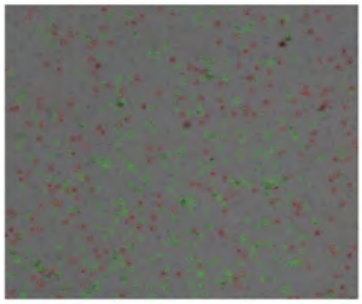


Fig3. Trypan blue staining for live cells

ABSTRACT 61

Engineering an Adipose Tissue Organ-on-a-chip Model for Therapeutic Drug Discovery

Presenter: Lindsey Huff (USA)
Affiliation: Carnegie Mellon University
Authors: Lindsey Huff, Charles Amurgis, Lauren E. Kokai, Rosalyn Abbott

INTRODUCTION: There is a critical need to develop pre-clinical models that closely mimic the physiology, metabolism, and immunology of organs. A model is especially needed for adipose tissue as it significantly contributes to metabolic diseases such as obesity and T2D. Organ-on-a-chip (OOC) models are used to emulate the natural physiology of the human body by recreating the mechanical forces experienced by cells through a microfluidics system. The goal of this OOC model is to develop an accurate vascularized tissue model for therapeutics testing and disease progression.

METHODS: The Micronit OOC platform (Fig. 1A) creates a 3D microenvironment that consists of two compartments, one containing human umbilical vein endothelial cells (HUVECs) in-line with the fluid flow and the other containing adipocytes suspended in a hydrogel protected from shear stresses. Mature adipocytes were isolated from primary human adipose tissue obtained from panniculectomies, suspended in a hyaluronic acid hydrogel, and seeded onto one side of the chip. A porous membrane separated the two compartments but allows for signaling between the two cell types. The OOC platform was perfused above and below the cell layers with adipocyte maintenance media (DMEM, 10% FBS, and 1% Pen/Strep) with a flow rate of 20 μl/hr for seven days. Viability was assessed with a LIVE/DEADTM Viability/Cytotoxicity Kit and the architecture of the cells through fixing and staining with BODIPY, DAPI, and phalloidin 555.

RESULTS: OOC trials with adipocytes remained viable (86% viability) while maintaining a healthy adipocyte morphology (unilocular lipid droplets) (Fig. 1B-C). OOC trials with HUVECs also remained viable (98% viability) (Fig. 1D). Ongoing work involves establishing co-cultures of the 2 cell types on the OOC platform and evaluating glycerol, triglyceride, DNA content, and LDH secretion.

CONCLUSION: The Micronit OOC platform supports adipocyte and endothelial cell viability. Our fat-on-a-chip model will allow for monitoring of vascularized adipose tissue responses to perturbations for longitudinally tracking therapeutic efficacy of novel drugs or drug combinations. Long-term we envision this model could be used to develop patient specific adipose models, explore effects of patient demographics, and study toxicity of lipophilic reagents or viruses that are sequestered in adipose tissue.

ABSTRACT 61 (images)

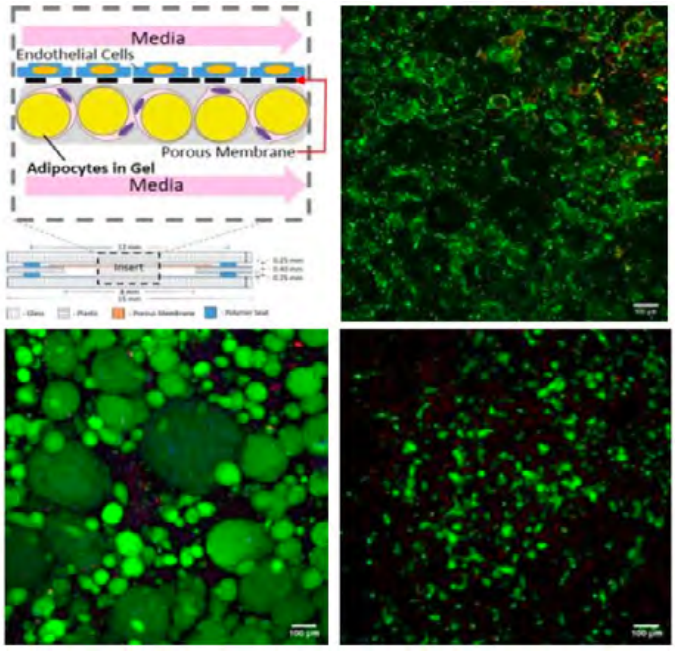


Figure 1. a) The organ-on-a-chip experimental setup where endothelial cells are in-line with the flow and adipocytes are in-set and shielded from shear stress, b) Day 7 Live (Calcein) Dead (Ethidium) image of adipocytes, c) Day 7 immunofluorescent image of adipocytes (BODIPY =green), DAPI =blue), phalloidin =red). d) Day 7 Live Dead image of HUVECs. Scale bar =100 μm.

ABSTRACT 62

Neuroprotective Effects of Adipose-derived Stromal Vascular Fraction on Acute Spinal Cord Injuries in Rats

Presenter: Céline Ertlen (France)
Affiliation: La Timone Hospital, Assistance Publique - Hôpitaux de Marseille
Authors: Céline Ertlen, Mostafa Seblani, Maxime Bonnet, Sara Belluco, Jean-Michel Brezun, Tanguy Marqueste, Nicolas Serratrice, Patrick Decherchi

INTRODUCTION: Spinal cord injuries (SCI) lead to functional alteration with important consequences such as motor and sensory disorders. The repair strategies developed to date remain ineffective. The adipose tissue-derived stromal vascular fraction (SVF) is composed of a cocktail of mesenchymal and hematopoietic stem cells with trophic, pro-angiogenic and immunomodulatory effects. Numerous therapeutic benefits were shown for tissue reconstitution, peripheral neuropathy and for the improvement of neurodegenerative diseases.

METHODS: Our strategy is based on an autologous injection of the SVF within 4 hours after SCI. To check our hypothesis, we conducted a pre-clinical study in adult male rats. Contusions performed at thoracic level T10 using an impactor, all the animals were paraplegic. The epididymal fat removed in a second time, then the autologous SVF is purified (>90% of viability), before 1 million of cells are reinjected into the peri-medullary space in front of the lesion.

RESULTS: Autologous SVF implantation promotes 1) locomotor recovery (BBB test, Ladder rung walking test), 2) H-reflex normalization, 3) and ventilatory frequency adjustment to an isometric exercise. 4) In vivo 7T MRI, shows a significant regeneration and vascularization in the periphery of the spinal contusion. 5)These results were also confirmed by immunohistology analysis (neurofilament labeling, signs of neuronotrophic and neuritogenic), that also revealed axonal regeneration within the SCI at 3 months.

CONCLUSION: However, our results are not complete, some biochemical analyses are still being processed, these encouraging results demonstrate significative recovery at 3 months post-autologous SVF implantation that could be improved by treadmill exercise.

ABSTRACT 63

Cancer Cell Migration Depends on Adjacent Stromal Cells in 3D Bioprinted Breast Cancer Models

Presenter: Hannes Horder (Germany)
Affiliation: University of Würzburg
Authors: Horder H; Böhringer D; Grummel N; Hildebrand L; Schweinitzer S; Teßmar J; Groll J; Bauer-Kreisel P; Fabry B; and Blunk T

INTRODUCTION: Engineering physiologically relevant cancer models that mimic cell-cell as well as cell-matrix interactions with the help of biofabrication techniques has recently attracted increased attention. In breast cancer, adipose tissue as an important part of the tumor microenvironment promotes different stages of tumor development and progression. Therefore, we developed two biofabricated co-culture models with the focus on the impact of stromal cells on cancer cell migration utilizing bioprinted human adipose-derived stromal cell (ASC) and adipocyte spheroids, as well as MDA-MB-231 breast cancer cells.

METHODS: As a prerequisite for 3D bioprinting, human ASC spheroids were assembled in agarose molds and suspended in a thiol-modified hyaluronic acid bioink. Adipose spheroids, differentiated pre-printing, and undifferentiated ASC spheroids were printed in an annular geometry in two different approaches: Model I was adapted to a 24-well plate with a wound healing assay insert containing MDA-MB-231 single cells in its center. 2D cancer cell migration was determined after 48h. Model II was adapted to a 48-well plate with a collagen core containing MDA-MB-231 single cells. 3D cancer cell migration was assessed after 24h using a custom-made live cell imaging setup.

RESULTS: The two co-culture models were designed to study 2D and 3D migration of tumor cells, respectively. First, bioprinting of adipogenically differentiated ASC spheroids was established with the focus on cell viability and sustained functionality. Quantification of lactate dehydrogenase and triglyceride content, immunohistochemical staining, and adipogenic marker gene expression indicated that the printing process did not impair the quality of differentiated spheroids. Factors secreted by printed ASC/adipocyte spheroids were analyzed, revealing high levels of proteins involved in tumor progression. Examination of MDA-MB-231 2D cell migration (Model I) showed significant differences in migration speed depending on differentiated and non-differentiated ASC spheroids. First measurements of MDA-MB-231 3D cell migration (Model II) indicated increased motile fraction, speed and persistence.

CONCLUSION: Our 3D bioprinted breast cancer models enabled us to investigate stromal cell-induced stimulation of cancer cell migration, reflected in increased cell motility, speed and directed migration. In future studies, these models may provide the basis to further elucidate the complex cell-cell and cell–matrix crosstalk within the tumor microenvironment.

ABSTRACT 64

White and Beige/Brown Human Adipose Tissue-on-Chips at a Platform for Disease Modeling and Drug Discovery

Presenter: Trivia Frazier (USA)
Affiliation: Obatala Sciences
Authors: Trivia Frazier, Cecilia Sanchez, Katie Hamel

INTRODUCTION: Obesity, a driver of accelerated biological aging has become a major public health crisis in the United States, with an estimated economic burden of $100 billion annually. The economic and health burden is easily tripled when factoring the increased risks of developing associated co-morbidities such as type II diabetes, cardiovascular disease, cancer and complications as result of infectious diseases.

There is a need to develop platforms that provide earlier indicators of toxicity and drug efficacy using human-based systems to accelerate drug development and reduce the need for drug testing in animal models, which is time consuming, costly and often does not predict the adverse effects in humans.

METHODS: Stromal Vascular Fraction (SVF) Cell Isolation from lipoaspirate of subcutaneous adipose tissue. 3D culture in Obagel®, Obagel WAT and BAT Media and the effect of AMPK inhibitors on Lipolysis and glucose uptake was determined using 2D and 3D cultures. Bodipy/ Hoechst Staining for imaging analysis.

RESULTS: Our studies validated In vitro fat constructs for human adipose derived cells using 3D culture for SVF. Proliferation and spheroid formation was determined. ObagelTM cultures maintained the expression of myeloid (CD19), stem/progenitor (CD34), pre-adipocyte (CD36), hematopoietic (CLA; CD45), pericytic (CD146), and lymphoid (CD3) markers. After differentiation, ObagelTMCultures exhibit physiologically relevant functionality. Leptin and Adiponectin (AdN) secretions were measured from traditional 2D cultures compared to 3D adipose cultures (ObaCell) after 21 days.

CONCLUSION: We have developed a proprietary human scaffold -- ObagelTM -- that, when combined with primary human stromal vascular fraction, creates 3-D tissue engineered white or beige/brown adipose depots called "Fat-on-a-Chip," for disease modeling and drug discovery.
The molecular, functional/physiological, and cellular characterization of our "fat-on-a-chip" system demonstrates a scalable ability to manufacture these "WAT-on-a-chip" or "BAT-on-a- Chip" constructs for modeling white and brown adipose, respectively. Furthermore, we licensed the use of a matrix mimicry system for in vitro adipocyte hypertrophy, using fiber networks. These self-assembling adipose depots, maintained for extended culture periods with minimal effort, can be used to model human adipose tissue that is representative of individual donor demographics, including body mass index, age, gender, ethnicity, and metabolic disease status.

ABSTRACT 65

Development of 3D Human Neuronal and Vascular Microphysiological Models with Human Adipose Stem/Stromal Cells or Bone Marrow Stem/Stromal Cells as Mural Cell Types

Presenter: Susanna Miettinen (Finland)
Affiliation: Tampere University
Authors: Susanna Miettinen, Lotta Isosaari, Hanna Vuorenpää, and Susanna Narkilahti

INTRODUCTION: Both neuronal and vascular networks are fundamental elements in almost all tissues, and crucial for maintaining the homeostasis in humans. Here, the present human 3D neuro-vascular model that utilizes mural cells derived from either from human adipose stem cells (ASCs) or human bone marrow -derived stem/stromal cells (BMSCs) in vasculature formation and enables longer (>14d) culturing period in a microphysiological environment. A stable 3D neuro-vascular co-culture is needed for establishing more in vivo -like innervated and vascularized tissue models.

METHODS: We used human induced pluripotent stem cell (hiPSC) -derived neural progenitor cells, RFP/GFP tagged human umbilical vein endothelial cells (HUVECs) and either BMSCs or ASCs as a mural cell type to create 3D neuro-vascular co-culture model. Collagen-fibrin hydrogel was used in AIM Biotech 3D Cell Culture Chip to establish 3D cell culture. Interstitial flow was created to the chip by passive perfusion. Different cell culture media were tested to find an optimal microenvironment for the co-cultures. The constructs were cultured for 14 days or more. Cocultures were analyzed with time-lapse imaging and immunocytochemical staining and the activity of neurons was measured with Ca2+ imaging. Protein secretion of angiogenesis-related proteins was analyzed from cell culture media.

RESULTS: The studied cell types interacted with each other and formed a viable co-culture for at least 14 days. Both pericytic cell types supported formation of functional vessels with open lumens, however, BMSCs were more efficient in supporting formation of vascular network. Neuronal cells made connections with both pericytic cells and HUVECs and Ca2+ oscillations indicated neuronal activity in all co-cultures with both pericytic cell type. Yet, neuronal support was not enough to balance the difference in vasculogenic potency between ASC and BMSC. Protein secretion analysis showed increase of angiogenesis-related protein levels in response to adding neurons to cocultures, supporting the angiogenesis-supporting role of neurons.

CONCLUSION: Here, we present a novel human neuro-vascular model that is applicable in creating in vivo-like tissue models and in revealing mechanisms in neuronal-vascular interactions.

ABSTRACT 66

Establishment of a 3D-biofabricated Tumor Model Suitable for Analyzing Tumor Progression, Cell-cell-and Cell-matrix-interactions

Presenter: Annika Kengelbach-Weigand (Germany)
Affiliation: University Hospital Erlangen
Authors: A. Kengelbach-Weigand, R. Schmid, S. K. Schmidt, R. Detsch, H. Horder, T. Blunk, S. Schrüfer, D. W. Schubert, L. Fischer, I. Thievessen, D. Schneidereit, O. Friedrich, A. Grüneboom, Pamela L. Strissel, Reiner Strick, R. E. Horch, A. K. Bosserhoff, A. Arkudas

INTRODUCTION: It is well-known that the tumor microenvironment with its numerous tumor-adjacent cells plays one major role in tumor initiation and progression. Precise simulation of this complex system with in vitro and in vivo models is of utmost importance for studying pathophysiology of tumors and becomes possible by using the methodology of biofabrication.

METHODS: Given the fact that adipose tissue could be one crucial factor for tumor progression, we analyzed interactions of tumor and normal mammary epithelial cells and adipose-derived stem cells (ADSC) within breast cancer. Stimulation of breast cancer cells in co-cultures and with ADSC secretome was evaluated using functional analyses and on mRNA level. In a further step, a printable hydrogel consisting of alginate, hyaluronic acid and gelatin was developed for extrusion-based bioprinting. Its suitability for mimicking the tumor ECM including ADSC and melanoma cells was evaluated by different cell assays and analysis of its mechanical properties in vitro and in a sophisticated in vivo approach, using the arteriovenous (AV) loop model in the rat.

RESULTS: ADSC as part of the tumor-adjacent tissue significantly increased tumor cell proliferation, viability, migration and invasion. ADSC secretome contained elevated levels of growth factors and cytokines stimulating invasion and upregulating gene expression including MMPs and ECM receptors.
The newly developed bioink showed good printability and shape-fidelity. It had mainly elastic properties with a storage modulus of 10.5 kPa at 1 rad/s using dynamic mechanical analysis. The stiffness of the bioink can be controlled by varying the alginate content making it a perfect tool to analyze cell-matrix interactions. Printed melanoma cells and ADSC survived and proliferated over weeks while ADSC were successfully differentiated into the adipogenic and osteogenic lineage within the bioink. In the in vivo AV loop model a solid and vascularized melanoma could be grown. Over four weeks reliable metastases developed thereby simulating closely the human pathophysiology.

CONCLUSION: The tumor microenvironment, and especially ADSC as part of it, might play a significant role in tumor progression. With our newly established 3D-biofabricated tumor model this complex tumor microenvironment can be closely resembled and used for studying tumor progression and developing therapies.

Acknowledgement: The work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Project number 326998133, TRR 225 (subprojects C03 and A01, A07, B06, B08, C02, C04).

ABSTRACT 67

Efficacy of Microfragmented Adipose Tissue for Treatment of Symptomatic Knee Osteoarthritis: A Randomized, Placebo-controlled Study

Presenter: Joshua Harrison (USA)
Affiliation: University of New Mexico, School of Medicine
Authors: Joshua Harrison, Anil Shetty, Robert Schenck, Dustin Richter

INTRODUCTION: Knee osteoarthritis (OA) is a debilitating joint disorder affecting tens of millions of people worldwide. Nonoperative treatment options have variable efficacy. This study evaluates pain relief and functional improvement after knee OA treatment with a novel therapeutic intervention, microfragmented adipose tissue, in comparison to a saline placebo and current standard, corticosteroid injections.

METHODS: Patients with radiographic knee OA, a minimum pain score of 3 on the visual analog scale (VAS), and absent history of knee injections were eligible for inclusion. Patients were randomized to, microfragmented adipose tissue (MFAT), corticosteroid (CS), or saline placebo (P) injection. Both the practitioner and patient were blinded to the injection in the CS and P groups. For the MFAT group, lipoaspiration was performed in clinic under local anesthesia. The VAS pain scale, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and the Knee Injury and Osteoarthritis Outcome Score scale (KOOS) were recorded pre-procedure and at specified time points post-procedure up to one year.

RESULTS: 75 patients have been enrolled (95% follow-up). Patients were randomized to the three groups, with 25 patients in each group. A linear mixed effects model was used to quantify changes in outcomes over the 1-year post-procedure period, the MFAT group demonstrated a consistently positive improvement across all five outcomes measures, whereas no consistent trend was noted in the placebo group and a negative trend was noted in the CS group after the initial 2-week improvement. Patients with more severe radiographic knee OA had poorer outcome scores in all groups. No complications were noted in any of the study patients with the exception of mild expected donor site morbidity of minor pain and ecchymosis in the MFAT group.

CONCLUSION: Nonoperative knee OA treatment options are limited with variable efficacy. It is critical to evaluate patient outcomes rigorously prior to instituting novel procedures or treatments. This data indicates microfragmented adipose tissue injection consistently provides the largest improvement in outcome scores at 6-12-month follow-up compared with the placebo and corticosteroid groups.

ABSTRACT 68

Understanding the Role of Subcutaneous Adipose Tissue Extracellular Matrix in Wound Healing Complications Following Caesarean Deliveries

Presenter: Louise Croizat-Vallet (New Zealand)
Affiliation: University of Otago
Authors: Louise Croizat-Vallet, Gabriella Stuart, Michael Stitely, and Lyn Wise

INTRODUCTION: Caesarean sections or C-sections are common surgical procedures requiring a deep incision through the skin including its deepest and largest layer: the subcutaneous adipose tissue (SAT). This results in a profound wound that frequently presents with complications during the recovery process that can result in wound non-closure. The frequency of these complications increases with patient-related factors such as obesity and the presence of scar tissue following repeated C-sections. As changes to the extracellular matrix (ECM) can occur during obesity and scarring, we hypothesized that modifications to the SAT ECM could may contribute to C-section healing impairments. This study investigated the SAT ECM composition in C-section patients relative to body mass index and C-section history. The impact of ECM polymers on healing responses in pre-adipocytes from the same patients was also explored.

METHODS: SAT samples were obtained during elective C-sections from otherwise healthy volunteers and were processed to isolate stromal vascular fraction cells or subjected to histological analysis and hydroxyproline/dimethylmethylene blue assays to investigate their ECM composition. Selected ECM polymers identified in the SAT ECM were then tested for their impact on pre-adipocyte function in vitro. Differentiation was assessed by quantifying intracellular lipid accumulation over 14 days incubation period. Migration was monitored using under-agarose migration assay during 24h incubation period.

RESULTS: Glycosaminoglycans (GAG) content was 2.37-fold higher in patients with previous C-section history (unpaired t-test, $p=0.0307$, $n=16$ repeated C-section, $n=9$ no C-section). In those presenting with scar tissue, the GAG content was 2.86-fold higher than for those without ($p=0.0068$, $n=12$ presenting scar tissue, $n=9$ no scar tissue). Collagen I enhanced pre-adipocyte differentiation by 1.22-fold (one-way ANOVA, Šidák post-hoc test, $p=0.0042$, $n=6$). Gelatin and fibronectin enhanced pre-adipocyte migration by 1.52-fold ($p=0.0019$, $n=6$) and 1.35-fold ($p=0.0236$, $n=6$), respectively.

CONCLUSION: These findings indicate that GAGs within the SAT may contribute to healing impairments in patients with previous C-section and scarring history, while collagen, gelatin and fibronectin may enhance healing responses in cells from the same tissue. These results help understanding as to how the SAT ECM composition under different clinical conditions may impact healing outcomes, and may guide the development of therapies targeting deep wound complications.

ABSTRACT 69

Management of Deep Tunneling Wounds with Novel Allograft Adipose Matrix: A Natural Off-The-Shelf Treatment Modality

Presenter: Chitang Joshi (USA)
Affiliation: Northwestern University
Authors: Chitang Joshi, Robert D Galiano

INTRODUCTION: Chronic non-healing wounds present a substantial economic burden with a reduction in patient quality of life, and often result in limp amputations or even premature deaths. This burden may increase with a larger proportion of elderly and increasing prevalence of life-style diseases such as obesity and diabetes.

There is a lack of clinical options for the treatment of deep tunneling chronic wounds, when debridement, skin grafting, or vacuum-assisted closure dressings fail. Autologous fat grafting has only recently been applied to chronic wounds that fail conservative management. This study aims to evaluate the efficacy of Allograft Adipose Matrix (AAM), an aseptically processed "off-the-shelf", ready to use allograft, to provide a scaffold and cushion to support the wound bed.

METHODS: This case series examined patients with deep tunneling and cavitation chronic wounds. AAM was injected subcutaneously to fill the adipose tissue deficits within the wound bed. Patients were evaluated for possible complications related to wound healing and infection.

RESULTS: The eleven patients (seven males; four females) that were treated had an average age of 65 years (ranging from 35-78 years). Of the 11 wounds in this case series, the etiologies were post-surgical (n=7), traumatic (n=2), and foot ulcer (n=2). Ten patients had only one application of the AAM, and one patient had two treatments. The volume of AAM applied was 2cc to fill the adipose tissue deficit and the average follow-up after AAM application was 6.6 weeks. Observations indicated that tunneling and cavitation in the wound subsided. Seven patients (63%) went on to achieve complete wound closure.

CONCLUSION: AAM is a novel use of an off-the-shelf allograft tissue, targeted towards a very frequently occurring pathology. AAM does not present the challenges associated with autologous fat grafting. The use of AAM provided a physical cushion and scaffold for deep tunneling and cavitation within these chronic wounds. This increased padding with the wound bed ultimately support wound closure.

ABSTRACT 70

Activin A Mediates Inflammatory Cell-induced Acquisition of Myofibroblast Phenotype by Adipose Mesenchymal/Stromal Cells

Presenter: Dmitry Traktuev (USA)
Affiliation: University of Florida College of Medicine
Authors: Sahana Manohar-Sindhu, Stephanie Merfeld-Clauss, Keith L. March, Dmitry O. Traktuev

IINTRODUCTION: Many diseases are associated with tissue ischemia and inflammation; prolonged inflammation limits tissue recovery, which is often attributed to progressive loss of microvasculature and fibrosis. Many ischemic conditions are characterized by significant increases in Activin A, which frequently correlates with disease severity. Resident mesenchymal stromal cells, including adipose stromal cells (ASC), are prevalent in the vascular niche and influence tissue homeostasis as well as pathologic progression. In this study, the effects of inflammatory cells and factors on modulation of ASC phenotype and role of Activin A in this process were investigated.

METHODS: Peripheral blood mononuclear cells from healthy human donors were activated with LPS (aPBMC) and presented to ASC monolayers. Expression of markers of smooth muscle phenotype was evaluated in ASC by immunofluorescence and Western Blotting; accumulation of several cytokines in incubation media was assessed by ELISA. A panel of inflammatory cytokines known to be secreted by aPBMC were tested to identify factor(s) responsible for aPBMC effects. Neutralizing antibodies, inhibitors to receptors, and silencing RNA constructs were used to dissect the precise mechanism of aPBMC signalling.

RESULTS: Five-day exposure of ASC to aPBMC upregulated several smooth muscle markers in ASC, including αSMA, SM22α, and Calponin I. Similar effects resulted from exposure of ASC to IL-1β, while IL-1β neutralizing IgG prevented αSMA induction in ASC when co-cultured with aPBMC. Concurrently, ASC exposure to either aPBMC or IL-1β led to 20- or 15-fold induction of Activin A, respectively, but no upregulation in TGFβ1-3 expression. Additionally, aPBMC induced a 3-fold induction of connective tissue growth factor (CTGF), a factor known to mediate fibrosis, in ASC. IL-1β neutralizing IgG abrogated aPBMC-induced Activin A expression, whereas silencing of Activin A with neutralizing IgG or siRNA prevented aPBMC-induced αSMA and CTGF expression.

CONCLUSION: aPBMC induce expression of Activin A in ASC, which is mediated by IL-1β. Both aPBMC and IL-1β induce an ASC transition toward a myofibroblast phenotype via Activin A autocrine activity. In addition, Activin A may promote fibrosis via induction of CTGF. These data suggest that chronic inflammatory induction of Activin A in perivascular MSC is a key and novel mechanism promoting fibrosis.

ABSTRACT 71

Molecular and Functional Characterization of Phosphodiesterase 10A in Human White Adipocytes

Presenter: Mohammed Hankir (Germany)
Affiliation: Würzburg University Hospital
Authors: Mohammed Hankir

INTRODUCTION: Phosphodiesterases (PDEs) play a key role in regulating adipose tissue function through modulating intracellular cyclic nucleotide levels. Here, we aimed to characterize for the first time the PDE10A isoforms expressed in human white adipocytes and the signaling pathways it controls.

METHODS: Human subcutaenous white preadipocytes (HWPs) were purchased from Promocell and differentiated to human white adipocytes (HWAs) according to a standard protocol. Oil Red O staining was employed to determine lipid droplet formation.

Western blot was performed to determine PDE10A protein expression in HWPs and HWAs. Fractionation and immunofluorence were performed to determine whether cytosolic (PDE10A1) or membrane-bound (PDE10A2) isoforms of PD10A are expressed in HWAs. Western blot was performed to determine whether inhibition of PDE10A with MP-10 activates protein kinase A (PKA) and/or protein kinase G (PKG) signalling through phosphorylation of cyclic response element binding protein (CREB) and vasodilator-stimulated phosphoprotein (VASP) at S133 and S239, respectively. Glycerol assay was performed to determine if inhibition of PDE10A with MP-10 triggers lipolysis in HWAs through PKA and PKG signalling. RT-qPCR was performed to assess the impact of pro-inflammatory cytokines (TNF-alpha, Il-1beta, and IFN-gamma) on PDE10A mRNA expression in HWAs.

RESULTS: HWPs were successfully differentiated to HWAs as determined by Oil Red O staining and Western blot for the adipocyte marker fatty acid binding protein 4 (FABP4). Western blot analysis revealed that both the cytosolic (PDE10A1) and membrane-bound (PDE10A2) isoforms of PDE10A are highly expressed in HWP-ds, predominantly in the former subcellular compartment. This was confirmed by fractionation and immunofluorescence analyses. Inhibition of PDE10A with MP-10 (0.5µm-5µM) dose-dependently increased PKA and PKG signaling as revealed by phosphorylation of CREB and VASP at S133 and S239, respectively. Consistently, the stimulation of lipolysis by MP-10 was fully prevented by combined pre-incubation of HWP-ds with selective PKA and PKG inhibitors. Finally, mimicking the adipose tissue microenvironment in obesity by applying pro-inflammatory cytokines onto HWP-ds caused an early (4-hour) and sustained (24-hour) increase in PDE10A mRNA expression.

CONCLUSIONS: These results provide new insight into the molecular character, function, and regulation of PDE10A in human adipocytes and further justify the use of selective inhibitors for the treatment of metabolic disease through targeting adipose tissue in a more efficient and targeted manner.

ABSTRACT 72

Adipocyte Differentiation and Collagen Secretion in Nf1-Deficient Breast Cancer

Presenter: Menusha Arumugam (USA)
Affiliation: Van Andel Institute
Authors: Menusha Arumugam, Elizabeth A. Tovar, Curt J. Essenburg, Ian T. Beddows, Lisa Turner, Corinne Esquibel, Patrick S. Dischinger, Megan E. Callaghan, Eve E. Gardner, Ghassan Mouneimne, Kirk C. Hansen, Carrie R. Graveel, Matthew R. Steensma

INTRODUCTION: Neurofibromin 1, encoded by the NF1 gene, functions as a negative regulator of RAS and is frequently mutated in cancer. Women with Neurofibromatosis Type I (NF1) – a tumor predisposition syndrome caused by germline NF1 mutation – have an increased risk of developing aggressive breast cancer with poorer prognosis. The mechanism of how NF1 mutation leads to aggressive breast cancer is unknown. Given the role of the tumor microenvironment (TME) in breast cancer progression, we hypothesize that NF1 mutation in stromal cells contributes to poor prognosis and aggressive cancer in NF1 patients. Despite adipocytes making up a large portion of the mammary gland, TME studies heavily focuses on fibroblast and immune cells. We have not extensively studied adipocytes and their contribution to breast cancer.

METHODS: We developed a Nf1-deficient breast cancer rat model using CRISPR-Cas9. Using RNA-seq, we are quantifying gene expression changes in different cell populations of an Nf1-mutant rat mammary gland. We are also isolating rat adipose-derived stem cells (ASC) and measuring their in vitro differentiation ability. To analyze global extracellular matrix (ECM) changes and specific adipocyte-derived ECM secretion, we are conducting mass spectrometry experiments on whole mammary gland and isolated mature adipocytes, respectively. Additionally, to investigate how Nf1 mutation alters mammary collagen deposition and architecture, we are using polarized microscopy of picrosirius red (PSR)-stained tissues and second harmonic generation (SHG) imaging.

RESULTS: Nf1-mutated rats have accelerated mammary development and develop multifocal, aggressive breast cancer. RNA- seq of Nf1-mutant mature adipocytes before tumor formation indicates the presence of cancer-associated adipocytes (CAA) characterized by (1) increased collagen expression, (2) decreased adipocyte markers, (3) increased fibroblast and preadipocyte markers, and (4) increased collagen modification genes. Additionally, the Nf1-mutant mature adipocytes have upregulated of the Hippo pathway gene, which is known to promote fibrosis, increase matrix remodeling, and regulate adipogenesis. Isolated ASC have reduced in vitro differentiation ability into adipocytes and remain in a preadipocyte-/fibroblast-like phenotype. Mass spectrometry experiments, polarized microscopy of PSR-stained tissues, and SHG imaging are currently in progress.

CONCLUSION: Nf1-deficient mature adipocytes before tumor formation have properties of CAA. Nf1 mutation restricts ASC differentiation to preadipocytes with fibroblast-like properties and creates a protumorigenic ECM.

ABSTRACT 73

Immunomodulatory Functions of Adipose Mesenchymal Stromal/Stem Cells Derived from Donors with Type2 Diabetes and Obesity on CD4+ T cells

Presenter: Marwa Mahmoud Mohamed El Shahat (Egypt)
Affiliation: National Research Centre
Authors: Marwa Mahmoud*, Laura Kummola, Miia Juntunen, Amna Adnan, Ilkka S. Junttila, Minna Kääriäinenj, Tuula Tyrväinen, Abeer Abd El Fattahl, Khalda Amr, Alaa Mohamad El erian, Mimmi Patrikoski, Susanna Miettinen*

INTRODUCTION: Type 2 diabetes (T2D) is a hyperglycemic state in which imbalanced metabolic and inflammatory pathways are integrated. Adipose stromal/stem cells (ASC) are multipotent cells with immunosuppressive and anti-inflammatory properties. Controversial knowledge is available about the impact of T2D on biology and the function of these cells. This point is of great importance for autologous application, since allogenic ASC transplantation may be hampered by immune rejection. Thus, it is important to study the effect of T2D, associated with obesity, on ASC immunomodulation to develop an effective ASC -based therapy.

METHODS: In our study, ASC were obtained from obese, T2D (dASC) or normal weight, non-diabetic (ndASC) donors and cultured with anti-CD3/CD28 stimulated allogeneic CD4 T cells. ASC were studied for expression of immunomodulators, CD54, CD274, and indoleamine 2, 3 dioxygenase 1 (IDO), in inflammation. In the monocultures and cocultures, CD4 T cell proliferation, activation surface markers, and apoptosis, frequency of regulatory T cells (Tregs; CD4+ CD25high FOXP3+), and expression of intracellular cytokines were detected using a flow cytometer. Modulation of T cell subsets' cytokines was explored via ELISA.

RESULTS: In inflammation, the expression of CD54, CD274, and IDO were significantly upregulated in ASC, with no statistical differences between ndASC and dASC. dASC retained the potential to significantly suppress CD4 T cell proliferation, with a slightly weaker inhibitory effect than ndASC, associated with significantly reduced abilities to decrease IL-2 production and to increase IL-8 levels. Such attenuated potentials were significantly correlated with increasing body mass index. dASC and ndASC comparably mitigated CD4 T cell viability, HLA-DR expression, and interferon-gamma (IFN-γ) production, conversely, increased CD69 expression, Tregs percentage, and IL-17A production. The immunomodulators prostaglandin E2 and IL-6 were detected in the conditioned medium of the cocultures in considerable amounts.

CONCLUSION: These findings suggest that ASC obtained from T2D obese donors are receptive to the inflammatory environment and are able to modulate CD4 T cells accordingly. However, pre-conditioning strategies may be needed to improve the therapeutic efficacy of dASC, in vivo, considering the disease state and body mass index of the patient.

ABSTRACT 74

Effects of Weight Loss on Adipose Stem/Stromal Cell Immunomodulation and Mitochondrial Respiration Capacity

Presenter: Amna Adnan (Finland)
Affiliation: Tampere University
Authors: Amna Adnan, Mimmi Patrikoski, Miia Juntunen, Tuula Tyrväinen, Minna Kelloniemi and Susanna Miettinen

INTRODUCTION: Adipose stem/stromal cells (ASC) are multipotent cells possessing excellent proliferation, differentiation, and immunomodulation capacity. The expansion of adipose tissue in obesity causes mitochondrial dysfunction of ASC and impacts the adipokine secretion regulating the functions of immune cells. This study aimed to investigate the effect of weight loss on ASC mitochondrial respiratory capacity and immunomodulatory functions especially macrophage polarization and cytokine secretion.

METHODS: ASC were isolated from adipose tissue samples obtained from donors undergoing bariatric surgery, before (obASC) and after (wlASC) weight loss. Pro-inflammatory (M1), anti-inflammatory (M2), and regulatory macrophages (Mreg) with characteristic morphology, immunophenotype, and cytokine secretion were polarized from peripheral blood mononuclear cells. Polarization of macrophages in mono- and co-culture with wlASC and obASC was analyzed using flow cytometry (CD86, CD11C, CD163, CD206 and HLA-DR). Anti- (IL-1RA, MDC, TARC, IL-10 and IL-4) and pro-inflammatory cytokine (MCP-1, MIP-1a, IL-6, IL-1B, TNF-α and IL-12p70) secretion was analyzed to characterize different cell responses during mono- and co-cultures (V-plex). Metabolic analysis of obASC vs wlASC was done to study the effect of weight loss on ASC cellular respiration by measuring the mitochondrial respiration after 48hrs of cell culture (seahorse analysis).

RESULTS: The expression of M1 markers CD86, CD11c and HLA-DR, decreased in co-culture with both ASC. In monocultures, anti-inflammatory cytokines MDC and TARC were secreted more by wlASC compared with obASC, while pro-inflammatory IL-6, IL-1B, IL-12p70 were secreted more by obASC. In co-cultures, both ASC decreased secretion of IL-1RA and MDC by M2, TARC and MDC by M1 and TARC by Mreg, while ASC increased secretion of IL-6, IL-1B and IL-12p70 by M2, IL-4, MCP-1, IL-6, IL-1B and IL-12p70 by M1 and IL-4, MCP-1, IL-6, IL-1B and IL-12p70 by Mreg. The cellular respiratory capacity was lower in obASC when compared to wlASC.

CONCLUSIONS: Our results show that weight loss may have some effect on ASC. After weight loss, ASC secrete more anti-inflammatory and less pro-inflammatory cytokines than before weight loss. Also, the respiratory capacity was improved. In co-culture studies, both ASC did regulate cytokine secretion, but we did not see a difference between the cells obtained before and after weight loss.

ABSTRACT 75

Novel Kinase Inhibitor Screening for Inhibitors of Adipogenic Differentiation in Adipose-derived Stem Cells from Lean and Obese Patients

Presenter: Caroline Rinderle (USA)
Affiliation: The University of North Texas Health Science Center at Fort Worth
Authors: Caroline Rinderle, Bruce Bunnell

INTRODUCTION: Obesity is an endocrine disorder characterized by excess adipose tissue accumulation and a body mass index of 30kg/m2 or greater. Over 40% of Americans are obese. Obesity leads to an increased prevalence of heart disease, type 2 diabetes, and cancer and ultimately results in worse clinical outcomes. Adipose tissue consists primarily of adipocytes, but the stromal vascular fraction (SVF) contains various cell types, including but not limited to adipose-derived stem cells (ASCs). ASCs are self-renewing, multipotent, mesenchymal stem cells of great interest for their potential role in regenerative medicine. After increased caloric intake, ASCs differentiate into adipocytes, and these cells accumulate over time. If excess caloric intake is maintained, obesity results. While the genes that control the differentiation of an ASC into an adipocyte are relatively well known, much less is known about the kinases involved in this process. Suppose the differentiation of ASCs into adipocytes can be reversed or entirely blocked via kinase inhibition of specific kinases. In that case, the obesity epidemic can be solved, and worse outcomes associated with excess weight gain can be prevented.

METHODS: Pools of ASCs from lean and obese donors were treated with 100nM of KCGS Drug Library kinase inhibitors obtained from the SGC at UNC Chapel Hill. After 72 hours, the drug was removed and replaced with adipogenic differentiation media. The cells were differentiated for 11 days and stained with an Oil Red O lipid droplet stain. After imaging and drying overnight, the stain was removed and quantified.

RESULTS: Ten kinase inhibitor drugs affected ASCs from both lean and obese donors. In addition, seven drugs seem to partially inhibit adipogenesis, while three seem to have completely inhibited differentiation.

CONCLUSION: Obesity causes changes to a cell's biology, ultimately making the body more susceptible to disease and resulting in worse outcomes. Determining which kinases are responsible for the differentiation of ASCs into mature adipocytes will give insight into mechanisms of molecular intervention to cure obesity and the role kinases play in normal ASC biology. In the future, qRT-PCR and western blots will be performed to confirm the phenotypic changes observed after kinase inhibition via gene and protein expression changes.

ABSTRACT 75 (images)

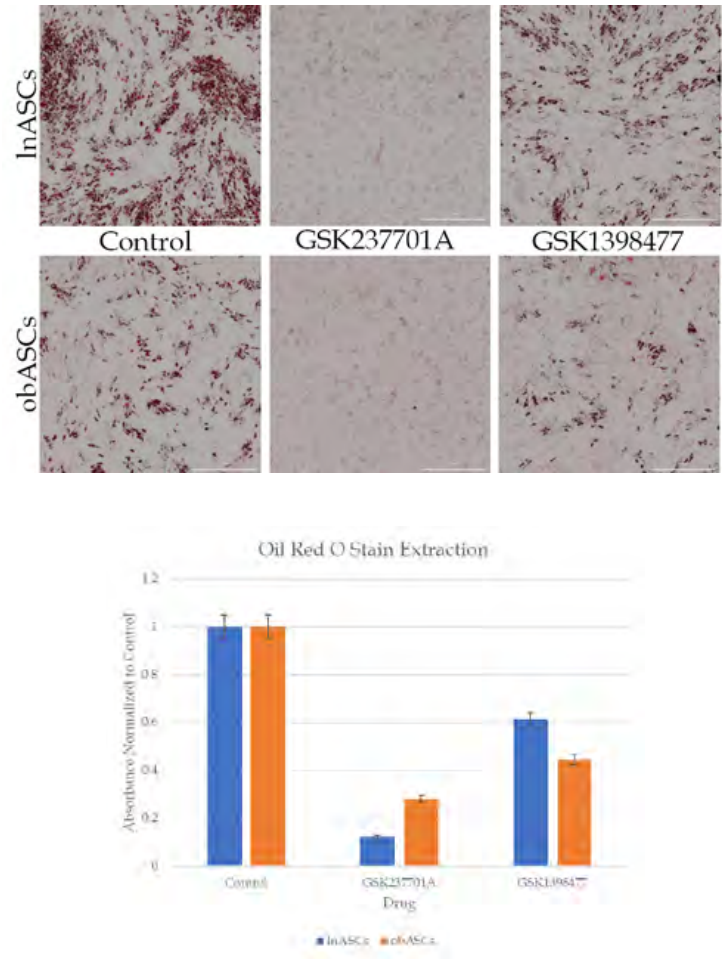


Figure 1: A) Oil Red O staining of drug-treated ASCs from both lean and obese patients. Staining was performed 72 hours after drug treatment and 11 days post-adipogenic induction. B) Oil Red O stain extraction of drug-treated ASCs from both lean and obese patients confirms the differences in stain present in the images from A.

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Notes

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IFATS Special Edition: Stem Cells and Development

The cover and caption for this IFATS Special Issue were provided by Lauren Kokai, of the Departments of Plastic Surgery and Bioengineering, University of Pittsburgh, Pennsylvania, USA, on behalf of the co-authors of the original research report, 'Comparison of Clinically Relevant Adipose Preparations on Articular Chondrocyte Phenotype in a Novel In Vitro Co-Culture Model' (Kokai et al., pp. xxx-xx). Pioneering clinical use of adipose-derived therapeutics for repairing musculoskeletal injuries led Kokai et al., to explore the impact of adipose processing methods on chondrocyte phenotype. The results suggest interesting adipose-processing differences in anabolic vs. catabolic chondrocyte gene expression.

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