

18TH ANNUAL IFATS CONFERENCE FORT LAUDERDALE - FLORIDA



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Presidential Note



On behalf of the IFATS Board of Directors, I have the pleasure to welcome you to our 18th Annual Meeting in beautiful Ft. Lauderdale, Florida.

In 2002, IFATS was founded by four scientific visionaries following the historical discovery of mesenchymal stem cells in human subcutaneous adipose tissue. Since then, the IFATS annual meeting has presented leading professionals in the exciting field of regenerative

medicine with the opportunity to both present and learn about cutting edge scientific and clinical research. A robust group of plastic surgeons, cell biologists, research scientists and physicians in other complementary domains gather at this meeting each year where they exchange the most current knowledge on basic, translational, and clinical research in adipose-derived products, including adipose-derived stem cells (ASC's).

IFATS works closely with other leading scientific organizations to organize collaborating panels for attendees that will be an important part of our 18th annual event. We will once again present the Industry Showcase, and this year it will take part of our podium presentations. In addition, several of our industry leaders will offer "Lunch and Learn" Tables available with reserved seating on Thursday.

The IFATS annual meeting provides all our attendees the opportunity to learn about state-of-the-art technology and clinical practice, as well as, cutting-edge products developed by our exhibiting companies, and further, to interact with the brightest minds in the field. For the first time, this year will be offered as a hybrid meeting. Those who cannot attend due to travel restrictions or schedules may join us "Live" on line streaming. The 2021 meeting features a Thursday evening casual gathering on the hotel pool deck overlooking beautiful Fort Lauderdale beach during which attendees will enjoy time with colleagues and a chance to interact with international leaders in the field.

We are very pleased that you will join us at the IFATS annual meeting this year and we are sure you will benefit from exciting new ideas and valuable tools for your research and clinical practice.

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Invited Speakers & Session Moderators

Rosalyn Abbott, PhD Katarina Andjelkov, MD, PhD Fabrizio Bembo, PhD Mark Berman, MD **Torsten Blunk, PhD** Robert Bowen, MD, FCCP, RVPI **Bruce Bunnell**, PhD **Bill Cimino, PhD** Sherry Collawn, MD, PhD Jason Dragoo, MD Jaime Garza, MD Alexandra Conde-Green, MD Julie Fradette, PhD **Trivia Frazier, PhD** Pietro Gentile, MD Jeffrey M. Gimble, MD, PhD

Farshid Guilak, PhD Marco Helder, PhD LaTonya Hickson, PhD Aaron W. James, MD Adam J. Katz, MD Roger Khouri, MD Lauren Kokai, MD Fred Kolle, MD, PhD Benjamin Levi, MD Ramon Llull, MD, PhD Marc Long, PhD Mandi J. Lopez, DVM, MS, PhD, DACVS Jeremy Magalon, MD Keith March, MD Kacey Marra, PhD Susanna Miettinen, PhD

Doug Oliver, MSW Kentaro Onishi, DO Graham Parker, PhD Ivona Percec, MD, PhD Robert Rehnke, MD Kyle Richardson Camilo Ricordi, MD Ricardo Rodriguez, MD J. Peter Rubin, MD Gordon H. Sasaki, MD Bernard Siegel, MD Filip Stilaert, MD Stuart Williams, PhD Kotaro Yoshimura, MD

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> Filip Stillaert, MD University Hospital Ghent

Dmitry Traktuev, PhD University of Florida College of Medicine

> Stuart Williams, PhD University of Louisville

Carnegie Mellon University
Katarina Andjelkov, MD, PhD

University of Belgrade & BelPrime Clinic

Rosalyn Abbott

Petra Bauer-Kreisel, PhD University of Wuerzburg

> Torsten Blunk, PhD University of Wuerzburg

Robert Bowen, MD, FCCP, RVP Private Practice

Bruce Bunnell, PhD University of North Texas Health Science Center at Fort Worth

> Louis Casteilla, PhD University of Toulouse

Evangelia Chnari, PhD MTF Biologics

Sherry Collawn, MD, PhD University of Alabama at Birmingham

• THURSDA	Y - NOVEMBER 18	
7:00 - 8:00 am	Continental Breakfast in Exhibit Hall	Las Olas Foyer
8:00 - 8:30 am	Welcome Remarks and Overview Ivona Percec, MD, PhD - IFATS President	Las Olas Ballroom
8:30 - 9:15 am	Keynote Speaker - Jeremy Magalon, MD The (r)evolution of Point of Care Regenerative Medicine Moderator: Ivona Percec, MD, PhD	Las Olas Ballroom
9:15 - 9:30 am	Guest Speaker - Graham Parker, PhD, Stem Cells and Development Journal Editor Moderator: Jeffery M. Gimble, MD, PhD	Las Olas Ballroom
9:30 - 10:30 am	Plenary Session 1 - BEST PAPERS (Award Eligible) Moderator: Torsten Blunk, PhD and Bruce Bunnell, PhD	Las Olas Ballroom
9:30 am	1 (p.16) - MESENCURE—AN ENHANCED ALLOGENEIC ADIPOSE-DERIVED CELL THERAPY DEVELOPED FOR TREAT RESPIRATORY DISTRESS IN COVID-19: FROM BENCHTOP TO BEDSIDE Presenter: Tomer Bronshtein (Israel) Affiliation: Bonus BioGroup Ltd. Authors: BEN DAVID D; NOVAK A; KIVITY V; HAMOUD S; HAYEK T; MERETZKI S	TING ACUTE
9:38 am	2 (p.16) - DEVELOPMENT OF A 3D BIOPRINTED BREAST CANCER-STROMA CO-CULTURE MODEL UTILIZING ADIPOSE-DERIVED STROMAL CELL SPHEROIDS Presenter: Hannes Horder (Germany) Affiliation: University of Wuerzburg Authors: GUAZA LASHERAS M; GRUMMEL N; NADERNEZHAD A; HERBIG J; ERGUN S; TESSMAR J, GROLL J; FABRY B; BAUER-KREISEL P, BLUNK T	
9:46 am	3 (p.17) - VASCULARIZED ADIPOSE TISSUE GRAFT USING A DECELLULARIZED LUNG FOR SOFT TISSUE RECONSTRUCTION Presenter: Rosalyn Abbott (USA) Affiliation: Carnegie Mellon University Authors: DEBARI MK; NG WH; KOKAI LE; MARRA KG; RUBIN JP; REN X	
9:54 am	4 (p.17) - ADIPOSE STEM CELL-BASED THERAPIES FOR FIBROSIS AND SCARRING OF THE VULVA ADIPOSE STEM CELL-BASED THERAPIES FOR FIBROSIS AND SCARRING OF THE VULVA Presenter: Aurora Almadori (Italy) Affiliation: UCL - Royal Free Hospital Authors: BOYLE D; HANSEN E; REID W; MACLEAN A; BUTLER PE	
10:02 am	5 (p.18) - CHANGES IN EXPANSION PRESSURES IN EXPANDER-BASED POST-MASTECTOMY DEFECT RECONSTRUCTION: THE EFFECT OF FAT GRAFTING IN NORMAL AND RADIATED TISSUES Presenter: Donald Browne (USA) Affiliation: Wake Forest Baptist Health Authors: MONSERRAT J; MATAS A; SESE B; LLULL R	
10:10 am	6 (p.19) - STROMAL VASCULAR FRACTION MITIGATES RADIATION-INDUCED GASTRO-INTESTINAL SYNDROME Presenter: Lydia Bensemmane (France) Affiliation: IRSN Authors: SQUIBAN C; DEMARQUAY C; MATHIEU N; BENDERITTER M; MILLIAT F; LINARD C	
10:18 - 10:25 am	Discussion	Las Olas Ballroom
10:25 am	Honoring Catherine Foss	
10:30 - 11:00 am	Coffee Break in Exhibit Hall	Las Olas Ballroom

11:00 - 12:00 pm	Free Papers 1 - Exosomes Moderators: Ramon Llull, MD, PhD, and Marco Helder, PhD		
11:00 am	7 (p.19) - IMMUNOMODULATORY AND REGENERATIVE EFFECTS OF THE FULL AND FRACTIONED ADIPOSE TISSUE DERIVED STEM CELLS SECRETOME IN SPINAL CORD INJURY Presenter: Antonio Salgado (Portugal) Affiliation: University of Minho Authors: CIBRÃO J.; PINHOE A.; LIMA R; GOMES E; SERRA S; LENTILHAS-GRAÇA J; RIBEIRO C; LANCEROS-MENDEZ S; TEIXEIRA F; MONTEIRO S; SILVA N		
11:08 am	8 (p.20) - MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES IN AUTOLOGOUS FAT GRAFTING – LESSONS FROM A METHODOLOGICAL ANALYSIS Presenter: Mohammad Ghiasloo (Belgium) Affiliation: Ghent University Authors: DE WILDE L; SINGH K; TONNARD PL; VERPAELE A; DE WEVER O; HENDRIX A; BLONDEEL PN		
11:16 am	9 (p.20) - INVESTIGATING THE EFFECT OF FACTORS SECRETED FROM ADIPOSE TISSUE ON MYOFIBROBLAST DIFFERENTIATION Presenter: Samuel Higginbotham (United Kingdom) Affiliation: The University of Sheffield Authors: WORKMAN VL; GREEN NH; GIBLIN V; LAMBERT DW; HEARNDEN V		
11:24 am	10 (p.21) - HUMAN PLATELET LYSATE CONCENTRATION IN CULTURE MEDIA AFFECT ADIPOSE-DERIVED STROM POTENCY Presenter: Jesper Svalgaard (Denmark) Affiliation: Stemmedical Authors: BROOKS P; BALLESTEROS O; MUNTHE-FOG L; FISCHER-NIELSEN A; HAASTRUP E	MAL/STEM CELL	
11:32 am	11 (p.22) - DEVELOPMENT OF AN ORGANOID MODEL DERIVED FROM HUMAN ADIPOGENIC STEM/PROGENITOR CELLS TO STUDY WHITE ADIPOSE TISSUE PHYSIOLOGY Presenter: Markus Mandl (Austria) Affiliation: Research Institute for Biomedical Aging Research Authors: VIERTLER HP; BRUCKER C; HATZMANN FM; WALDEGGER P; RAUCHENWALD T; MATTESICH M; ZWIERZINA M; PIERER G; ZWERSCHKE W		
11:40 - 12:00 pm	Discussion		
12:00 - 1:00 pm	Lunch & Learn Tables - Stem Cell & Development, MTF Biologics, Obatala Sciences	Las Olas Ballroom	
1:00 - 2:00 pm	Guest Speaker - Gordon H. Sasaki, MD PRP, Exosomes, and Fat Hair Follicle Growth Moderator: Ricardo Rodriguez, MD	Las Olas Ballroom	
2:00 - 3:00 pm	Industry Showcase Moderators: Marc Long, PhD and Bill Cimino, PhD	Las Olas Ballroom	
2:00 pm	Scaling up the Revolve ™ Platform: Introducing Science and Performance Data for REVOLVE ENVI ™ 600 Presenter: Maryellen Sandor, PhD, Associate Director, Biological Research Affiliation: Allergan Aesthetics an AbbVie company		
2:10 pm	Fat Quality Using a Start-to-Finish Closed Autologous Grafting System (LipoGrafter®) Presenter:Anouska Dasgupta Affiliation: MTF Biologics		
2:20 pm	Your Website: Does It Trigger Scrutiny By Enforcement Agencies? Presenter: Douglas Oliver, MSW Affiliation: Regenerative Outcomes		
2:30 pm	Designing Adipogenic Microphysiological Systems with ObaGel Presenter: Trivia Frazier, PhD Affiliation: Obatala Sciences, Inc.		

2:40 pm	Microfragmented Fat:Methods, Mechanisms and Regulations Presenter: Nathan Katz Affiliation: Jointechlabs	
2:50 pm	Autologous Fat Grafting Accelerates Healing of Recalcitrant Chronic Ulceration on the Diabetic Foot, Non-healing Pressure Sores and Other Problem Wounds of the Lower Limb Presenter: Tilman Stasch, MBChB, MRCS(Ed.), F EBOPRAS, MD Affiliation: CAREstream America	
3:00 - 3:30 pm	Coffee Break in Exhibit Hall	Las Olas Foyer
3:30 - 4:30 pm	Free Papers 2 Moderators: Jeremy Magalon, MD and Robert Bowen, MD FCCP, RVPI	Las Olas Ballroom
3:30 pm	12 (p.22) - HUMAN-PLATELET LYSATE SERUM AS A POTENTIAL CLINICAL-TRANSLATABLE SUPPLEMENT TO SUPPO ADIPOSE-DERIVED STEM CELLS NEUROTROPHIC PROPERTIES Presenter: Martino Guiotto (Switzerland) Affiliation: Centre Hospitalier Universitaire Vaudois - CHUV Authors: PALOMBELLA S; HIGGINS G; APPLEGATE L; RAFFOUL W; HART A; CHERUBINO M; RIEHLE M; DI SUMMA	DRT HUMAN P
3:38 pm	13 (p.23) - UPDATED PLATELET-RICH PLASMA UNDERSTANDINGS AND THERAPEUTIC CONSIDERATIONS Presenter: Peter Everts (USA) Affiliation: Gulf Coast Biologics Authors: FABIO LANA J; MAUTNER K; ONISHI K; JAYARAM P	
3:46 pm	14 (p.24) - AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE (LIPOGEMS): THE ROLE OF MESENCHYMAL STE TREATMENT OS RECURRENT PERIANAL FISTULAS. MULTICENTRIC - (BRAZIL - ITALY) Presenter: Ana Luiza Silva (Brazil) Affiliation: Universidade de Mogi das Cruzes Authors: ROTTA CM; ELBETTI C; GIANI I; BURG MB; TRAGO S	IM CELLS FOR THE
3:54 pm	15 (p.25) - INVESTIGATION INTO USE OF SOLUBLE AND SUSTAINED RELEASE LATANOPROST FOR REDUCING AD Presenter: Lauren Kokai (USA) Affiliation: University of Pittsburgh Authors: SUKINIK J; LEE P; GUERRERO D; SEMAN S; NERONE WV; LODER S; SU S; KOKAI LE	IPOSE VOLUME
4:02 pm	16 (p.25) - HORMONAL, ADIPOGENIC AND ANGIOGENIC ALTERATIONS IN LIPEDEMA ADIPOSE TISSUE Presenter: Eleni Priglinger (Austria) Affiliation: Ludwig Boltzmann Institute Traumatology Authors: HOFMANN M; STROHMEIER K; JACAK J; WEIGL M; HACKL M; HOLNTHONER W; GRILLARI J; REDL H; SAN WOLBANK S	NDHOFER M;
4:10 - 4:20 pm	Discussion	
4:20 - 5:20 pm	Panel 1 - Clinical Applications in Soft Tissue Reconstruction Moderators: Alexandra Conde-Green, MD and Roger Khouri, MD Panelists: Roger Khouri, MD; Robert D. Rehnke, MD; Ricardo Rodriguez, MD	Las Olas Ballroom
5:20 - 5:30 pm	Discussion	
5:30 - 6:30 pm	Guest Speaker - Farshid Guilak, PhD Osteoarthritis and Fat: The Good, The Bad, and The Ugly Moderator: Jeffrey M. Gimble, MD, PhD	Las Olas Ballroom
6:30 pm	Meeting Adjourns for the day	
7:00 - 9:00 pm	Conference Reception & Dinner - Hotel Pool Terrace Pre-registration is required	

• FRIDAY -	NOVEMBER 19		
7:00 - 8:00 am	Continental Breakfast in Exhibit Hall		Las Olas Foyer
8:00 - 9:30 am	Plenary Session 2 - Regenerative Therapies for Sports Medicine Moderators: J. Peter Rubin,MD and Mandi J. Lopez, DVM, M Panelists: Kentaro Onishi, DO; Jaime Garza, MD; Farshid Gui MD; Kyle Richardson	Orthopedics & S, PhD, DACVS lak, PhD; Jason Dragoo,	Las Olas Ballroom
Ma	ain Auditorium - <i>Las Olas Ballroom</i>		Room 2 - <i>Bonnet</i>
9:30 - 10:30 am	Panel 2 - Advances in Bone Regeneration Moderators: Marco Helder, PhD and Susanna Miettinen, PhD Panelists: Marco Helder, PhD; Susanna Miettinen, PhD; Fabrizio Bembo, PhD; Benjamin Levi, MD	9:30 - 10:30 am	Free Papers 7 - Clinical Topics II Moderators: Sherry Collawan, MD, PhD and LaTonya Hickson, PhD
		9:30 am	42 (p.39) - VALIDATION OF A NOVEL, SIMPLE AND INEXPENSIVE SCANNING PROCESS FOR THE THREE- DIMENSIONAL ASSESSMENT OF THE GLUTEAL REGION Presenter: Carlo Oranges (Switzerland) Affiliation: Basel University Hospital Authors: RICKLIN A; BENITEZ B; SCHARMA N; SCHAEFER DJ; KALBERMATTEN DF; THIERINGER FM
		9:38 am	43 (p.40) - ENRICHMENT OF THE FACIAL FAT GRAFT FOR INCREASED VOLUME RETENTION, A SYSTEMATIC REVIEW Presenter: Jan Aart Schipper (Netherlands) Affiliation: University Medical Centre Groningen Authors: VRIEND L; TUIN AJ; DIJKSTRA PU; SCHEPERS RH; VAN DER LEI B; JANSMA J; HARMSEN MC
		9:46 am	44 (p.40) - ENHANCED UTILITY AND OPERATIVE EFFICIENCY OF THE NOVEL PUSH-TO-SPIN HANDHELD (P2S) FAT GRAFT PROCESSING DEVICE Presenter: Shawn Loder (USA) Affiliation: University of Pittsburgh Authors: LEE PL; KOKAI L; RUBIN JP; GUSENOFF BR; GUSENOFF JA
		9:54 am	45 (p.41) - MSC MECHANICAL DISSOCIATION SINCE 2006: THE PARADIGMA SHIFT AND THE NEW CLINICAL APPLICATIONS Presenter: Hebert Lamblet (Brazil) Affiliation: Vikaara Klinik
		10:02 - 10:20 am	Discussion
10:20 - 11:00 am	Coffee Break in Exhibit Hall		Las Olas Foyer

Main Auditorium - Las Olas Ballroom			Room 2 - <i>Bonnet</i>
11:00 - 12:30 pm	Free Papers 3 - Cell Activity and Senescence Moderators: Lauren Kokai, MD and Trivia Frazier, PhD	11:00 - 12:30 pm	Free Papers 8 Musculoskeletal Applications Moderators: Susanna Miettinen, PhD and Fred Kølle, MD, PhD
11:00 am	17 (p.26) - QUIESCENCE, STEMNESS AND ADIPOGENIC DIFFERENTIATION CAPACITY IN HUMAN DLK1-/CD34+/ CD24+ ADIPOSE STEM/PROGENITOR CELL Presenter: Florian Hatzmann (Austria) Affiliation: University of Innsbruck Authors: EJAZ A; WIEGERS GJ; MANDL M; BRUCKER C; LECHNER S; RAUCHENWALD T; ZWIERZINA M; BAUMGARTEN S; WAGNER S; MATTESICH M; WALDEGGER P; PIERER G; ZWERSCHKE W	11:00 am	47 (p.42) - 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: Atara Novak (Israel) Affiliation: Bonus BioGroup LTD. Authors: NOVAK A; BRONSHTEIN T; KIVITY V; TZUR E; ROZEN N; MERETZKI S
11:08 am	18 (p.26) - SENESCENCE DID NOT ALTER THE CHONDROPROTECTIVE EFFECT OF EXTRACELLULAR VESICLES FROM ADIPOSE MESENCHYMAL STROMAL CELLS Presenter: Jérémy Boulestreau (France) Affiliation: Inserm Authors: NOEL D; MAUMUS M; ROZIER P; JORGENSEN C	11:08 am	48 (p.42) - INTRA ARTICULAR INJECTION OF AUTOLOGOUS MICROFAT AND PLATELET-RICH PLASMA IN THE TREATMENT OF KNEE OSTEOARTHRITIS: A DOUBLE BLIND RANDOMIZED COMPARATIVE STUDY Presenter: Jeremy Magalon (France) Affiliation: Culture and Cell Therapy Unit INSERM CBT1409 C2VN Research Unit Authors: LOUIS ML; GRAVIER DUMONCEAU R; JOUVE E; COHEN M; DJOURI R; RICHARDET N; JOURDAN E; GIRAUDO L; DUMOULIN C; GRIMAUD F; DIGNAT GEORGE F; VERAN J; SABATIER F; MAGALON J
11:16 am	19 (p.27) - DETECTION, QUANTIFICATION AND ELIMINATION OF SENESCENCE IN AGED AND/OR CULTURE EXPANDED HUMAN ADIPOSE DERIVED STEM CELLS Presenter: Sudheer Ravuri (USA) Affiliation: Steadman Philippon Research Institute Authors: MULLEN MM; BILLINGS JB; MITCHELL JM; HAMBRIGHT SH; HUARD JH	11:16 am	49 (p.43) - A NOVEL METHOD TO PROVIDE 3D MRI IMAGING FOR EVALUATING HUMAN KNEE CARTILAGE: CLINICAL TRAIL OF SVF THERAPY FOR HUMAN KNEE OA Presenter: Xin Xiao Zheng (China) Affiliation: Wuhan University Zhongnan Hospital Authors: LIU R; REN B; XIAO F; XU J; LI L; LI T; RUAN Z; BAO Y; LING J; ZHAO J; LIAO W; PAN Z; RUBIN P; XU H; TIAN J; CAI L
11:24 am	20 (p.27) - ADIPOSE DERIVED STEM CELLS ACCUMULATE SENESCENCE WITH AGE AND SERIAL PASSAGING Presenter: Michael Mullen (USA) Affiliation: Steadman Philippon Research Institute Authors: HUARD J; HAMBRIGHT WS; RAVURI S	11:24 am	50 (p.43) - IMMUNOREGULATORY PROPERTIES OF A HUMAN LYOPHILIZED 3D SCAFFOLD FREE TISSUE ENGINEERED PRODUCT FOR BONE RECONSTRUCTION Presenter: Carmen Brenner (Belgium) Affiliation: Novadip Biosciences SA Authors: EPISKOPOU H
11:32 am	21 (p.28) - IDENTIFICATION AND HISTOLOGICAL MAPPING OF SENESCENT STROMAL CELLS IN ADIPOSE TISSUE: A PATH TOWARDS TISSUE DESENESCENCE Presenter: Anthony Elias (USA) Affiliation: Wake Forest Baptist Medical Center Authors: JUSTICE JN; LLULL R	11:32 am	51 (p.44) - BONOFILL: A NOVEL TISSUE-ENGINEERED AUTOLOGOUS BONE GRAFT FROM ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS FOR MAXILLOFACIAL BONE TISSUE REGENERATION Presenter: Dror Ben David (Israel) Affiliation: Bonus BioGroup LTD. Authors: BEN-DAVID D; BRONSHTEIN T; ROZEN N; KIVITY V; TZUR E; MERETZKI S
11:40 - 11:50 am	Discussion	11:40 - 11:50 am	Discussion

Ma	ain Auditorium - <i>Las Olas Ballroom</i>	Room 2 - <i>Bonnet</i>		
11:50 am	22 (p.28) - INVOLVEMENT OF STROMAL AGE IN BREAST CANCER ENDOCRINE RESPONSE Presenter: Katie Hamel (USA) Affiliation: Louisiana State University Authors: CAVALIER MB; LIIMATTA KQ; ROZANSKI GL; KING CT; BELGODERE JA; BRATTON MR; BUNNELL BA; MARTIN EC	11:50 am	52 (p.44) - MECHANICALLY DERIVED VASCULAR FRACTION ACTS ANTI-INF CHONDROCYTES IN VITRO Presenter: Lucienne Vonk (Netherland Affiliation: University Medical Center Authors: VAN BOXTEL J; VAN DONGE STEVENS HP	TISSUE-STROMAL LAMMATORY ON s) Groningen N J; VONK LA;
11:58 am	23 (p.29) - IN VITRO PBMC ASSAYS TO EVALUATE IMMUNOMODULATORY CELL PRODUCT POTENCY AND PREDICT IN VIVO THERAPEUTIC RESPONDERS Presenter: Elaine Richards (USA) Affiliation: University of Florida Authors: SAVA R; ZAMAN MO; HANDBERG EM; PEPINE CJ; MARCH KL	11:58 am	53 (p.45) - BONE-MARROW DERIVED STEM/STROMAL CELLS HAVE ENHAN VASCULOGENIC POTENCY OVER ADI MESENCHYMAL STEM/STROMAL CEL VITRO CULTURES Presenter: Susanna Miettinen (Finland Affiliation: Tampere University Authors: MYKULIAK A; YRJÄNÄINEN J VUORENPÄÄ H	D MESENCHYMAL CED POSE DERIVED LLS IN PERFUSED IN d) A; GEBRAAD A;
12:06 pm	24 (p.29) - PERSONAL CELL THERAPY – THE BLACK SWAN OF REGENERATIVE MEDICINE CAN WE HEAL LYMPHEDEMA? Presenter: Mark Berman (USA) Affiliation: University of Southern California	12:06 pm	54 (p.45) - ISOLATION OF HUMAN AI STROMAL CELLS USING SUCTION-AS ULTRASOUND-ASSISTED LIPOASPIRA THERAPEUTIC POTENTIAL IN CARTILA ENGINEERING Presenter: Jian Wang (China) Affiliation: Suzhou Hospital of Anhui I Authors: CAI CH; ZHOU XU	DIPOSE-DERIVED SISTED OR TION AND THEIR AGE TISSUE Medical University
12:14 pm	25 (p.30) - CHARACTERIZATION AND POTENTIAL USE OF NOVEL PLURIPOTENT ADIPOSE STEM CELLS (PASCS) IN REGENERATIVE MEDICINE AND CELL THERAPY Presenter: Gregorio Chazenbalk (USA) Affiliation: UCLA Authors: CASANUEVA DE ROSA P; GIMENO M	12:14 pm	55 (p.46) - AN ALLOGENIC TISSUE-EN PRODUCT FOR BONE REGENERATION INDUCTION OF ENDOCHONDRAL OS INHIBITION OF OSTEOCLASIA Presenter: Hara Episkopou (Belgium) Affiliation: Novadip Biosciences SA Authors: THEYS N; THIRION G	NGINEERED N ENABLING SIFICATION AND
12:22 - 12:30 pm	Discussion	12:22 - 12:30 pm	Discussion	
12:30 - 1:30 pm	Lunch & Learn with Allergan Aesthetics an AbbVie co	ompany		Las Olas Ballroom
1:30 - 2:00 pm	Late Breaking Abstracts Moderators: Bruce Bunnell, PhD and Jeffery M. Gimble, MD,	PhD		Las Olas Ballroom
1:30 - 1:38 pm	A (p.57) - AUTOLOGOUS FAT GRAFTING AS TREATMENT OF Presenter: Martin Sollie, MD (Denmark) Affiliation: Research Unit for Plastic Surgery, Odense Univers Authors: Sollie M, Toyserkani N, Bille C, Thomsen JB, Sørense	POST-MASTECTOMY P. ity Hospital, DK n JA	AIN SYNDROME: A RANDOMIZED CON	ITROLLED
1:38 - 1:46 pm	B (p.57) - ENHANCED POTENCY OF A CELL-FREE STEM CELL Presenter Yansheng Du (USA) Affiliation Indiana University School of Medicine Authors Xin Yi, Huiying Gu, Brian H Johnstone, Michael Cole	- THERAPY FOR TREATI man, Keith L March, Yai	MENT PF ISCHEMIC STROKE BY DEPLE	TION OF MCP
1:46 - 1:54 pm	C (p.58) - EVALUATION OF THE SAFETY AND EFFICACY OF A Presenter: Robert Harman, DVM, MPVM (USA) Affiliation: Personalized Stem Cells, Inc. Authors: Rogers C, Harman R, Sheinkop M, David T, Hanson	N AUTOLOGOUS ADIP	DSE SVFTTREATMENT OF KNEE OSTEO D, Desai R, Malanga G, Bowen J, Amba	ARTHRITIS ack M

1:54 - 2:00 pm **Discussion**

Main Auditorium - Las Olas Ballroom		Room 2 - <i>Bonnet</i>		
2:00 - 3:00 pm	Free Papers 4 - Characterization and Behavior Moderators: Kacey Marra, PhD and Rosalyn Abbott, PhD	2:00 - 3:00 pm	Free Papers 9 - Radiation Injury Moderators: Julie Fradette, PhD and Pietro Gentile, MD	
2:00 pm	26 (p.30) - EXADEX: TOWARDS A MICROPHYSIOLOGICAL SYSTEM MODELING HUMAN ADIPOSE PROGENITOR CELL EXPANSION IN AN OBESE ADIPOSE TISSUE MICROENVIRONMENT Presenter: Christian Dani (France) Affiliation: iBV Authors: DANI V; BRUNI-FAVIER S; CARRIÈRE A; DEVINEAUX L; KÉOPHIPHATH M; CHIGNON-SICARD B; CASTEILLA L; DOGLIO A; DANI C	2:00 pm	56 (p.46) - ADIPOSE MESENCHYMAL STEM CELLS TREATMENT LIMITS CHRONIC RADIATION CYSTITIS Presenter: Clement Brossard (France) Affiliation: IRSN Authors: LEFRANC AC; DOS SANTOS M; DEMARQUAY C; BUARD V; TARLET G; SQUIBAN C; LINARD C; MATHIEU N; SIMON JM; MILLIAT F; CHAPEL A	
2:08 pm	27 (p.31) - REGULATION OF ADIPOGENIC POTENTIAL IN DEDIFFERENTIATED LIPOSARCOMAS Presenter: Elizabeth Floyd (USA) Affiliation: Pennington Biomedical Research Center Authors: DANG TN; TIONGCO RP; BROWN LM; TAYLOR JL; LYONS JM; LAU FH	2:08 pm	57 (p.47) - ALLOGENEIC ADIPOSE DERIVED STEM CELLS MITIGATE ACUTE RADIATION SYNDROME Presenter: Somaiah Chinnapaka (USA) Affiliation: University of Pittsburgh Authors: YANG S; SAMADI Y; EPPERLY M; HOU W; GREENBERGER J; EJAZ A; RUBIN P	
2:16 pm	28 (p.31) - MONOCYTE CHEMOATTRACTANT PROTEIN- 1-SUPPLEMENTED PLASMA ENHANCE ADIPOGENESIS THROUGH IL-33/DPP4 Presenter: Lee-Wei Chen (Taiwan) Affiliation: Kaohsiung Veterans General Hospital Authors: LI M; CHEN P	2:16 pm	58 (p.47) - CLINICAL TRIAL EVALUATING THE EFFICACY OF MESENCHYMAL STROMAL CELL INJECTIONS FOR THE TREATMENT OF CHRONIC PELVIC COMPLICATIONS INDUCED BY RADIATION THERAPY Presenter: Alain Chapel (France) Affiliation: IRSN Authors: SEMONT A; LINARD C; MATHIEU N; DEMARQUAY C; SQUIBAN C; VOSWINKEL J; ROUARD H; LATAILLADE JJ; MARTINAUD C; BENDERITTER M; GORIN NC; SIMON JM; MOTHY M	
2:24 pm	29 (p.32) - CHARACTERIZATION OF ADIPOSE TISSUE AND ADIPOSE-TISSUE DERIVED STEM CELLS IN LIPEDEMA Presenter: Sara Al-Ghadban (USA) Affiliation: University of North Texas Health Science Center Authors: HERBST KL; BUNNELL BA	2:24 pm	59 (p.48) - ALLOGENEIC ADIPOSE TISSUE-DERIVED MATRIX MITIGATE RADIATION-INDUCED FIBROSIS (RIF) Presenter: Asim Ejaz (USA) Affiliation: University of Pittsburgh Authors: CHINNAPAKA S; KATHERINE Y; EPPERLY M; WEN U; GREENBERGER J; RUBIN P	
2:32 pm	30 (p.32) - VITAMIN D AS A METABOLIC LIPOPROTECTANT: PHARMACOLOGIC ENHANCEMENT OF FAT GRAFT SURVIVAL Presenter: Phoebe Lee (USA) Affiliation: University of Pittsburgh School of Medicine Authors: LODER SL; LEFTWICH PA; NERONE WN; SINGH- VARMA AS; MARRA KM; RUBIN PR; KOKAI LK	2:32 pm	60 (p.48) - ADIPOSE-DERIVED REGENERATIVE CELLS AND LIPOTRANSFER IN ALLEVIATING BREAST CANCER- RELATED LYMPHEDEMA: AN OPEN-LABEL PHASE I TRIAL WITH 4-YEARS OF FOLLOW-UP Presenter: Mads Jørgensen (Denmark) Affiliation: Odense University Hospital Authors: TOYSERKANI NM; JENSEN CH; ANDERSEN DC; SHEIKH SP; SØRENSEN JA	
2:40 - 3:00 pm	Discussion	2:40 - 3:00 pm	Discussion	
М	ain Auditorium - <i>Las Olas Ballroom</i>		Room 2 - <i>Bonnet</i>	
3:00 - 4:00 pm	Free Papers 5 - Clinical Topics I Moderators: Ivona Percec, MD, PhD and Filip Stillaert, MD	3:00 - 4:00 pm	Free Papers 10 - Matrix Moderators: Rosalyn Abbott, PhD and Aaron W. James, MD	

3:00 pm	31 (p.33) - 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 Tor Vergata	3:00 pm	61 (p.49) - DIFFERENCES OF EMBEDDING ADIPOSE- DERIVED STROMAL CELLS IN NATURAL AND SYNTHETIC SCAFFOLDS FOR DERMAL AND SUBCUTANEOUS DELIVERY Presenter: Frederik Mamsen (Denmark) Affiliation: StemMedical Authors: MUNTHE-FOG L; KRING MK; DUSCHER D; TAUDORF M; KATZ AJ; KØLLE SF
3:08 pm	32 (p.33) - STUDY INTO THE EFFECT OF FAT GRAFTING ON SKIN CONTRACTION IN VITRO Presenter: Victoria Workman (United Kingdom) Affiliation: University of Sheffield Authors: GIBLIN V; GREEN NH; MACNEIL S; HEARNDEN V	3:08 pm	62 (p.50) - SKIN-DERIVED EXTRACELLULAR MATRIX HYDROGELS LOADED WITH ADIPOSE TISSUE-DERIVED STROMAL CELL- SECRETED FACTORS AS TREATMENT FOR ENHANCING WOUND REGENERATION AND VIABILITY OF SKIN FLAPS: A RAT MODEL Presenter: Cristina Camargo (Netherlands) Affiliation: University and Medical Center Groningen Authors: VAN DONGEN JA; VAN DER LEI B; HARMSEN MC; VRIEND L
3:16 pm	33 (p.34) - SKIN REJUVENATION USING CONCENTRATE STROMAL VASCULAR TISSUE: AN INNOVATIVE TECHNIQUE FOR DIFFICULT AREAS Presenter: Sophie Menkes (Switzerland) Affiliation: Nescens Clinique de Genolier	3:16 pm	63 (p.51) - THE EFFECT OF STROMAL VASCULAR FRACTION CELLS MIXED WITH DIFFERENT TYPES OF HYALURONIC ACID FILLERS IN IMMUNODEFICIENT MICE Presenter: Heetae Koo (SouthKorea) Affiliation: Un Seoul National University Hospital Authors: JIN US
3:24 pm	34 (p.34) - TISSUE STROMAL VASCULAR FRACTION TO PREVENT DERMAL SCARRING: A PROSPECTIVE RANDOMIZED MULTICENTER CLINICAL TRIAL Presenter: Joris van Dongen (Netherlands) Affiliation: University Medical Center Groningen Authors: VAN BOXTEL J; UGUTEN M; BROUWER LA; VERMEULEN KM; MELENHORST WB; NIESSEN FB; HARMSEN MC; STEVENS HP; VAN DER LEI B	3:24 pm	64 (p.51) - 3D PRINTED MICRONIZED FAT-LADEN COLLAGEN CONSTRUCTS FOR TREATMENT OF CHRONIC WOUNDS Presenter: Nathan Katz (USA) Affiliation: Jointechlabs Inc Authors: SCHMITT T.; KISHORE V.
3:32 pm	35 (p.35) - PRECISE SUBDERMAL FAT GRAFT INJECTION TECHNIQUE FOR SAFE GLUTEAL AUGMENTATION Presenter: Ricardo Rodriguez (USA) Affiliation: Cosmeticsurgnet	3:32 pm	65 (p.52) - EXTRACELLULAR MATRIX-DERIVED HYDROGELS TO AUGMENT DERMAL WOUND HEALING: A SYSTEMATIC REVIEW Presenter: Martin Harmsen (Netherlands) Affiliation: University and Medical Center Groningen Authors: SINKUNJAS V; VRIEND L; VAN DER LEI B; VAN DONGEN JA
3:40 - 4:00 pm	Discussion	3:40 - 4:00 pm	Discussion
4:00 - 4:30 pm	Coffee Break in Exhibit Hall		Las Olas Foyer
Ма	ain Auditorium - <i>Las Olas Ballroom</i>		Room 2 - <i>Bonnet</i>
4:30 - 5:30 pm	Free Papers 6 - Adipose Tissue Processing Moderators: Kotaro Yoshimura, MD and Fred Kølle, MD, PhD	4:30 - 5:30 pm	Free Papers 11- Skin and Scars and Pain Moderators: Alexandra Conde-Green, MD and Ivona Percec, MD, PhD

4:30 pm	36 (p.35) - TOWARDS STANDARDIZATION IN MECHANICALLY ISOLATED STROMAL VASCULAR FRACTION RESEARCH – PRELIMINARY RESULTS OF A METHODOLOGICAL ANALYSIS Presenter: Ann-Sophie Madeleyn (Belgium) Affiliation: Ghent University Authors: DÍAZ JM; LOBATO RC; BUELVAS BUSTILLO AM; SINGH K; BLONDEEL PN; TONNARD PL; VERPAELE A; GHIASLOO M	4:30 pm	66 (p.52) - STROMAL VASCULAR FRACTION-ENRICHED FAT GRAFTING AS TREATMENT OF ADHERENT SCARS: STUDY DESIGN OF A PROSPECTIVE COHORT STUDY Presenter: Linda Vriend (Netherlands) Affiliation: University and Medical Center Groningen Authors: VAN DONGEN JA; PIJPE A; NIEUWENHUIS MK; JONGEN SJ; HARMSEN MC; VAN ZUIJLEN PM; VAN DER LEI B
4:38 pm	37 (p.36)- IMPROVING THE TECHNIQUE OF NON- ENZYMATIC METHOD FOR OBTAINING THE STROMAL- VASCULAR FRACTION Presenter: Natalia Khramtsova (Russia) Affiliation: EA Vagner Perm State Medical University Authors: PLAKSIN SA; SOTSKOV AY; PONOMAREV DN; GULYAEVA NI	4:38 pm	67 (p.53) - DIRECT COMPARISON OF BIOLOGICAL- BASED THERAPIES ON HUMAN FOLLICLE DERMAL PAPILLA CELL FUNCTION Presenter: John Cole (USA) Affiliation: PHTC Authors: COLE MA
4:46 pm	38 (p.36) - TO LYSE, OR NOT TO LYSE? Presenter: Gabriela Aguilo-Seara (USA) Affiliation: Wake Forest School of Medicine Authors: SHANG H; NORTHRUP S; LLULL R; KATZ AJ	4:46 pm	68 (p.54) - AUTOLOGOUS FAT GRAFTING AS TREATMENT OF POSTHERPEUTIC NEURALGIA - RESULTS OF A DOUBLE-BLINDED PLACEBO-CONTROLLED RCT Presenter: Martin Sollie (Denmark) Affiliation: Odense University Hospital Authors: THOMSEN JB; SØRENSEN JA
4:54 pm	39 (p.37) - CELL VIABILITY IS NOT COMPROMISED IN MECHANICALLY SHEARED CELL-DENSE STROMAL CELL AGGREGATES Presenter: Ramon Llull (USA) Affiliation: Wake Forest School of Medicine Authors: SESE B; MONSERRAT J; SHANG H; SANFILLIPO WA; KATZ AJ; AGUILO SEARA G	4:54 pm	69 (p.55)- REVIEW OF ADIPOSE GRAFTING AND PLATELET RICH PLASMA FOR VOLUMIZATION AND SCAR RELEASE Presenter: Sherry Collawn (USA) Affiliation: UAB Authors: JAFFE TJ; TEKUMALLA ST
5:02 pm	40 (p.38) - LONG TERM CLINICAL RESULTS OF MECHANICAL STROMAL-CELL TRANSFER (MEST) AND ADJUSTABLE REGENERATIVE ADIPOSE TRANSFER (ARAT) BY USING ULTRA-SHARP BLADES Presenter: Eray Copcu (Turkey) Affiliation: MEST Authors: OZTAN S	5:02 pm	70 (p.56)- TRANSDERMAL NANOFAT DELIVERY ACCELERATES SKIN RESURFACING RECOVERY FOLLOWING CO2 LASER AND MINIMALLY INVASIVE PERCUTANEOUS COLLAGEN INDUCTION TREATMENT Presenter: Brannon Claytor (USA) Affiliation: Claytor/Noone Plastic Surgery
5:10 pm	41 (p.39) - DEVELOPMENT AND VALIDATION OF A FULLY GMP-COMPLIANT PROCESS FOR MANUFACTURING STROMAL VASCULAR FRACTION: A COST-EFFECTIVE ALTERNATIVE TO AUTOMATED METHODS Presenter: Pauline Francois (France) Affiliation: C2VN Aix marseille univ INSERM 1263 INRA 1260 Authors: MAGALON J; GIRAUDO L; VERAN J; BERTRAND B; DUMOULIN C; ABOUDOU H; GRIMAUD F; VOGTENSPERGER M; VELIER M; ARNAUD L; LYONNET L; SIMONCINI S; GUILLET B; DIGNAT-GEORGE F; SABATIER F	5:10 pm	71 (p.56) - SYNGENEIC FAT GRAFTING ATTENUATES HYPERSENSITIVITY IN A LEWIS RAT NERVE CRUSH INJURY MODEL Presenter: Benjamin Scott (USA) Affiliation: Massachusetts General Hospital Authors: MCCORMACK MC; BEJAR MK; AUSTEN WG
5:18 - 5:30 pm	Discussion	5:18 - 5:30 pm	Discussion
5:30 - 6:30 pm	Guest Speaker - Camilo Ricordi, MD Micro-fragmented Tissue in Regenerative Surgery: A Cost-effective Opportunity for the Future of Healthcare Moderator: Stuart Williams, PhD	Transformational	Las Olas Ballroom
6:30 pm	Meeting Adjourns for the day		

• SATURDA	Y - NOVEMBER 20	
7:30 - 8:30 am	Continental Breakfast in Exhibit Hall	Las Olas Foyer
8:00 - 9:00 am	IFATS Members Meeting	Las Olas Ballroom
9:00 - 10:00 am	Guest Speaker - Arnold Caplan, PhD MSCs: Bigger and Better than Ever Moderator: Ricardo Rodriguez, MD	Las Olas Ballroom
10:00 - 10:30 am	Coffee Break in Exhibit Hall	Las Olas Foyer
10:30 - 11:30 am	Plenary Session 3 - Regulatory Affairs Moderators: Adam Katz, MD and Keith March, MD Panelists: Mark Berman,MD, Bernard Siegel, MD, Keith March, MD, Doug Oliver, MSW	Las Olas Ballroom
11:30 - 11:45 am	Closing Remarks - Torsten Blunk, PhD	Las Olas Ballroom
11:45 am	Meeting Adjourns	

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ABSTRACT 1

MESENCURE—AN ENHANCED ALLOGENEIC ADIPOSE-DERIVED CELL THERAPY DEVELOPED FOR TREATING ACUTE RESPIRATORY DISTRESS IN COVID-19: FROM BENCHTOP TO BEDSIDE

Presenter: Tomer Bronshtein (Israel) Affiliation: Bonus BioGroup Ltd. Authors: BEN DAVID D; NOVAK A; KIVITY V; HAMOUD S; HAYEK T; MERETZKI S

Mesenchymal stromal cells (MSC) have attracted much attention for treating ARDS in Covid-19. These efforts are, nonetheless, overshadowed by earlier studies that showed marginal efficacy attributable to loss of MSC potency. Also, concerns were raised regarding the hemocompatibility of MSCs in coagulopathic Covid-19 patients. Relying on years of experience and manufacturing capacity of clinical-grade MSCs, Bonus BioGroup developed MesenCure—an enhanced allogeneic adipose-derived MSC product for IV injection professionalized to treat ARDS in Covid-19.

MesenCure is based on technologies for the efficient and standardized isolation and cultivation of adipose MSCs yielding 600k p0 MSCs per ml fat and up to 45k doses per donor cell bank under 20 PDLs. In addition, the cells are formulated for cold storage, avoiding potency loss associated with cryopreservation while providing sufficient shelf-life for global supply. Importantly, MesenCure cells are professionalized to treat ARDS by exposure to a combination of biological and physical priming conditions that enhance their potency and safety.

Compared to unprimed MSCs, MesenCure cells express higher levels of immunomodulatory and regenerative genes, including EDIL3, IL6R, and FGF7. In vitro, MesenCure inhibited the proliferation of activated PBMCs twice more effectively than unprimed MSCs. MesenCure, but not unprimed MSCs, alleviated edema in an acute lung injury model by 60% and reduced the leukocytes' counts in the lung fluids by 40% while reversing the pneumonia pathological presentation. MesenCure cells are also more hemocompatible with 50% less Tissue Factor at the mRNA, protein, and activity levels and >2-fold higher level of Tissue Factor Pathway Inhibitor compared to unprimed MSCs.

MesenCure is tested in a Phase II study in severe Covid-19 patients hospitalized at the Rambam Health Care Campus (Haifa, Israel). Results from a Phase I/II study on ten such patients with high-risk comorbidities (90%) demonstrated significant improvements in all objective and subjective health parameters following MesenCure treatment with no IMP-related AEs. Patients were discharged within 1 day following treatment, requiring no respiratory support and pointing to MesenCure's potential for treating ARDS and other conditions.

ABSTRACT 2

DEVELOPMENT OF A 3D BIOPRINTED BREAST CANCER-STROMA CO-CULTURE MODEL UTILIZING ADIPOSE-DERIVED STROMAL CELL SPHEROIDS

 Presenter: Hannes Horder (Germany)

 Affiliation: University of Wuerzburg

 Authors:
 GUAZA LASHERAS M; GRUMMEL N; NADERNEZHAD A;

 HERBIG J; ERGUN S; TESSMAR J, GROLL J; FABRY B; BAUER-KREISEL

 P, BLUNK T

Introduction: In breast cancer, stromal cells associated with the tumor microenvironment, such as adipose-derived stromal cells (ASCs) and adipocytes, contribute to various stages of tumor development, progression, and metastasis. To investigate the complex interplay between tumor and stromal cells in vitro in a more physiological context, we developed a 3D breast cancer-stroma model utilizing bioprinted human ASC spheroids and MDA-MB-231 breast cancer cells.

Methods: ASC spheroids were generated on a large-scale and dispersed in a thiol-modified hyaluronic acid bioink for the use in an extrusion-based bioprinting setup. Viability and differentiation capability of the printed spheroids were assessed. Adipogenically differentiated and undifferentiated spheroids were co-cultured with MDA-MB-231 breast cancer cells in printed 3D constructs. Cancer-induced alterations of the lipid content and ECM composition of the spheroids were analyzed after 9 days of co-culture.

Results: Bioink composition and the printing process were optimized with regard to homogeneous spheroid distribution and cell viability. The printing process had no negative impact on the differentiation behavior of spheroids, as shown regarding triglyceride accumulation and adipogenic marker gene expression (PPARY, C/EBPa, FABP4). Differentiated spheroids developed into adipose microtissues, exhibiting a native adipose tissue-like ECM composition, with highly elevated levels of laminin and collagen IV, enhanced collagen I and VI, and a marked reduction of fibronectin. In the printed co-culture model, differentiated spheroids revealed a significant reduction of lipid content and ECM remodeling with increased collagen I, VI and fibronectin, as compared to the monoculture control. Undifferentiated spheroids also showed ECM remodeling, with increased levels of tenascin c and collagen I.

Conclusions: A 3D-bioprinted breast cancer model was developed as proof-of-concept that recapitulates characteristic cancer cellinduced alterations in stromal cells known from in vivo studies, such as reduction of lipid content in adipocytes and ECM remodeling. The biofabricated 3D model may facilitate to further decipher the complex tumor-stroma crosstalk in breast cancer in a defined, native-like environment.

VASCULARIZED ADIPOSE TISSUE GRAFT USING A DECELLULARIZED LUNG FOR SOFT TISSUE RECONSTRUCTION

Presenter: Rosalyn Abbott (USA) Affiliation: Carnegie Mellon University

Authors: DEBARI MK; NG WH; KOKAI LE; MARRA KG; RUBIN JP; REN X

Introduction: Innovation is needed to engineer critically sized adipose tissue implants that can fill large tissue defects, rapidly integrate into the host vascular system and maintain volume. This will result in minimal donor site morbidity, can be implemented in lean patients, and eliminates the need for immunosuppressive therapy. By using a decellularized lung matrix (DLM), perfusable, pre-vascularized, large-volume adipose grafts can be developed using patient-derived cells.

Materials & Methods: Rat lungs were isolated and decellularized. Mature adipocytes and adipose derived stem cells (ADSCs) were isolated from primary human adipose tissue obtained from liposuction/ panniculectomies. Human umbilical vein endothelial cells were seeded into the DLM through the vasculature two hours before the cell solution was injected into the alveoli through the trachea. The lung was perfused with adipocyte maintenance media (DMEM, 10% FBS, and 1% Pen/Strep) through the vasculature for seven days. The lungs were then fixed and stained (Adipored and CellTracker green dye). Separate lungs were seeded with ADSCs and cultured statically in differentiation media (DMEM. 10% FBS, 1 μM insulin, 0.5 mM IBMX, 1 μM dexamethasone, 0.05 mM indomethacin) to initiate differentiation into adipocytes. After five weeks of culture, the lungs were fixed, stained (Adipored, DAPI, Phalloidin), and imaged using confocal microscopy.

Results: DLMs seeded with adipocytes and ADSC remained viable for 5 weeks in static culture (Fig. 1A-B). ADSCs cultured using this perfusion technique differentiated into uniocular adipocytes and experienced increased lipogenesis (increase in triglyceride content) compared to DLMs in static culture (data not shown). The 3D perfusion system also stimulated greater lipolysis and resulted in decreased concentrations of lactic acid dehydrogenase, demonstrating enhanced function and reduced cell toxicity, respectively. Adipocytes remained in alveolar space while endothelial cells were successfully seeded along vasculature (Fig. 1C).

Discussion: Decellularized lung matrices supported adipocyte and adipose derived stem cell viability, function, differentiation, and growth by providing an established, perusable vascular system.

ABSTRACT 3 Image:



Figure 1. The DLM supports adipocytes (A) and differentiation of ADSCs (B) for 5 weeks. Staining: AdipoRed (red), DAP1 (blue), and Phalloidin (green, *B only*). Scale=100 μ m. Rat DLM seeded with adipocytes (red = AdipoRed) (C) in the alveolar compartment & endothelial cells (green = CellTracker Green Dye) in the



ABSTRACT 4

ADIPOSE STEM CELL-BASED THERAPIES FOR FIBROSIS AND SCARRING OF THE VULVA

Presenter:Aurora Almadori (Italy)Affiliation:UCL - Royal Free HospitalAuthors:BOYLE D; HANSEN E; REID W; MACLEAN A; BUTLER PE

Introduction: Minimally invasive regenerative therapies including autologous fat transfer, adipose-derived stem cells (ASCs) and plateletrich plasma (PRP) have been proposed as new option to treat women with fibrosis and scarring in the vulvar and perineal area. The aim of the study was to evaluate the outcome in a mixed cohort of women presenting fibrosis and scarring of the vulva due to lichen sclerosus, post-partum episiotomy/lacerations, and female genital mutilation.

Materials & Methods: A series of 50 patients was included and prospectively assessed. The outcome evaluation included: clinical signs of disease assessed with the Vulvar Architecture Severity Scale (VASS), symptoms (Visual Analogue Scale), sexual function (Female Sexual Function Index), sexual distress (Female Sexual Distress Scale), anxiety and depression (Hospital Anxiety and Depression Scale), and romantic relationship (Relationship Assessment Scale).

Results: The clinical score showed an overall improvement in the treated areas, particularly in labia minora, clitoral area and posterior fourchette (p<0,001). A significant improvement was reported in the VAS for itching (p<0,05) and soreness (p<0,05). Sexual function was improved after treatment (p<0,05), as well as the distress associate with sexuality (p<0,05). In addition, the patients reported a significant improvement in the level of anxiety (p<0,05) and depression (p<0,05).

Conclusion: Regenerative surgery represents a potentially transformative therapy for vulvar scarring. It reverses skin fibrosis, ameliorates symptoms and patients' quality of life. In vitro studies are required to better understand the mechanism of action and to allow the optimization of the surgical technique.

CHANGES IN EXPANSION PRESSURES IN EXPANDER-BASED POST-MASTECTOMY DEFECT RECONSTRUCTION: THE EFFECT OF FAT GRAFTING IN NORMAL AND RADIATED TISSUES

 Presenter:
 Donald Browne (USA)

 Affiliation:
 Wake Forest Baptist Health

 Authors:
 MONSERRAT J; MATAS A; SESE B; LLULL R

Introduction: The impact of fat grafting on the viscoelasticity of irradiated tissues is poorly defined. We investigate the effect of subcutaneous fat grafting on post-mastectomy tissue expansion in patients undergoing delayed breast reconstruction. We hypothesize fat grafting changed the trophic and viscoelastic properties of the breast soft tissue envelope.

Methods: Post-mastectomy defects delayed more than two years and reconstructed with sub-pectoral tissue expanders were prospectively studied. Control (no irradiation, no fat grafting, n=7), fat grafted control (no irradiation, fat grafting n=8), and radiated mastectomy (irradiation, fat grafting, n=9) groups were included (Figure 1). Hydrostatic pressure of the tissue was measured before and immediately after expansion, and again post expansion day 1 and prior to the following expansion session (Figure 2). Pressure changes calculated as "post expansion-relaxation interval": difference between maximal pressure at each expansion and the minimal pressure prior to each expansion session.

Results: Hydrostatic pressure plots reflect the soft tissue envelope's ability to accommodate sequential expansion. Fat grafted breasts demonstrated a statistically significant increased "post expansion-relaxation interval" versus the non-grafted control group (P< 0.0001, Figure 3). Viscoelastic changes impact the overall expansion time: the fat grafted group achieved total expansion two weeks earlier than the nongrafted control group (P< 0.05). The fat grafted, radiated group completed expansion in similar time interval as non-grafted control group. Complications were not significantly different between the irradiated, grafted breasts and control breasts.

Conclusions: Observed viscoelastic changes impact the overall expansion time. Fat grafting in non-radiated mastectomy defects allows for shorter expansion period. Fat grating in radiated mastectomy defects appears to allow safe expansion, and expansion durations equivalent to non-radiated, non-fat grafted control defects.

ABSTRACT 5 Images:



STROMAL VASCULAR FRACTION MITIGATES RADIATION-INDUCED GASTRO-INTESTINAL SYNDROME

Presenter: Lydia Bensemmane (France) Affiliation: IRSN Authors: SQUIBAN C; DEMARQUAY C; MATHIEU N; BENDERITTER M; MILLIAT F; LINARD C

Introduction: The intestine is particularly sensitive to moderate-high radiation dose and the development of gastrointestinal syndrome (GIS) leads to the rapid loss of intestinal mucosal integrity, resulting in bacterial infiltration, sepsis that comprise patient survival, the death occurs between 10 and 15 days post-exposure. There is an urgent need for effective and rapid therapeutic countermeasures. The stromal vascular fraction (SVF) derived from adipose tissue is an easily, rapidly accessible source of cells with angiogenic, anti-inflammatory and regenerative properties. We studied the therapeutic impact of SVF and its action on the intestinal stem cell compartment.

Methods: Mice exposed to the abdominal radiation (18 Gy) received a single intravenous injection of stromal vascular fraction (SVF) (2.5*106 cells), obtained by enzymatic digestion of inguinal fat tissue, on the day of irradiation. Mortality was evaluated as well as intestinal regeneration by histological analyses and absorption function.

Results: The SVF treatment limited the weight loss of the mice and inhibited the intestinal permeability and mortality after abdominal irradiation. Histological analyses of intestine showed that SVF treatment stimulated the regeneration of the epithelium by promoting numerous enlarged hyperproliferative zones. SVF restored CD24+/ lysozyme- and Paneth cell populations in the intestinal stem cell compartment with the presence of Paneth Ki67+ cells. SVF has an anti-inflammatory effect by repressing pro-inflammatory cytokines, increasing the presence of anti-inflammatory M2 macrophages in the ileum and anti-inflammatory monocyte subtypes CD11b+Ly6clowCX3CR1high in the ileum and the spleen. Conclusions: Through the pleiotropic effects that contribute to limiting radiation-induced lethality, SVF opens up attractive prospects for the treatment of emergency GIS.

ABSTRACT 7

IMMUNOMODULATORY AND REGENERATIVE EFFECTS OF THE FULL AND FRACTIONED ADIPOSE TISSUE DERIVED STEM CELLS SECRETOME IN SPINAL CORD INJURY

Presenter:	Antonio Salgado (Portugal)
Affiliation:	University of Minho
Authors:	CIBRÃO J.; PINHOE A.; LIMA R; GOMES E; SERRA S;
	LENTILHAS-GRAÇA J; RIBEIRO C; LANCEROS-MENDEZ S;
	TEIXEIRA F; MONTEIRO S; SILVA N

Spinal cord Injury (SCI) is a dramatic pathology, with a high number of new cases emerging every year. The injury itself triggers several biological events such as the activation of apoptotic pathways, the release of inflammatory cytokines and also the formation of a glial scar that primarily contains further damage, but also releases biomolecules that inhibit axons outgrowth, causing motor and sensory functional loss below the level of the spinal cord which translates in poor living conditions to the patients.

In this study, we intended to dissect the role of the secretome of adipose tissue derived stem cells (ASCs), as well as its individual vesicular and proteic individual fractions, in in vitro and in vivo models of axonal growth, inflammation and spinal cord injury, respectively. In vitro experiments revealed that the unfractionated secretome had a significant effect growth, when compared o its protein or vesicular fractions, on axonal growth and neuroinflammatory profile of microglial cells. Following on this data we then evaluated the impact of ASCs secretome on the histological and functional recovery of a severe compression-based SCI model in mice. Results of these experiments revealed that ASCs secretome induced a significant improvement (p<0.05) of the locomotor performance of SCI mice, when compared to untreated animals, as assessed by the Basso Mouse Scale test (BMS) , Open Field and Beam Balance tests. This was particular evident in the animals that were injected systemically (IV through the tail veins) with ASCs secretome, when compared to a local delivery. Additionally, the histological analysis has indicated that this motor improvement is closely related with a consistent reduction of the lesion volume, as well as a decreased activation of inflammatory cells (microglia) activation after treatment.

Overall, our results indicate the full secretome of ASCs induces important histological and motor improvement in SCI and can represent an interesting strategy to mitigate the current limitations in the field.

ABSTRACT 7 Image:



MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES IN AUTOLOGOUS FAT GRAFTING – LESSONS FROM A METHODOLOGICAL ANALYSIS

Presenter: Mohammad Ghiasloo (Belgium) Affiliation: Ghent University Authors: DE WILDE L; SINGH K; TONNARD PL; VERPAELE A; DE WEVER O; HENDRIX A; BLONDEEL PN

Introduction: The mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) are a promising cell-free modality in autologous fat grafting (AFG). However, the study of EVs mandates a rigorous methodology and transparent reporting —as established by the MISEV2018 guidelines— thereby facilitating reproducibility. We methodologically evaluated studies investigating MSC-EV-enriched AFG in preclinical studies.

Methods: A systematic review was performed according to the PRISMA guidelines. Embase, PubMed and CENTRAL databases were used. Included studies were submitted to EV-TRACK (http://evtrack.org) –a crowdsourcing knowledgebase– thereby producing the EV-METRIC of the experiments within the individual studies. The EV-METRIC is expressed as a percentage of fulfilled components from a list of nine –evaluating the EV isolation method, as well as biophysical and biochemical analysis of separated EVS– with each component deemed as indispensable for unambiguous interpretation and independent replication of EV experiments.

Results: Of 2474 studies, 61 underwent full text screening. Finally, seven studies were included, consisting of 12 experiments. The EV-METRIC was 15-38% (Figure 1). Six different EV isolation methods were observed, mainly based on a combination of (differential)(ultra) centrifugation and filtration. No density gradient centrifugation was used, nor were separated EVs analyzed for their density. Biophysical analysis could only be reproduced in 5/12 (42%) and 2/12 (17%) experiments, respectively. Western blotting was used in 9 experiments to analyze the EV-enriched –positive marker– and non EV-enriched proteins –negative marker– in the EV mixtures. None of the experiments specified the EV lysate preparation method. Positive markers were used in 9/12 (75%) experiments, whereas negative markers were controlled in only 4/12 (33%) experiments. Finally, only 3/10 (30%) provided sufficient data on the used antibodies.

Conclusion: MSC-EV-enrichment appears to be a novel approach in improving AFG survival. However, a lack of transparency in reporting has limited the value of previous reports. Therefore, early adherence to the MISEV2018 guidelines and EV-TRACK knowledgebase should be encouraged, thus allowing knowledge gaps to be identified at an early stage.

ABSTRACT 8 Image:



ABSTRACT 9

INVESTIGATING THE EFFECT OF FACTORS SECRETED FROM ADIPOSE TISSUE ON MYOFIBROBLAST DIFFERENTIATION

Presenter: Samuel Higginbotham (United Kingdom) Affiliation: The University of Sheffield Authors: WORKMAN VL; GREEN NH; GIBLIN V; LAMBERT DW; HEARNDEN V

Aberrant activation of fibroblasts to myofibroblasts is a key event in the formation of hypertrophic scars. Dysregulated differentiation leads to excessive production of extracellular matrix (ECM) components, such as collagen, resulting in thick, inflexible, painful scars for which there is no current effective treatment (1). Autologous fat grafting is an exciting new surgical option that has been shown to regenerate and improve the appearance and function of scar tissue (2). Despite this reported effect, we still have a limited understanding of how adipose tissue exerts these effects at a bio-molecular level and there is a gap between our scientific understanding and clinical observations. The aim of this study was to determine how adipose tissues and factors secreted from them affected fibroblast to myofibroblast differentiation and scar behaviour in vitro.

Adipose tissue was processed into clinically relevant formulations and used to condition tissue culture medium. This was added to human dermal fibroblasts in combination with transforming growth factor beta-1 (TGF β -1, which stimulates myofibroblast differentiation). Following this, markers of a myofibroblast phenotype were measured to assess the effect adipose tissue factors had on differentiation.

ECM components (collagen and fibronectin) and the myofibroblast marker α -SMA were significantly reduced in treated cells demonstrating that factors secreted from adipose tissue can inhibit the differentiation of fibroblasts into myofibroblasts. Further analysis to elucidate the mechanisms behind this anti-fibrotic effect has been performed to identify potential target molecules.

Our data shows that adipose tissue can inhibit canonical TGF β -1 signaling in dermal fibroblasts. Increasing our understanding of the mechanisms responsible for scar improvement presents new opportunities to design and optimise future fat-based regenerative therapies to improve patient outcomes.

1. Darby I, Laverdet B, Bonté F, Desmoulière A. Fibroblasts and myofibroblasts in wound healing. Clinical, Cosmetic and Investigational Dermatology 4, 7, 2014;

2.Klinger M, Marazzi M, Vigo D, Torre M. Fat injection for cases of severe burn outcomes: a new perspective of scar remodeling and reduction. Aesthetic Plastic Surgery 119, 5, 2008;

ABSTRACT 9 Images:



Aberrant activation of fibroblasts to myofibroblasts is a key event in the formation of hypertrophic scars. Dysregulated differentiation leads to excessive production of extracellular matrix (ECM) components, such as collagen, resulting in thick, inflexible, painful scars for which there is no current effective treatment (1). Autologous fat grafting is an exciting new surgical option that has been shown to regenerate and improve the appearance and function of scar tissue (2). Despite this reported effect, we still have a limited understanding of how adipose tissue exerts these effects at a bio-molecular level and there is a gap between our scientific understanding and clinical observations. The aim of this study was to determine how adipose tissues and factors secreted from them affected fibroblast to myofibroblast differentiation and scar behaviour in vitro.

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ABSTRACT 10

HUMAN PLATELET LYSATE CONCENTRATION IN CULTURE MEDIA AFFECT ADIPOSE-DERIVED STROMAL/STEM CELL POTENCY

 Presenter:
 Jesper Svalgaard (Denmark)

 Affiliation:
 Stemmedical

 Authors:
 BROOKS P; BALLESTEROS O; MUNTHE-FOG L; FISCHER-NIELSEN A; HAASTRUP E

Background: Adipose-derived stromal/stem cells (ASCs) are increasingly being explored for treatment of a wide range of diseases. Pooled human platelet lysate (pHPL) has replaced fetal bovine serum (FBS) as the preferred growth supplement because of its xeno-free origin and improved cell proliferation. Much has been done toward reducing the concentration of the hugely expensive pHPL required when expanding ASCs. However, the consequences of reducing the concentration of pHPL have not received much attention, nether has the potential beneficial effects of increasing the concentration. Objectives:

Objectives: This study investigated the effects of culturing ASCs in media containing 5, 10, or 20% pHPL on ASC proliferation and surface marker expression, including chemokine receptors CXCR2, CXCR3, CXCR4, and VLA-4. Adipogenic and osteogenic differentiation potential, as well as immunosuppressive properties, including the ability to induce indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1) and suppress T cell proliferation, were also examined.

Results: We observed a reduction in population doubling time and a corresponding increase in ASC cell yield (approx. 2-fold), when doubling the concentration of pHPL in the growth media. ASCs maintained expression of phenotypic surface markers, however, a reduction in CXCR3 and CXCR4 was observed. The ability to induce IDO1 and suppress T cell proliferation was not affected by the pHPL concentration. Adipogenesis and osteogenesis, however, seem to be increased at higher cconcentrations of pHPL (20% > 10% > 5%).

Conclusions: Increasing the pHPL concentration to 20% could be used to optimize culture conditions when producing cells for clinical treatments and may even be used to enhance beneficial ASC properties depending on the desired therapeutic effect.

DEVELOPMENT OF AN ORGANOID MODEL DERIVED FROM HUMAN ADIPOGENIC STEM/PROGENITOR CELLS TO STUDY WHITE ADIPOSE TISSUE PHYSIOLOGY

Presenter: Markus Mandl (Austria)

Affiliation: Research Institute for Biomedical Aging Research Authors: VIERTLER HP; BRUCKER C; HATZMANN FM; WALDEGGER P; RAUCHENWALD T; MATTESICH M; ZWIERZINA M; PIERER G; ZWERSCHKE W

The success of autologous white adipose tissue (WAT) grafting depends on survival, proliferation and adipogenic capacity of adipose stem/progenitor cells (ASCs). To study these processes several cell and animal models exist whereas none matches human physiology sufficiently. To mitigate this problem, we established a WAT organoid model employing isolated human ASCs which were forced to selfaggregate by a hanging-drop technique. To avoid plastic-adherence and dis-aggregation, spheroids were transferred into agar-coated cell culture plates followed by the induction of adipogenic differentiation. Morphometric analysis during the course of adipogenesis revealed a statistically significant increase of organoid size until day 18. Immunohistochemistry analysis confirmed the differentiation of ASCs into mature adipocytes. Whole mount staining of organoids using specific lipophilic dyes showed large fat deposits in differentiated cells. On the molecular level, adipogenesis was monitored by RT-qPCR and Western blotting. Marker of terminal differentiation such as FABP4 and Adiponectin were profoundly induced on mRNA and protein level with a peak expression on day 12. In addition, secreted Adiponectin was detected by ELISA at the same time point thereby confirming the physiological role of mature adipocytes. Moreover, Colony formation assays of collagenase-digested organoids revealed the maintenance of a significant fraction of ASCs within this system upon day 18. In conclusion, we provide a reliable WAT organoid model which enables the accurate analysis of adipogenic differentiation and adipocyte physiology on the molecular level. This system will contribute to future studies regarding adipose tissue biology and fat grafting.

ABSTRACT 12

HUMAN-PLATELET LYSATE SERUM AS A POTENTIAL CLINICAL-TRANSLATABLE SUPPLEMENT TO SUPPORT HUMAN ADIPOSE-DERIVED STEM CELLS NEUROTROPHIC PROPERTIES

 Presenter:
 Martino Guiotto (Switzerland)

 Affiliation:
 Centre Hospitalier Universitaire Vaudois - CHUV

 Authors:
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Introduction: Autologous nerve graft is the gold standard in peripheral nerve repair, despite donor site morbidity and unpredictable functional recovery. We investigate a completely xenogeneic-free expansion method for adipose-derived stem cells (hADSC) to maintain their properties and enhancing their neurotrophic potential. Moreover, we focus on the therapeutic potential of mimicking the Extra Cellular matrix (ECM)-components (Laminin (LN), Fibronectin (FN)) in a combinatorial strategy with hADSC expanded in the human platelet lysate (hPL-hADSC) to develop a reliable and safe strategy to be extended to nerve regeneration in vivo.

Material & Methods: We compared isolation methods (with collagenase or only mechanical) and supplement media (fetal bovine serum (FBS) or human-platelet lysate (hPL)) on hADSC morphology, proliferation rate, immunophenotype, lineage differentiation potential and neurogenic commitment (nerve growth factors secretion). Functional analysis of hADSC expanded in both medium conditions and on different ECM-proteins was performed using an in vitro co-culture model with rat dorsal root ganglia (DRG) explants, following neurite outgrowth. Co-cultures were performed in direct contact with neurons or "indirect", where neurons were treated with conditioned medium of the hADSC grown on the ECM molecules.

Results: We found no significant difference between the means of cell isolation and the supplements except for a higher proliferation rate and more elongated, spindle-shape morphology of hPL-hADSC. Neurotrophic factor secretion by hPL-hADSC showed statistically higher levels in all three growth factors (*p<0.05) compared with FBS-hADSC. DRG showed significantly longer neurite length and higher axonal area when co-cultured with hPL-hADSC (direct co-culture) or with their conditioned medium (indirect). LN positively impacted on the DRG sprouting in all hPL-culture conditions.

Conclusion: We show that hPL provides a clinically translatable means to support hADSC growth in vitro: increasing cell proliferation, maintaining stem cell phenotype and enhancing secretion of neurotrophic factors. Considering the ECM role, hPL-hADSC act synergistically with LN, strengthening cells proliferation and promoting their neurotrophic properties.

UPDATED PLATELET-RICH PLASMA UNDERSTANDINGS AND THERAPEUTIC CONSIDERATIONS

Presenter: Peter Everts (USA) Affiliation: Gulf Coast Biologics Authors: FABIO LANA J; MAUTNER K; ONISHI K; JAYARAM P

Emerging autologous cellular therapies that utilize platelet-rich plasma (PRP) applications have the potential to play adjunctive roles in a variety of regenerative medicine plastic reconstructive surgical procedures. There is a global unmet need for tissue reconstructive and repair strategies in soft tissue reconstructions, bone augmentation, and in patients with chronic complex and recalcitrant wounds who are candidates for surgical intervention.

Unfortunately, after decades of clinical PRP applications, both the clinical and scientific communities are not able to agree on terminology, standardization, and classification. Resulting in the availability of (too) many different PRP products and formulations, originating from human, in vitro, and animal studies. However, recommendations from in-vitro and animal research often lead to different clinical outcomes because it is difficult to translate nonclinical study outcomes and methodology recommendations to the implementation of human clinical treatment protocols.

General consensus is accomplished that PRP therapy is based on the fact that platelet growth factors (PGFs) and other cytokines support the three phases of wound healing and repair cascade (inflammation, proliferation, remodeling).

In recent years, progress has been made in better understanding PRP technology and the concepts for non-generic PRP bioformulations, with the suggestion for more specific research directives and new clinical indications have been suggested.

In this presentation, we will discuss recent developments that have emerged from the literature regarding new essentials in PRP preparation, bio-formulation, and options in cellular composition based on two-spin cellular density separation of whole blood.

We will address effective platelet concentrations and dosing, the various leukocyte activities concerning innate and adaptive immunomodulation, painkilling effects and the role of peripheral platelet serotonin.

Furthermore, we discuss PRP mechanisms related to inflammation and angiogenesis in tissue repair and regenerative processes. Lastly, we will review the effect of certain drugs on PRP activity, like NSAIDs and interactions with anti-platelet medications.

ABSTRACT 13 Images:





AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE (LIPOGEMS): THE ROLE OF MESENCHYMAL STEM CELLS FOR THE TREATMENT OS RECURRENT PERIANAL FISTULAS. MULTICENTRIC - (BRAZIL - ITALY)

 Presenter: Ana Luiza Silva (Brazil)

 Affiliation: Universidade de Mogi das Cruzes

 Authors:
 ROTTA CM; ELBETTI C; GIANI I; BURG MB; TRAGO S

Introduction: The aim of the study is to evaluate the efficacy of autologous micro-fragmented and minimally manipulated adipose tissue injection (LIPOGEMS) associated with reconstructive surgery in the treatment of recurrent perianal fistulas. The success of the procedure was estimated through the closure and/or non-secretion of the external orifice during the follow up.

Methods: Between December 2016 and november 2020, all patients affected by recurrent perianal fistula with an important scar retraction and with a rigid internal fistulous orifice, were evaluated for a possible treatment with Lipogems.

Exclusion criteria were: age < 18 years, pregnancy, previous local radiotherapy, diagnosis of neoplasia.

Results: 6 patients were inserted in the study and underwent surgery. The average age were 57.5 years (range 47-72), only 1 was woman. 6 patients were treated with a closure of the internal orifice with a Z-stitch with Vicryl 0 (5/8 needle) and with Lipogems injection (12 ml). The other one patient, because an extreme rigidity of the internal orifice was treated with placement of a loop draining seton and at the same time Lipogems injection. No intraoperative or postoperative complications were recorded. 1 case of abdominal pain due to liposuction was registered.

The results were as follows: at one month 1 out 4 patients already had the external orifice close, 1 patient (the one with the draining loop) showed the seton entrapped by the sclerosis due to the healing around the internal orifice. Therefore it was decided to remove the draining loop and continue to monitor the patient. At 3 months of follow-up, 6 out of 7 patients had the external orifice closed.

Conclusion: The results obtained, even if on a limited number of patients and with a short follow-up, indicate that Lipogems represents a high interesting tool in the care of patients affected by recurrent perianal fistula.

It will be a challenge for the near future to establish specific treatment protocols that envision the specific characteristics of the treated cases and consider a short-term surgical closure of the internal orifice to take advantage of the healing process triggered by Lipogems.

ABSTRACT 14 Images:







ABSTRACT 15 INVESTIGATION INTO USE OF SOLUBLE AND SUSTAINED RELEASE LATANOPROST FOR REDUCING ADIPOSE VOLUME

Presenter: Matthew Cannon (USA) Affiliation: University of Pittsburgh Authors: SUKINIK J; LEE P; GUERRERO D; SEMAN S; NERONE WV; LODER S; SU S; KOKAI LE

Autologous facial fat grafting is increasingly common used alone or in combination with rhytidectomy for facial rejuvenation. However, there is concern that injected subcutaneous fat ages differently compared to native tissue and may result in contour deformities over time. Obesity research has long-shown that adipocytes have "memory," whereby lifelong nutritional and hormonal factors induce epigenetic changes in all adipose-derived cells, and alters cell metabolism and gene expression. Therefore, transplanted fat can grow and hypertrophy over time while cheeks sink and skin loosens, requiring revision surgeries and mini liposuction to correct. Such procedures are effective if the skin has retained elasticity, however many patients are not good candidates for revision or elect to avoid secondary surgical procedures. Therefore, injectable solutions to reduce facial fat deposits would be valuable secondary options that clinicians could offer. Kybella (deoxycholic acid), a cytolytic drug that is FDA approved to reduce submental fat, represents one such option. However, Kybella has known iatrogenic effects such as injection site ulceration, necrosis and alopecia, and induction of dysphagia. In this study, we investigated latanoprost, a prostaglandin F2 α analogue, as an alternative for small volume fat pad reduction.

Methods: To compare efficacy, single administrations of Kybella, latanoprost or liposome encapsulated latanoprost were injected into C57bl/6 mouse inguinal fat pads. Study outcomes included mouse weight, inguinal fat pad volume, adipose stromal cell concentration and tissue architecture as assessed histologically. Mechanistic effects of latanoprost were investigated with 7 and 14 day in vitro cultures of adipose particles in media containing drugs.

Results: Soluble latanoprost significantly decreased animal weight at 14 days and increased adipose stromal cell concentration, while Kybella significantly reduced inguinal fat pad volume. H&E showed that latanoprost did not induce tissue inflammation while Kybella caused dermal ulcerations, adipocyte lysis and increased tissue inflammation.

Conclusion: Our results suggest that latanoprost has potential applications in reducing small fat volumes without inducing cell lysis and associated inflammation.

ABSTRACT 16

HORMONAL, ADIPOGENIC AND ANGIOGENIC ALTERATIONS IN LIPEDEMA ADIPOSE TISSUE

 Presenter:
 Eleni Priglinger (Austria)

 Affiliation:
 Ludwig Boltzmann Institute Traumatology

 Authors:
 HOFMANN M; STROHMEIER K; JACAK J; WEIGL M; HACKL M; HOLNTHONER W; GRILLARI J; REDL H; SANDHOFER M; WOLBANK S

Lipedema is a chronic, progressive disease of adipose tissue with unknown etiology and lack of consistent diagnostic criteria. We have previously identified that stromal vascular fraction (SVF) secrets small extracellular vesicles (sEV) contained microRNA (miRNA) profiles (miR-16-5p, miR-29a-3p, miR-24-3p, miR-454-p, miR-144-5p, miR-130a-3p and let-7c-5p) characteristic of lipedema tissue. Based on the relevance of SVF cell population in lipedema and the potential link between extracellular miRNA profiles and disease phenotypes, we performed a thorough characterization of the different SVF contained cell types in lipedema. For this study, we investigated the whole adipose tissue, the SVF and the sorted endothelial cells (ECs), pericytes (PCs) and adiposederived stromal/stem cells (ASCs) thereof on a protein and mRNA level. We found that expression profiles in SVF cell types are altered in lipedema adipose tissue including factors relevant for interaction with hormones, adipogenesis, angiogenesis, ECM deposition, inflammation, and hypoxia. In addition, endothelial permeability assay and immunofluorescence of endothelial cells, treated with either lipedema or healthy secretome gave insights into impaired vessel and endothelial barrier functionality. Since the cellular mechanism and composition in lipedema is largely unknown, our findings might contribute to a better understanding of its etiology.

QUIESCENCE, STEMNESS AND ADIPOGENIC DIFFERENTIATION CAPACITY IN HUMAN DLK1-/CD34+/CD24+ ADIPOSE STEM/ PROGENITOR CELLS

Presenter: Florian Hatzmann (Austria)

Affiliation: University of Innsbruck

Authors: EJAZ A; WIEGERS GJ; MANDL M; BRUCKER C; LECHNER S; RAUCHENWALD T; ZWIERZINA M; BAUMGARTEN S; WAGNER S; MATTESICH M; WALDEGGER P; PIERER G; ZWERSCHKE W

Human adipose stem/progenitor cells (ASCs) are crucial for adipose tissue homeostasis, regeneration, expansion, and aging, but their biological properties are not precisely known. We explore the status of quiescence, stemness and adipogenic differentiation capacity in defined ASC populations directly after their isolation from human abdominal subcutaneous white adipose tissue (sWAT) by sorting freshly isolated stromal vascular factions (SVF) in cell surface DLK1+/ CD34-, DLK1+/CD34dim and DLK1-/CD34+ cells. We demonstrate that the known proliferation and adipogenesis competent DLK1-/ CD34+ cell population express the bonafide guiescence markers p21Cip1, p27Kip1 and p57Kip2 but neither proliferation markers nor the senescence marker p16Ink4a. Pluripotency markers are barely detectable while the somatic stemness factors c-MYC and KLF4 and the early adipogenic factor C/EBPbeta are highly expressed. Further sorting of ASCs into DLK1-/CD34+/CD24- and DLK1-/CD34+/CD24+ fractions shows that KLF4 and c-MYC are higher expressed in DLK1-/ CD34+/CD24+ cells correlating with higher colony formation capacity and considerably lower adipogenic activity. Moreover, we show that ASCs routinely isolated by plastic-adherence are DLK1-/CD34+/CD24+. Intriguingly, CD24 knock-down in these cells reduces proliferation and adipogenesis. In conclusion, DLK1-/CD34+ ASCs in human sWAT exist in a quiescent state and express high levels of somatic stemness factors and the early adipogenic transcription factor C/EBPbeta. Moreover, stemness is higher and adipogenic capacity lower in DLK1-/CD34+/ CD24+ ASCs relative to the DLK1-/CD34+/CD24- subpopulation, and CD24 is necessary for adequate ASC proliferation and adipogenesis. The role of CD24 in ASCs will be further discussed during the presentation.

Relevant papers: Hatzmann et al. Cells 2021, 10, 214. https://doi. org/10.3390/cells10020214

ABSTRACT 18

SENESCENCE DID NOT ALTER THE CHONDROPROTECTIVE EFFECT OF EXTRACELLULAR VESICLES FROM ADIPOSE MESENCHYMAL STROMAL CELLS

 Presenter:
 Jérémy Boulestreau (France)

 Affiliation:
 Inserm

 Authors:
 NOEL D; MAUMUS M; ROZIER P; JORGENSEN C

Introduction. Age is the most important risk factor in degenerative osteoarthritis (OA) and is associated with the accumulation of senescent cells that contribute to functional decline of joint. We previously demonstrated that extracellular vesicles (EVs) from adipose mesenchymal stromal cells (ASCs) largely mediate the therapeutic effect of parental cells in OA. Here, we assessed the impact of senescence on EV properties in an in vitro model of OA.

Method. ASCs were induced to senescence using 25µM etoposide for 24 hours. Senescence was assessed by quantifying proliferation rate, SA- β Gal activity, nuclear γ H2AX foci number, phalloidin staining and expression of p21 (RT-qPCR). ASC-EVs were isolated by differential ultracentrifugation and characterized by size, concentration, total protein content, structure (cryo-TEM) and immunophenotype. In vitro OA model used chondrocytes isolated from OA patients, which were stimulated with IL1 β for 48h before culture with ASCs or ASC-EVs for 7 days. Gene expression and SASP factors were quantified by RT-qPCR and ELISA, respectively.

Results. Senescence-induced ASCs experienced growth arrest and increase of SA- β Gal staining, p21, γ H2AX foci, stress fibers and SASP factors (IL6, IL8, MMP3) confirming the expression of main senescence features. Senescent ASCs produced 4-fold more EVs than healthy ASCs and senescent ASC-EVs were larger. In vitro, both healthy and senescent ASCs decreased fibrotic markers (type III COLLAGEN), catabolic a markers (MMP3, MMP13, AP) in OA chondrocytes. By contrast, healthy ASCs decreased the expression of IL6 while senescent ASCs highly increased IL6. Looking at the role of ASC-EVs on OA chondrocytes, we found out that both healthy and senescent ASC-EVs were able to increase the expression of AGG and type II COLLAGEN while they decreased the expression of MMP13, AP, type X COLLAGEN and IL6. Finally, healthy and senescent ASC-EVs decreased the number of SA- β Gal positive chondrocytes.

Conclusions. Our results indicated a chondroprotective effect of ASC-EVs, independently of the senescent state of parental cells and suggested that EVs might act through different mechanisms than ASCs, which warrants further investigation.

DETECTION, QUANTIFICATION AND ELIMINATION OF SENESCENCE IN AGED AND/OR CULTURE EXPANDED HUMAN ADIPOSE DERIVED STEM CELLS

Presenter: Sudheer Ravuri (USA) Affiliation: Steadman Philippon Research Institute Authors: MULLEN MM; BILLINGS JB; MITCHELL JM; HAMBRIGHT SH; HUARD JH

Introduction: ADSCs may require culture expansion for the potential treatment of any musculoskeletal injury. Similar to aging, prolonged in vitro expansion of ADSCs results in a significant accumulation of cellular senescence. The loss in the therapeutic potential of ADSCs due to aging and/or culture expansion has given rise to the idea of senotherapeutics that could eliminate senescence and produce an enriched stem cell population. Our group has previously shown that immunosuppressors like rapamycin can reduce cellular senescence while improving myogenic and chondrogenic differentiation potential of muscle-derived stem cells from progeroid mice. It is hypothesized that fisetin, a senolytic agent, can reduce cellular senescence in ADSCs from aged and/or culture-expanded ADSCs.

Methods: Adipose Derived Stem Cells: ADSCs isolated from a 10-yearold male (YM), 27-year-old female (YF), 75-year-old male (OM), and 79-year-old female (OF) were purchased and cultured with normal growth media (DMEM/F:12, 10% FBS, and 1% penicillin/streptomycin). Fisetin Treatment: To eliminate natural and induced senescence, ADSCs were treated with fisetin for 48 hours. Immunofluorescence: γ -H2AX and H3K9 antibodies were used to detect senescence-associated heterochromatin foci. Flow Cytometry: Senescent ADSCs were detected and quantified by using 33 uM of C12FDG stain and flow cytometry.

Results: Following treatment with 1, 25, and 50 uM of fisetin for 48 hours and assayed with the CellTiter 96® AQueous Cell Proliferation reagent it was found that fisetin was not toxic to ADSCs. 50 uM of fisetin was used to treat low passage (p4) and high passage (p18) YF and OF ADSCs. After treatment, a reduction in H3K9 and γ -H2AX positive cells was observed for all groups. Flow cytometry was used to quantify C12FDG in YM and OM cells and the number of cells positive for C12FDG was significantly reduced in YM low and high passage groups.

Discussion: This study shows that fisetin is a senotherapeutic agent to mitigate senescence in aged and/or culture-expanded ADSCs via a single dose of senolytic treatment. Further characterization of senescence-associated secretory phenotypes (SASPs) and functional assays is in progress to evaluate the therapeutic potential of fisetin-treated ADSCs.

ABSTRACT 20

ADIPOSE DERIVED STEM CELLS ACCUMULATE SENESCENCE WITH AGE AND SERIAL PASSAGING

Presenter:Michael Mullen (USA)Affiliation:Steadman Philippon Research InstituteAuthors:HUARD J; HAMBRIGHT WS; RAVURI S

Introduction: Aging is associated with a depletion of functional stem cells primarily due to cellular senescence and increased proinflammatory signaling. To be considered a potential therapy for musculoskeletal repair, ADSCs require culture expansion before treatment. Similarly to aging, prolonged in vitro expansion of ADSCs results in a significant accumulation of senescent cells. However, the mechanisms by which these findings occur is not clearly shown. With this knowledge, we wished to determine the causes and markers of senescence in aged and culture expanded ADSCs.

Methods: Banked ADSCs isolated from varying ages and donors were purchased and cultured with normal growth media (DMEM/F:12, 10% FBS, and 1% penicillin/streptomycin). To characterize the senescent accumulation, cells were passaged up to 20 times. The PrestoBlue reagent or Countess Cell counter were used to determine cell proliferation and growth rate. gamma-H2AX and H3K9 antibodies were used to stain senescence-associated heterochromatin foci. The C12FDG fluorescent substrate was used to determine cells positive for beta galactosidase. Milliplex Adipokine kits were used for all senescence associate secretory phenotype (SASP), and SYBR Green and the Step-One PLUS system was used for quantitative PCR.

Results: Serial passaging was found to decrease the proliferation and growth rate of all cells. Furthermore, we found a significant increase in b-galactosidase with serial passaging as noted by an increase of C12FDG detection. Next, we found that while not all SASPs were upregulated at each passage, IL-6 and MCP-1 were upregulated between high and low passaging. Lastly, we found p21 and p16 to directly correlate with increased passage number. All these results correlated with a decrease in cell function as noted by decreased Oil Red O staining and qPCR markers (PPARg, CEBPG, and FABP4).

Discussion and Conclusion: The findings described here promote the research of optimized culture expansion methods for ADSCs. Moving forward there is a need to further characterize senescence accumulation and a push to find senolytics that may be used in vitro to attenuate these findings. We plan to expand on these experiments with assays to determine how senolytics may be used to improve ADSC function.

IDENTIFICATION AND HISTOLOGICAL MAPPING OF SENESCENT STROMAL CELLS IN ADIPOSE TISSUE: A PATH TOWARDS TISSUE DESENESCENCE

Presenter: Anthony Elias (USA) Affiliation: Wake Forest Baptist Medical Center Authors: JUSTICE JN; LLULL R

Cellular senescence – a cell cycle dysregulated state – has widely been considered to be detrimental to tissue turnover and thus contributory to histotypical aging. Therefore, weeding out tissue resident senescent cells, hence "desenescing" aging tissues, may improve tissue upkeep while rendering senescent-rich populations. We hypothesize that the distribution of these cells within tissue is determined by their developmental progression: as precursor cells accumulate around the PERIvascular region, their descendant postmitotic cells – which are more likely to be senescent – are likely displaced further out from the vascular axles (the PARAvascular region). p16INK4a, a cyclin-dependent kinase inhibitor, is considered to be a cellular marker for senescence. By quantifying the population of p16INK4a-expressing cells in these regions, we aim to confirm the unequal distribution of senescent cells within adipose tissue.

Adipose tissue samples (n=3) were needle biopsied and stained with anti-p16INK4a monoclonal antibody. Equivalent samples were enzymatically dissociated, and their resulting isolated cell suspensions were cytospun, anti-p16INK4a stained, and quantified by manual count and image cytometry.

p16INK4a+ stromal cells were readily present in enzymatically isolated cell suspensions (Figure 1): mononuclear bodies of 20-35 ø with a small nuclear/cytoplasm ratio, homochromatic nucleus, and clear cytoplasm. Initial manual cell count ranged between 1-3% in high power magnification fields, similar to preliminary image cytometry readings of approximately 4%. A number of large p16INK4a+ cluster structures were also identified; their cellular composition remains under study. In biopsied tissue samples, p16INK4a+ stromal cells were present with a density of 62.85 ± 20.67 cells/mm3. Interestingly, p16INK4a+ cells (in green, Figure 2) were preferentially located in PARAvascular regions and sparsely in PERIvascular regions (in red) (81.23 \pm 9.05% PARAvascular, P= 0.0011, unpaired t test; Figure 3).

Senescent stromal cells within adipose tissue are identifiable in lipoaspirated samples as p16INK4a+ cells. They are predominately housed within the paravascular regions, and enzymatic dissociation appears to effectively extract p16INK4a+ cells.

ABSTRACT 21 Images:



ABSTRACT 22

INVOLVEMENT OF STROMAL AGE IN BREAST CANCER ENDOCRINE RESPONSE

Presenter: Katie Hamel (USA)

 Affiliation: Louisiana State University

 Authors:
 CAVALIER MB; LIIMATTA KQ; ROZANSKI GL; KING CT; BELGODERE JA; BRATTON MR; BUNNELL BA; MARTIN EC

In disease states such as cancer, endocrine and paracrine signals from adipose tissue contribute to cancer progression and drug resistance. Young individuals diagnosed with luminal estrogen receptor-alpha positive (ER- α +) breast cancer have exhibit resistance to endocrine therapies. This suggests that an alternative estrogen signaling pathway is active within these cells. Analysis of TCGA data from young and aged ER- α + breast tumors revealed enrichment of matrix and paracrine factors in young patients compared to aged tumor samples. Analyzing cell infiltrate of young and aged $ER-\alpha$ + breast tumors revealed significant differences in several immune cell populations. To determine if the tumor stroma alters estrogen signaling, adiposederived stem/stromal cells (ASCs) from healthy young and aged donors were evaluated for alterations in matrix production and paracrine secreted factors. Aged and young ASCs were not phenotypically different, nor did they demonstrate alterations in matrix production as evident through gene expression, proliferation and differentiation assays. Young and aged ASCs demonstrated differences inflammatory cytokines, as evident through elevated levels of pro-inflammatory cytokines in young ASC donors. Through a series of condition media-based experiments, we demonstrate that ASC donor age is a contributing factor to an elevated endocrine response in ER- α + breast cancer cell lines. Secreted factors from young ASCs enhanced ER-a regulated genes including progesterone receptor (PGR) and stromalderived factor 1 (SDF-1) in the MCF-7 ER- α + breast cancer cell line compared to MCF-7 cells treated with secreted factors from aged ASCs. Western blot analysis demonstrated increased activation of p-ER ser167 in the MCF-7 cell line treated young ASC secreted factors This increase in ER ser-167 was not observed in aged donors. These results are important in understanding the mechanisms of estrogen signaling in young breast cancer patients, as well as unveiling underlying factors that contribute to endocrine signaling in young breast cancer patients. Furthermore, this study also provides compelling evidence suggesting that the age-dependent difference in the stromal regulation of the ER- α + breast cancer response is primarily via the cytokine secretome.

IN VITRO PBMC ASSAYS TO EVALUATE IMMUNOMODULATORY CELL PRODUCT POTENCY AND PREDICT IN VIVO THERAPEUTIC RESPONDERS

 Presenter:
 Elaine Richards (USA)

 Affiliation:
 University of Florida

 Authors:
 SAVA R; ZAMAN MO; HANDBERG EM; PEPINE CJ; MARCH KL

Introduction: Mesenchymal stem cell (MSC), including adipose stem cell (ASC) therapeutic approaches (cells or secretomes) demonstrate potential to manage inflammatory conditions. Therapy is safe but expensive, and individual responses vary. Questions include potency of cell or secretome preparations and which patients will respond to treatment. We hypothesized that a robust, inexpensive, practical in vitro assay using PBMCs of potential subjects could test cell potency and predict responder status to therapy.

Methods: PBMCs from 8 healthy controls and 3 women with symptoms and/or signs of ischemia and no obstructive coronary artery disease (INOCA) were evaluated for cytokine and chemokine responses in 3 experimental groups using PBMCs: untreated; LPS to stimulate monocyte/macrophage (M/M) pathways; CD3/CD28-coated beads to stimulate lymphocyte (L) pathways. Ability of co-cultured ASCs from a healthy individual to repress responses was evaluated by ELISA of PBMC-conditioned media.

Results: Neither PBMCs, ASCs, PBMC+ASC, ASC+M/M, nor ASC+Lstimulation secreted detectable TNFa. M/M-stimulated PBMCs secreted TNFa (2032 \pm 398 pg/mL, n=8), that was repressed by 70±13% by ASCs (n=4, p=0.028); the response was dose-dependent. In control PBMCs, M/M-stimulation also produced IL-12p40 and 70, IL1A, IL1B, MIP1B, eotaxin, CCL1, C6kine, and IL-10; ASCs inhibited each response. L-stimulation induced more TNFa production than M/M, 3084±982 vs 1512±170 pg/mL, but repression by ASC was similar here 56±10% vs 55±17% (n=3). PBMCs from INOCA subjects had marked individual variability with distinct phenotypes: 1) higher baseline inflammatory cell hyperactivity manifested by TNFa production vs controls; 2) enhanced M/M-but low L-induced TNFa production; and 3) greater L than M/M-induced TNFa. ASC lowered TNFa production in PBMCs of subjects by 22, 48 and 65%, but high baseline TNFa secretion of one was unchanged. This subject's PBMCs also produced \sim 9x more TNF α after L-stimulation.

Conclusions: Practical in vitro PBMC assays to personalize cell based therapies have been developed, with potential to predict non-responders and help determine optimal MSC/ASC products to reduce inflammatory factors, by identification of the dominant inflammatory pathways and their repressibility.

ABSTRACT 24

PERSONAL CELL THERAPY – THE BLACK SWAN OF REGENERATIVE MEDICINE CAN WE HEAL LYMPHEDEMA?

Presenter: Mark Berman (USA) **Affiliation:** University of Southern California **Authors:**

Background: Lymphedema commonly follows lymph node biopsies and chemotherapy for a variety of cancer treatments. It can be mild but also very unsightly and debilitating. There are no known cures or completely effective treatments.

Materials & Methods: Following mastectomy for breast cancer along with lymph node resection and chemotherapy a 50 year-old female developed Stage 2-3 left arm lymphedema. Over a two-month period, she underwent a series of conservative physical therapy maneuvers along with an extended period of diuretics without significant improvement. Following a "mini-liposuction" procedure under straight local anesthesia, about 35ml of condensed fat was sent to a GMP facility to culture expand her MSCs and then cryopreserve them for later use. 8 weeks following her initial surgery and chemotherapy, she received her banked MSCs as an intravenous infusion and direct injection around a few lymph node sites from her right arm, axilla and inframammary area. About 40 million MSCs were diluted in 10ml of saline and distributed in lots of 2-4 million in 5 injection sites and the remaining 5ml of solution was given via an IV infusion in 100ml of normal saline.

Results: Within 8 hours of deployment of her expanded autologous adipose mesenchymal stem cells she first experienced about 4 hours of a burning dysesthesia on her fingers and toes. After that cleared up, she then experienced a diuresis of 3 liters of fluid and the lymphedema also resolved.

Conclusions: We believe the use of MSCs not only helped clear up the lymphedema but by understanding MSC physiology it helps to further elucidate the pathophysiology of this condition. More intervention and data are needed to validate these findings, but we're fairly certain that MSCs will become a featured avenue for repair of lymphedema.

CHARACTERIZATION AND POTENTIAL USE OF NOVEL PLURIPOTENT ADIPOSE STEM CELLS (PASCS) IN REGENERATIVE MEDICINE AND CELL THERAPY

Presenter: Gregorio Chazenbalk (USA) Affiliation: UCLA Authors: CASANUEVA DE ROSA P; GIMENO M

Pluripotent stem cells have the ability to repair and replace any diseased and damaged tissues, prompting research groups worldwide to investigate the use of such cells in regenerative medicine and cell therapy for prevalent diseases. Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells remain the gold standard of pluripotent stem cells, however both cell types exhibit tumorigenesis in vivo which limits their potential for clinical treatment. In 2013, a novel population of pluripotent stem cells was discovered in adipose tissue, named Pluripotent Adipose Stem Cells (PASCs), previously known as Muse-AT cells. PASCs were isolated under severe cellular stress conditions including long-term exposure to the proteolytic enzyme collagenase, nutrient deprivation, low temperature, and hypoxia. PASCs express pluripotent stem cell markers (SSEA3/4, Nanog, SOX2, Oct4, TR1-60) and are able to differentiate into cells of all three germ layers. PASCs display non-tumorigenic activity in vivo as well as a stable karyotype in culture. Because PASCs are highly capable of migrating directly to damaged tissues, simple intravenous injection of PASCs may be used for tissue regeneration. In addition, PASCs do not prompt immunorejection because they express high levels of human leukocyte antigen (HLA) class I vs low levels of HLA class II, which supports their feasible usage in allogeneic transplantation. PASCs engage in anti-inflammatory activities which downregulate the secretion of pro-inflammatory cytokines. PASCs are easily accessible from lipoaspirate material and can be obtained at a low cost after a simple procedure. Safety studies performed in C57BL/6 mice after 21 days of intravenous PASCs (106 cells/animal) injection show intact cellular architecture in the lung, heart, kidney, liver, and spleen, similar to that which was observed in placebo. Preliminary studies performed in patients with neurodegenerative disorders under compassionate treatment show safety and no adverse events after 6 months of PASCs treatment. PASCs are very promising pluripotent stem cells in the field of regenerative medicine and cell therapy.

ABSTRACT 26

EXADEX: TOWARDS A MICROPHYSIOLOGICAL SYSTEM MODELING HUMAN ADIPOSE PROGENITOR CELL EXPANSION IN AN OBESE ADIPOSE TISSUE MICROENVIRONMENT

Presenter:	Christian Dani (France)
Affiliation:	iBV
Authors:	DANI V; BRUNI-FAVIER S; CARRIÈRE A; DEVINEAUX L; KÉOPHIPHATH M; CHIGNON-SICARD B; CASTEILLA L; DOGLIO A; DANI C

Lipids accumulate in non-adipocyte cells when the adipose tissue expansion limit is reached, causing lipotoxic damage that contributes to obesity-associated disorders. Many factors are involved in the regulation of adipose tissue expansion, including the generation of new adipocytes from adipose progenitors (APCs). Understanding how to promote the expansion of APCs in obese adipose tissues could help to protect against metabolic disease. The critical role of CD26+-APCs in the generation of adipocytes has recently been described. CD26expressing cells act as early progenitor cells giving rise to committed CD54+-preadipocytes. CD26-APCs were depleted in visceral adipose tissues relative to the subcutaneous fat, strongly suggesting a link between the abundance of CD26-APCs and the pathologic remodeling of adipose tissue. An ex vivo physiological model of adipose tissue that would enable us to investigate the regulation of APC expansion in response to environmental signals would be of great value, but is yet to be developed.

We present a microphysiological adipose tissue model generated from subcutaneous lipoaspirates. This model of mini-adipose tissue, named ExAdEx, recapitulates the 3D structure and microenvironment of the native tissue. Its viability was assessed measuring LDH release and cellular respiration. After 8 weeks of culture, no cytotoxic effect was observed and a biological oxygen consumption was measured. ExAdEx secrete adipokines, respond to exogenous signaling and engraft into mice. Adipose tissue inflammation and fibrosis were modeled by a 7 day-treatment with TNFa, LPS, or TGFb1. These treatments were marked by increased IL6 secretion, inhibition of Glut4 expression and adiponectin secretion. In the same way, when ExAdEx was cultured in presence of human activated macrophages conditioned media, an increase in IL-6 secretion paired with a decrease in adiponectin levels were observed compared to the control condition. Data revealed that the obese-like microenvironment inhibited the CD26+ population while increasing the CD54+population.

ExAdEx could represent a microphysiological platform to identify factors modulating APC expansion and a powerful tool for the pharmaceutical development of small molecules to counteract obesity.

ABSTRACT 27 REGULATION OF ADIPOGENIC POTENTIAL IN DEDIFFERENTIATED LIPOSARCOMAS

 Presenter:
 Elizabeth Floyd (USA)

 Affiliation:
 Pennington Biomedical Research Center

 Authors:
 DANG TN; TIONGCO RP; BROWN LM; TAYLOR JL; LYONS JM; LAU FH

Introduction: Well-differentiated and dedifferentiated liposarcomas are tumors originating in adipose tissue that share genetic abnormalities but have significantly different metastatic potential. Dedifferentiated liposarcoma (DDLPS) is highly aggressive and has an overall 5-year survival rate of 30% as compared to 90% for well-differentiated liposarcoma (WDLPS). This discrepancy may be connected to their adipogenic potential, where WDLPS is highly adipogenic but DDLPS is adipogenic-impaired. Normal adipogenesis requires Seven-In-Absentia Homolog 2 (SIAH2) to promote the degradation of Zinc Finger Protein 521 (ZFP521) in conjunction with Early B-cell Factor 1 (EBF1) to upregulate Zinc Finger Protein 423 (ZFP423) expression, a marker of preadipocyte commitment that coactivates PPARG expression. The aim of our study was to determine if the adipogenic potential of dedifferentiated liposarcoma is dysregulated at steps controlling conversion of preadipocyte cells to adipocytes.

Methods: Human WDLPS and DDLPS paraffin-embedded and frozen tissues were used to assess the gene and protein expression of adipogenic regulators. In parallel, WDLPS and DDLPS cell lines were cultured, genetically modified, and induced to undergo adipogenesis in vitro.

Results: SIAH2 expression does not define a clear distinction related to adipogenesis in these liposarcomas. However, we observed adipogenic dysregulation at preadipocyte commitment, potentially between ZFP521 and ZFP423 activation. Furthermore, in primary tumor specimens, SIAH2 mRNA was consistently upregulated in DDLPS. This correlated with increased macrophage infiltration in DDLPS tissues.

Conclusions: We found that expression of ZFP423, a transcriptional regulator required for converting a preadipocyte to a mature adipocyte is downregulated in dedifferentiated liposarcomas that fail to form new adipocytes. This study provides a molecular understanding of the development of the well-differentiated and dedifferentiated liposarcomas that correlates with a clear difference in patients' survival. These data provide novel insights into the adipogenic regulation between WDLPS and DDLPS adipocytic tumor development and introduce SIAH2 as a promising molecular marker to distinguish between WDLPS.

ABSTRACT 28

MONOCYTE CHEMOATTRACTANT PROTEIN-1-SUPPLEMENTED PLASMA ENHANCE ADIPOGENESIS THROUGH IL-33/DPP4

Presenter: Lee-Wei Chen (Taiwan) Affiliation: Kaohsiung Veterans General Hospital Authors: LI M; CHEN P

Fat grafting has emerged as an important technique in soft-tissue reconstruction in plastic reconstructive surgery. Platelet-rich plasma (PRP) reportedly improves grafted fat retention and enhanced proliferation of adipose cells with mechanisms unclarified. Monocyte chemoattractant protein-1 (MCP-1) is a key chemokine that regulate migration of monocytes. To study effects and mechanisms of Monocyte chemoattractant protein-1 (MCP-1) with plasma on enhancing adipose cells proliferation, stromal vascular fractions (SVFs) were purified from inguinal adipose tissue of obese and diabetic (Leprdb/db) and control (Lepr+/+) mice followed by analysis adipogenesis gene expression. SVFs of Leprdb/db mice did not exhibit much difference of adiponectin, IL-33, CCAAT-Enhancer-binding Protein (C/EBP), peroxisome proliferator-activated receptor gamma (PPAR?), and platelet-derived growth factor (PDGFR) mRNA expression as compared with those from Lepr+/+ mice. Plasma of Lepr+/+ mice significantly increased PDGFR mRNA expression as compared with PBS. Plasma of Leprdb/db mice significantly increased IL-33, DPP4, and adiponectin as compare with those treated with plasma of Lepr+/+ mice. MCP-1 with plasma of Lepr+/+ mice treatment significantly increased adiponectin, IL-33, C/ EBP, PPAR?, DPP4, and PDGFR mRNA expression of adipose tissue in vitro and in vivo as compared with those treated with plasma of Lepr+/+ mice. Altogether, our data suggest that plasma treatment did not significantly change adipogenic gene expression of SVFs of adipose tissues. However, MCP-1 supplementation enhances adipogenesis of adipose tissue through the increase of IL-33 and DPP4. Adding MCP-1 in PRP could be a promising therapeutic strategy in enhancing fat graft retention in clinical practice.

CHARACTERIZATION OF ADIPOSE TISSUE AND ADIPOSE-TISSUE DERIVED STEM CELLS IN LIPEDEMA

Presenter:Sara Al-Ghadban (USA)Affiliation:University of North Texas Health Science CenterAuthors:HERBST KL; BUNNELL BA

Introduction: Lipedema is a loose connective painful adipose tissue (AT) disorder that occurs almost exclusively in women, with onset manifesting at puberty or at times of hormonal change. This disorder is characterized by a symmetrical increase of fat deposition in the legs and the arms, sparing the hands and the feet.

The goal of this study was to determine the histology of the lipedema fat tissue and characterize the adipose tissue-derived stem cells (ASCs) obtained from the stomal vascular fraction (SVF) of the thigh AT of non-lipedema and lipedema patients. Methods: Histological sections from AT were stained with H&E. Adipocyte area was quantified using ImageJ software. Markers for macrophages (CD68), mast cells (CD117) and endothelial cells (CD31, SMA) were investigated by immunohistochemistry. ASCs were characterized by the expression of stemness markers and their multi-differentiation potential in 2D monolayer and 3D spheroid cultures. Flow cytometry, differentiation, qRT-PCR and immunofluorescence assays were performed.

Results: The data show hypertrophic adipocytes, increased numbers of macrophages and blood vessels in thigh tissue of women with lipedema compared to non-lipedema patients. Additionally, at the cellular level, an increase in the adipogenic differentiation potential with no change in the expression of mesenchymal markers (CD73, CD90 and CD105) or extracellular markers (collagen, fibronectin and laminin) was detected in lipedema ASCs compared to non-lipedema ASCs.

Conclusion: Infiltration of immune cells, increase in adipocyte size and adipogenesis stimulates angiogenesis and fibrosis in lipedema AT. Defining the structure and the components of AT will provide insights into the pathophysiology of lipedema and will help researchers develop potential treatment for the disease.

ABSTRACT 30

VITAMIN D AS A METABOLIC LIPOPROTECTANT: PHARMACOLOGIC ENHANCEMENT OF FAT GRAFT SURVIVAL

Presenter: Phoebe Lee (USA)

Affiliation: University of Pittsburgh School of Medicine Authors: LODER SL; LEFTWICH PA; NERONE WN; SINGH-VARMA AS; MARRA KM; RUBIN PR; KOKAI LK

Introduction: Post-operative reabsorption of fat graft remains a significant drawback in achieving optimal and reliable results. Prior data suggests that an ideal pharmaceutical adjunct, a 'lipoprotectant,' will have the following characteristics: a) reduce post-grafting inflammation; b) increase regenerative cell survival during hypoxia; and c) promote adipogenesis following graft revascularization. We have previously demonstrated that the VD3 analog, calcitriol, significantly improved graft retention vs. traditional Coleman processing. Follow-up studies with VD3, cholecalciferol, suggest similar efficacy within the clinically relevant range. Here we demonstrate our pre-clinical analysis of VD3.

Methods: Lipoaspirate was either 1) cultured in McCoys 5A media containing calcitriol or VD3 (15.6, 62.5, or 250 nM) for 7 days or 2) injected into the bilateral murine flank at 0.3 cc/side and maintained for 12-weeks. Mice received an IP MWF injection schedule of either saline; 50 ng calcitriol; or 50, 500, or 5000 ng VD3. Group 1 samples were assessed at 7 days using a) trypan blue for viability; b) calcein/ PI cytometry for cell size and survival; and c) seahorse bioanalyzer for cell metabolism after cobalt chloride induced hypoxic stress. Group 2 mice were sacrificed at 12-weeks mice for graft volume, weight, and histology.

Results: In vitro concentrations of 62.5 nM and 250 nM VD3 significantly increased adipose stromal cell viability compared to controls ($85.3\pm2.9\%$ and 87.7 ± 3.7 vs $77.6\pm2.8\%$, respectively). After inducing stress to baseline conditions, we saw that VD3 significantly reduced oxygen consumption rates compared to vehicle controls. we are currently exploring further the impact of VD3 on stress metabolism in larger patient population samples. In vivo VD3 demonstrated 1.5-fold increase in both weight and volume vs. untreated controls (p=0.0040) and was equivalent to calcitriol. This effect was dose-dependent as log-fold decreases in dosing (0.8 and 0.08 IU/g) did not demonstrate statistically significant changes weight or volume.

Conclusion: Our data suggests that VD3 provided at safe, therapeutic doses may act as a lipoprotectant to preserve fat grafts by blunting metabolic flux, minimizing losses/resorption, and promoting sustained viability.

ABSTRACT 31 VITAMIN D AS A METABOLIC LIPOPROTECTANT: PHARMACOLOGIC ENHANCEMENT OF FAT GRAFT SURVIVAL

Presenter: Phoebe Lee (USA) Affiliation: University of Pittsburgh School of Medicine Authors: LODER SL; LEFTWICH PA; NERONE WN; SINGH-VARMA AS; MARRA KM; RUBIN PR; KOKAI LK

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Conclusion: Our data suggests that VD3 provided at safe, therapeutic doses may act as a lipoprotectant to preserve fat grafts by blunting metabolic flux, minimizing losses/resorption, and promoting sustained viability.

ABSTRACT 32

STUDY INTO THE EFFECT OF FAT GRAFTING ON SKIN CONTRACTION IN VITRO

Presenter: Victoria Workman (United Kingdom) Affiliation: University of Sheffield Authors: GIBLIN V; GREEN NH; MACNEIL S; HEARNDEN V

Introduction: Skin contraction resulting from skin grafting or impaired wound healing is a debilitating condition. Contraction of the skin, particularly in areas of high mobility such as the hands and neck, severely reduces the quality of life for individuals. The current gold standard of treatment includes pressure garments and z-plasty revision surgery, however, outcomes remain poor in many patients. Clinical evidence has shown that fat grafting can improve hypertrophic scar tissue appearance and pliability but its role in reducing or reversing skin contraction is limited. The aim of this study was to develop an in vitro model to study the interactions between contracting skin and adipose tissue to understand whether fat grafting has the potential to improve outcomes for patients and to further our understanding of the biological mechanisms involved.

Methods: Our group at the University of Sheffield previously developed a tissue engineered skin model (TESM) which has been shown to contract in a similar way to damaged skin and skin grafts [1]. The model, comprised of primary human keratinocytes and human dermal fibroblasts cultured on a decellularised dermis, contracted by approximately 55-60% over 14 days in culture. Human lipoaspirate was obtained from routine surgery following informed consent (NHS ethics approval 15/YH/0177), processed as Coleman fat and placed beneath the contracting skin to model fat grafting in vitro.

Results: The tissue engineered skin model (TESM) which had lipoaspirate tissue added showed a 50% reduction in skin contraction compared to the TESM without adipose tissue over 14 days in culture. Cultured adipose derived stromal/stem cells (ADSC) added to the contracting TESM were also able to reduce contraction to a similar degree suggesting that ADSCs within lipoaspirate are, at least in part, responsible.

Conclusions: In conclusion, we have developed an in vitro TESM to investigate skin contraction which has demonstrated the potential of fat grafting to reduce contraction by approximately 50%. Further studies are now underway to investigate the role of the different components of adipose tissue to further understand the molecular mechanism behind adipose tissue's regenerative effects. [1] https://doi.org/10.1089/ten.2006.12.3119

SKIN REJUVENATION USING CONCENTRATE STROMAL VASCULAR TISSUE: AN INNOVATIVE TECHNIQUE FOR DIFFICULT AREAS

Presenter: Sophie Menkes (Switzerland) Affiliation: Nescens Clinique de Genolier Authors: None

Introduction: We aimed to access whether a novel concentrate SVT procedure improves skin quality, while yielding a regenerative effect, and whether this novel technique can also achieve good results in upper lip, tear trough, and others difficult areas.

Objectives: Our goal is to show that a concentrate SVT can be effective in theses indications. This presentation aims to present our technique, analyzing effectiveness, patient satisfaction, and complications.

Methods: Fat was harvested from inner part of the knee, or medial thigh. Following aspiration and flushing into a Pure Graft pocket, microfat was obtained after washing with saline. Emulsification of the microfat to obtain a Stromal Vascular Tissue (SVT) suspension was achieved with the Tulip kit (Tulip Medical products, San Diego, CA). Finally the SVT suspension was centrifuged at 1200 g 3000 rpm for 3 minutes to remove oil, liquids and obtain a lipoconcentrate. From 10 ml of microfat we obtain 3 ml of concentrate SVT. The concentrate SVT was injected in the upper lip using a 30G needle, the tear trough area using a 25 g cannula, into the subcutaneous layer. Images were obtained before and after at 3, and 6 months. Number of cells, viability, cytometry, CFU-F were measured.

Results: 10 patients were included. Concentrate SVT contained significantly higher adipose-derived stem cells and endothelial cell density. The clinical results were apparent between 4 and 6 weeks after injection, and improvements were continuously observed until 6 months postoperatively. All patients confirmed an improvement in texture, elasticity, firmness, fine lines, skin hydration, and skin pigmentation, with a regenerative effect. Patients also exhibited considerable improvements in skin regularity. Only minor complications were noted, including redness and edema between 2 and 4 days, and some bruises and pain in donor site.

Conclusion: Skin rejuvenation with concentrate stromal vascular tissue injections at upper lip, tear trough and other difficult areas appears to be an effective method. Centrifugation of stromal vascular tissue increases the number of cells. This autologous procedure is able to provide a very good improvement in all this indications, although additional studies are necessary.

ABSTRACT 33 Image:



ABSTRACT 34

TISSUE STROMAL VASCULAR FRACTION TO PREVENT DERMAL SCARRING: A PROSPECTIVE RANDOMIZED MULTICENTER CLINICAL TRIAL.

Presenter: Joris van Dongen (Netherlands) Affiliation: University Medical Center Groningen Authors: VAN BOXTEL J; UGUTEN M; BROUWER LA; VERMEULEN KM; MELENHORST WB; NIESSEN FB; HARMSEN MC; STEVENS HP; VAN DER LEI B

Introduction: Ideally, a skin wound heals without a scar, however multiple factors contribute to pathological scar formation e.g. chronic inflammation or large wound size. Till now, no evidence-based treatment is available to stimulate wound healing that regenerates damaged skin without a scar. Tissue stromal vascular fraction (tSVF) of adipose tissue is a heterogenous mixture of cell types embedded in an extracellular matrix loaded with a large number of growth factors and cytokines that stimulate and modulate all wound healing-related processes including parenchymal proliferation, angiogenesis, matrix remodeling and inflammation. In this study, we hypothesized that tSVF increases dermal wound healing of post-surgical wounds and thus suppress subsequent scar formation.

Material & Methods: This prospective, double-blinded, placebocontrolled, randomized trial was conducted between 2016 and 2020. In total, 40 female mammoplasty patients were enrolled with 34 completing the follow-up. All patients received tSVF in the lateral 5 cm of the horizontal scar of one breast directly postoperative after a bilateral reduction mammaplasty. The other lateral 5 cm of the horizontal scar of the other breast received a placebo injection comprising 0.9% NaCl. tSVF was isolated by means of the fractionation of adipose tissue (FAT) procedure. Results were obtained using the patient and observer scar assessment scale (POSAS), photograph evaluation using a visual analogue scale (VAS) by blinded observers and number of complications up to 1 year postoperative.

Results: Injection of tSVF improved postoperative scar appearance as compared to a placebo injection evaluated using the POSAS questionnaire six months postoperative. This difference in scar appearance lost significance after twelve months. In both groups, postoperative scars were barely visible after twelve months. No improvement was seen based on the evaluation of photographs of postoperative scars between both groups.

Conclusion: Immediate postoperative injection of tSVF suppresses scar formation up to six months postoperative, although this effect seems to disappear after twelve months. This study indicates that tSVF accelerates early wound healing and coincides with barely visual scar formation.

ABSTRACT 35 PRECISE SUBDERMAL FAT GRAFT INJECTION TECHNIQUE FOR SAFE GLUTEAL AUGMENTATION

Presenter: Ricardo Rodriguez (USA) Affiliation: Cosmeticsurgnet Authors:

Introduction: Gluteal Augmentation by Fat Graft (GAFG) has the highest mortality rate of any esthetic Plastic Surgery procedure. Pathology studies of mortality cases show an intramuscular injection combined with a gluteal vein injury leading to a Fatal Pulmonary Fat Embolism (FPFE). The ASPS, ASAPS, ISAPS, ISPRES, and IFATS jointly issued an advisory that "fat may only be injected into the subcutaneous space". However, in many of these fatal cases surgeons allegedly intended to stay in the subcutaneous space but unwittingly injected in the sub-muscular plane. Inadvertent penetration of the muscle, even with use of recommended large cannula diameter, is inevitable in a certain number of cases because GAFG is a blind procedure and in any given GAFG procedure the cannula is advanced blindly through the subcutaneous space many times.

Methods: We describe a technique for large volume GAFG that uses explicit cannula tip awareness to reliably and securely deposit micro fat grafts in the superficial subcutaneous space. It relies on visualion and palpation of the cannula tip as it abuts the subdermal plane to ensure accurate position. Video demonstration of surgical procedure and anatomic demonstration will be provided. No ancillary techniques such as ultrasound to verify subcutaneous placement of the fat graft are needed.

Results: The author has used this technique in more than 400 of cases over 15 years. Average of 600cc/buttock injected. Only 7 minor complications (1 infection at donor site, 4 postop symptomatic hypovolemia, 1palpable cyst, 1 significant contour de-formity) were reported. No serums or palpable fat necrosisNo patient had major complications (DVT, PE) or was treated for cardiopulmonary events.

Conclusion: Primum Non Nocere should be the primary goal of every GAFG procedure. The precise superficial injection technique we propose uses well established principles of fat grafting as described by Coleman and Yoshimura (viability of interspersed small depots of fat). It has significant lower morbidity rate than a published meta analysis of GAFG (<2% vs 7%). Most importantly, it avoids the possibility of intramuscular injection and the specter of death.

ABSTRACT 35 Image:



ABSTRACT 36

TOWARDS STANDARDIZATION IN MECHANICALLY ISOLATED STROMAL VASCULAR FRACTION RESEARCH – PRELIMINARY **RESULTS OF A METHODOLOGICAL ANALYSIS**

Presenter:	Ann-Sophie Madeleyn (Belgium)
Affiliation:	Ghent University
Authors:	DÍAZ JM; LOBATO RC; BUELVAS BUSTILLO AM; SINGH K;
	BLONDEEL PN; TONNARD PL; VERPAELE A; GHIASLOO M

Introduction: Mechanically isolated adipose tissue-derived stromal vascular fraction (SVF) is a potent regenerative emulsion, increasingly implemented in clinical practice. However, insufficient experimental reporting has hampered comparison and optimization. We argue that the SVF field requires more transparent reporting, subsequently facilitating reproducibility and standardization. We performed a methodological evaluation of existing reports, describing all relevant steps from patient preparation and fat harvesting to SVF isolation and characterization.

Methods: A systematic review was performed according to the PRISMA guidelines. Embase, PubMed, WoS and CENTRAL were used. Studies describing mechanical SVF isolation for clinical implementation were included. Following data were extracted: infiltration solution, method of aspiration, cannula specifics, SVF isolation procedure, endto-start-volume ratio, SVF analysis.

Results: Of 4939 studies, 14 comparative and 28 non-comparative articles were included, comprising a total of 66 experiments. Infiltration solution was specified in 27 (40.9%) experiments. Cannula- and syringe-aspiration were performed in 48 (72.7%) and 18 (27.3%) experiments, respectively. Of those performing cannula-aspiration, 39 (81.3%) did not report the vacuum pressure during aspiration. Cannula diameter was reported in 38 (57.6%) experiments, whereas hole size was only reported in 15 (22.7%). Sixty-one different SVF isolation procedures were identified, with 38 (57.6%) using a commercial kit. Fifteen (22.7%) experiments reported sufficient details allowing to deduce the total SVF isolation duration and in only 22 (33.3%) the end-to-start-volume ratio could be calculated. While 55 (83.8%) performed cell counts, only 24 (36.4%) evaluated cellular vitality. Although 64 (96.9%) experiments performed some form of phenotypic analysis, this was mostly superficial and the markers were often arbitrary.

Conclusion: Identified knowledge gaps highlight pivotal opportunities for research design. The rate at which the SVF field is growing necessitates the introduction of rigor and standardization in a timely fashion. These results form the first step towards the formulation of SVF research guidelines, underlining the importance of evidence over hype.

IMPROVING THE TECHNIQUE OF NON-ENZYMATIC METHOD FOR OBTAINING THE STROMAL-VASCULAR FRACTION

 Presenter:
 Natalia Khramtsova (Russia)

 Affiliation:
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 Authors:
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Introduction: Methods of obtaining the stromal-vascular fraction include enzymatic and non-enzymatic approaches. The aim of the work was to improve the technique of the non-enzymatic method. Method. Lipoaspirate obtained by mechanical, water-jet or syringe lipectomy from the abdomen was processed, and then microscopy of formalin-fixed and May-Grunwald stained smears was performed. The cells with fibroblast morphology, including mesenchymal stromal cells, were counted.

Results: The following algorithm was developed. The lipoaspirate was washed from blood cells in a standard way using a saline, then mechanical filtration was performed using two syringes with a volume of 10.0 ml connected to each other by anaerobic cell transfers, alternately: 1.4 mm - 30 times, then - 1.2 mm - 30 times, then through emulsifying "nanofat" filter - 30 times.

The resulting emulsion of adipose tissue was placed in the specially developed original tube and centrifuged at 2000 rpm for 20 minutes. After centrifugation, the filtrate was divided into 2 fractions, the upper part contained fat, and the lower part - the stromal-vascular fraction with a high content of blood cells. Microscopy of fragments from both parts of the tube showed that most amount of the fibroblast-like cells were retained in the upper fraction and were tightly bound to adipocytes.

Therefore, in the next step, the saline was added to the resulting filtrate through a needle, the lower fraction was thoroughly mixed and centrifuged again at 2000 rpm for 15 min. As a result, white flakes settled at the bottom of the tube, which were taken with a syringe using a needle and placed to the another tube. As a result, about 0.1 ml of liquid containing the stromal-vascular fraction was obtained from 1 ml of fat emulsion.

Microscopy showed that fibroblast-like cells were located mainly in groups of 30-50 pieces and were interconnected by small fragments of connective tissue. No adipocytes were observed in the smears obtained. Cell counting showed that there were about 170 cells with fibroblast morphology per 1 ml of fluid.

Conclusions: The described technique of the non-enzymatic method of obtaining the stromal-vascular fraction allows to get about 17000 microscopically proved fibroblast-like cells from 1 ml of fat emulsion.

ABSTRACT 38 TO LYSE, OR NOT TO LYSE? Presenter: Gabriela Aguilo-Seara (USA) Affiliation: Wake Forest School of Medicine Authors: SHANG H; NORTHRUP S; LLULL R; KATZ AJ

Introduction: Stromal vascular fraction (SVF) cell preparations typically include some degree of erythrocyte (RBC) "contamination." Most protocols utilize a RBC lysate step, despite the fact that RBC contents are known to be inflammatory. This study aimed to evaluate the relative impact of intact RBCs and RBC lysate on SVF and ASC (adipose stromal cell) proliferation, as well as the effect of reduction of RBC burden on SVF cell populations.

Methods: RBCs isolated from fresh lipoaspirate (LA) were divided and suspended in equal volumes of either DMEM with 10% fetal bovine serum (D10) at three dilutions or ACK (ammonium-chloride-potassium) lysing buffer. RBCs undergoing lysis were then centrifuged, and the supernatant was diluted in D10 in similar fashion. Freshly isolated SVF cells and second passage ASCs were cultured in the media solutions for up to 7 days. Conditioned medium was collected on days 0, 3, and, 6/7 and evaluated for RBC derivatives, hemin and hemoglobin, using ELISA. Cell proliferation was evaluated after 6 or 7 days using Electric Cell-Substrate Impedance Sensing. Fresh LA was also washed and/ or treated with lysis buffer, and isolated SVF was evaluated by flow cytometry for blood-derived cell (BDC) populations.

Results: SVF cultured with all three RBC concentrations proliferated 2.5-4x more than cells grown in control D10 (p=0.0013, p=0.0003 for the two highest RBC concentrations) and 3.2-3.7x more than lysate cultures (p=ns). The degree of cell proliferation was inversely related to levels of free hemoglobin and hemin detected in medium. Washing of LA reduced RBC burden 3.7x compared to unwashed samples and those treated with lysing buffer alone (p=0.0036). Washing also decreased CD45+ BDCs 1.7x (p=0.0008), including CD66b+ neutrophils by 3.5x (p=0.03), while yielding higher CD206+ macrophage counts (p=0.016).

Conclusions: Toxic compounds from lysed RBCs were detected in culture media despite dilution as low as 2%, correlating with dose-dependent increases in cell death. No RBC lysate compounds were detected in cultures with intact RBCs, and SVF cells grown in the presence of intact RBCs proliferated up to 4x more than cells grown in control media. Washing of LA effectively reduces RBC burden, while decreasing the number of pro-inflammatory BDCs.

CELL VIABILITY IS NOT COMPROMISED IN MECHANICALLY SHEARED CELL-DENSE STROMAL CELL AGGREGATES

 Presenter:
 Ramon Llull (USA)

 Affiliation:
 Wake Forest School of Medicine

 Authors:
 SESE B; MONSERRAT J; SHANG H; SANFILLIPO WA; KATZ AJ; AGUILO SEARA G

Introduction: As mechanical disaggregation lyses adipocytes, it renders a subset of suspended, isolated stromal vascular cells (mSVF) and produces a tissue fraction containing undissociated cells of questionable viability. We hypothesize that undissociated stromal cells after shear force mechanical dissociation (SFMD) remain aggregated and do not withstand the mechanical trauma, leading to widespread cell death within the tissue residual.

Methods: To determine the viability of undissociated cells after SFMD, standardized human lipoaspirate (LA) samples (n=17) were exposed to moderate or severe SFMD. The resulting mechanical tissue residuals (mTRs) were whole mounted and stained with live/dead histochemical fluorescent probes. mTRs were also enzymatically digested, and the resulting isolated cells were quantified and tested for viability.

Results: Following SFMD, mTRs significantly reduced their mass, a 3x difference (13.95 g +/- 4.6 g vs 4.03 g +/- 2.5 g), at the expense of triglycerides from lysed adipocytes, as suggested by the resulting oil phase (average oil phase increased from 2 g to 56 g) and confirmed histologically. After eliminating the oil phase, mTRs increased in planar cell density per gram of initial LA 2x by histochemical quantification (903.7 +/- 172.2 cells/mm2 in controls vs 1785 +/- 227.3 cells/mm2 for moderate and 1736 +/- 717.8 cells/mm2 for severe shear). Cell viability was notably preserved despite degree of SFMD and differed insignificantly from controls (70.0 +/- 8.05%, 67.35 +/- 11.05%, and 69.39 +/- 14.92%, respectively, P ns). When mTRs were enzymatically digested, they reproducibly released into suspension at a 5x increase in isolated cells per gram of digested material compared to controls (40.7 +/- 26.2 x 105 cells per q of mTRs vs 8.8 +/- 7.04 x 105 cells per gram of LA, P<0.001), with equivalent viabilities (73.80% +/- 5.7% in controls vs 74.67% +/- 6.5% post-SFMD, P ns).

Conclusions: SFMD lyses lipid-laden adipocytes preferentially to stromal cells which appear to remain predominantly aggregated around vascular structures and concentrates the cell density in mTRs by releasing volume occupying triglycerides. Contrary to our initial hypothesis, cells remaining in or enzymatically isolated from stromal cell aggregates remain viable.



Fort Lauderdale, Florida

LONG TERM CLINICAL RESULTS OF MECHANICAL STROMAL-CELL TRANSFER (MEST) AND ADJUSTABLE REGENERATIVE ADIPOSE TRANSFER (ARAT) BY USING ULTRA-SHARP BLADES

Presenter: Eray Copcu (Turkey) Affiliation: MEST Authors: OZTAN S

Background: Adipose tissue is not only a very important source of filler but also the body's greatest source of regenerative cells.

Objectives: In this study, adipose tissue was cut to the desired dimensions using ultra-sharp blade systems to avoid excessive blunt pressure and applied to various anatomical areas-a procedure known as adjustable regenerative adipose-tissue transfer (ARAT). Mechanical stromal cell transfer (MEST) of regenerative cells from fat tissue was also examined.

Methods: ARAT, MEST, or a combination of these was applied in the body of a total of 211 patients who were followed for at least 24 months. The integrity of the fat tissue cut with different diameter blades is shown histopathologically. The number and viability of the stromal cells obtained were evaluated and secretome analyses were performed. Patient and surgeon satisfaction were assessed with a visual analog scale.

Results: With the ARAT technique, the desired size fat grafts were obtained between 4000- and 200-micron diameters and applied at varying depths to different aesthetic units of the face and other organs, and a guide was developed. In MEST, stromal cells were obtained from 10- 100 mL of condensed fat using different indication-based protocols with 93% mean viability and cell counts of 2.66 to 9.18×106 / cc condensed fat.

Conclusions: There are 2 main complications in fat grafting: visibility in thin skin and a low retention rate. The ARAT technique can be used to prevent these 2 complications. MEST, on the other hand, obtains a high rate of fat and viable stromal cells without applying excessive blunt pressure.Long-term is needed to show real results in both fat graft applications and stromal-cell applications. In this study, the long-term results of 2 different clinical methods we defined are presented. In addition, the results of the defined IPs (Indication-based Protocols) method are presented; It has been suggested to use the term Total Stromal-cell (TOST) instead of Stromal Vascular Fraction (SVF) for the final product in mechanical stromal cell isolation.

ABSTRACT 40 Images:







DEVELOPMENT AND VALIDATION OF A FULLY GMP-COMPLIANT PROCESS FOR MANUFACTURING STROMAL VASCULAR FRACTION: A COST-EFFECTIVE ALTERNATIVE TO AUTOMATED METHODS

Presenter: Pauline Francois (France)

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Production of stromal vascular fraction (SVF) using enzymatic digestion is a cell therapy that meets the criteria for advanced therapy medicine (ATMP) status. As such, its production must comply with the European good-manufacturing-practices (GMP), applicable in France. The study carried out here presents the development and the validation of a GMP-compliant process for the manufacturing of the SVF, called the LG method. The influence of each critical parameter of the production process was evaluated in terms of cell viability, recovery yield and the proportion of regenerative cells in the final ATMP. Once the better parameters identified, the LG protocol was validated in comparison to an automated process: the Celution device. First, an inter donor comparison demonstrated that LG method (n=6) allowed better viability et and higher recovery yield of the SVF, compared to the Celution method (n=33). Then, an intra-donor comparison (n=5) showed that both methods are comparable in terms of viability, recovery yield, cell subset distribution and percentage of colonyforming-units assay fibroblast. Furthermore, the angiogenic properties of the SVF obtained using the LG protocol or the Celution were deeply explored using in vitro angiogenic assays which demonstrated similar angiogenesis potential.

Finally, an innovative mouse model of ischemic cutaneous wound proved a similar wound healing ability of both SVF groups. The developed and validated LG protocol allows standardization of the manufacturing part of the GMP rules. In addition to the production, the GMP also supervise the quality control of the SVF, that is the next step to be standardizes.

ABSTRACT 42

VALIDATION OF A NOVEL, SIMPLE AND INEXPENSIVE SCANNING PROCESS FOR THE THREE-DIMENSIONAL ASSESSMENT OF THE GLUTEAL REGION

 Presenter:
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 Authors:
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Introduction: Three-dimensional photography is increasingly used to assess surgical changes of volume, tissue distribution and projection. Our group has recently validated an innovative, simple and inexpensive three-dimensional scanning process for tree-dimensional assessment of the breast. The aim of this study was to validate its use also for the gluteal region.

Material & Methods: The three-dimensional scanning process was performed using the newly introduced Structure Sensor 3D scanner (Occipital, Inc., Boulder, Colo.) connected to an iPad Pro (Apple, Inc., Cupertino, Calif.). A medical human female anatomy torso model of rigid plastic was employed to repeatedly capture surface images. Digital measurements of vector and surface gluteal distances were calculated using Mimics® Innovation Suite 20 medical imaging software (Materialise, Leuven, Belgium) and compared with those obtained using Computer Tomography (CT) scan and Vectra M5 Scanner (Canfield Scientific Inc., Parsippany, NJ, USA), two clinically established scanning processes. Analysis of variance (ANOVA) was performed to identify possible statistical significant differences among the methods.

Results: For all variables examined, there was no significant difference among measurements obtained using different scanning processes (p>0.05).

Conclusion: Our study was able to validate the use of Structure Sensor for three-dimensional gluteal photography in comparison to other clinically established scanners, demonstrating analogous practicability and reliability. This novel three dimensional technology will allow objective and simple evaluation of gluteal volume and morphology changes.

ENRICHMENT OF THE FACIAL FAT GRAFT FOR INCREASED VOLUME RETENTION, A SYSTEMATIC REVIEW

 Presenter:
 Jan Aart Schipper (Netherlands)

 Affiliation:
 University Medical Centre Groningen

 Authors:
 VRIEND L; TUIN AJ; DIJKSTRA PU; SCHEPERS RH; VAN DER LEI B; JANSMA J; HARMSEN MC

Introduction: Facial fat grafting for volume restoration is a technique that is already used for decades. One of the main challenges is the variable amount of volume retention. Therefore, new graft enrichments are investigated to increase volume retention in the face. The aim of this systematic review is to investigate which enrichments increase volume retention in fat grafting of the face.

Methods: A systematic literature search was conducted using the databases of Cochrane Central, MEDLINE, EMBASE, Web of Science Core Collection and Google Scholar. Relevant search terms used were synonyms of fat grafting, retention, enrichment therapies, stromal vascular fraction, adipose-derived stromal cells, platelet rich plasma and nanofat. Two reviewers independently assessed the quality of the studies using the Effective Public Health Practice Project tool. Studies were included when they reported human subjects that were treated with enriched facial fat grafting and reported outcomes of volume or patient satisfaction.

Results: After duplicates were removed 3724 studies were screened by title abstract. After reading 95 full-text articles, 27 studies were included for comparison. Studies included enrichments with platelet rich plasma (PRP), platelet rich fibrin, cellular or tissue stromal vascular fraction (SVF), nanofat, adipose-derived stromal cells or bone marrowderived stromal cells.

Conclusions: Most studies reported increased volume retention in the enriched group. Overall, the quality of the studies was low. Measurement methods are highly variable and poorly described. There is a need for standardization and (randomized) controlled studies to be able to compare outcomes.

ABSTRACT 44

ENHANCED UTILITY AND OPERATIVE EFFICIENCY OF THE NOVEL PUSH-TO-SPIN HANDHELD (P2S) FAT GRAFT PROCESSING DEVICE

Presenter: Shawn Loder (USA) Affiliation: University of Pittsburgh Authors: LEE PL; KOKAI L; RUBIN JP; GUSENOFF BR; GUSENOFF JA

Introduction: Small volume fat graft efficiency is a critical determinant of the cost- and material effectiveness of aesthetic fat grafting in both the operative and clinical space. While several techniques have been described, recent development of devices such as the Push-to-Spin (P2S) system have improved upon the process by yielding a rapid, handheld, multi-use system to minimize operative time and mess. Here we described further technical innovations on the P2S prototype which further improve operative ease-of-use and minimize operative time without sacrificing adipose viability or yield.

Methods: Abdominoplasty samples from elective body contouring procedures was obtained as discarded tissue. Lipoaspirate was collected utilizing a 3.0 mm liposuction cannula was collected and processed via 1 of 3 techniques: centrifugation (Coleman technique), the P2S benchtop (P2S-B) or the novel P2S handheld device (P2S-H). Operative processing time, spin-time, oil fraction, SVF yield and viability, and adipocyte viability were assessed to compare both efficacy and viability of each device/technique in a clinically relevant setting.

Results: Both P2S devices processed adipose significantly faster versus Coleman processing (p<0.05). The novel P2S-H device was additionally significantly faster vs. the benchtop prototype (p<0.05). Oil yield increased significantly time and number of rotation. Purification was not significantly different between the two P2S devices. No significant difference in SVF or adipocyte viability was noted between the two P2S devices.

Conclusion: The technical advancements to the P2S system which enable single-unit, handheld operation significantly improve operative time with the additional benefit of not requiring a stand or separate workspace for processing. This operative quality of life improvement comes at no cost to efficacy of oil extraction, cellular yield, or cell viability.

MSC MECHANICAL DISSOCIATION SINCE 2006: THE PARADIGMA SHIFT AND THE NEW CLINICAL APPLICATIONS

Presenter: Hebert Lamblet (Brazil) **Affiliation:** Vikaara Klinik **Authors:**

Goal / Purpose: Besides the fact that fat grafting gained popularity, isolation of ADCs (Adipose Derived Cells) and fat tissue manipulation still remains controversial. In 2001, a putative Stem cell population was isolated within the adipose stromal compartment. Since then, many studies exhibited and confirmed the abundance of adult mesenchymal cells, endothelial progenitor cells and growth factor-producing cells derived from fat tissue. Isolation of those cells, its activation and their immediate use for fat transplant still remain a challenge. The purpose of this study is to show our evolution from chemical to mechanical dissociation of those cells from the fat tissue stroma since 2002

Methods / Technique: Adipose tissue is collected from the abdomen of patients undergoing liposuction. The fat is harvested and processed using two selective methods. Chemical: Half of the collected fat is left to decant, the other half is submitted to the collagenase isolation method. The stromal vascular fraction is centrifuge and the infranated pellet is added to the fresh fat tissue. Mechanical: After the washing process with a saline solution, a collagenase free Mechanical shear Force maneuver is made, generating a gradient force that detaches the adcs from the fat tissue stroma. The presence of mesenchymal stem cells isolated in the pellet was confirmed by Indirect Immunofluorescence and Flow Cytometer analysis in a selective sample data in both methods.

Results / Complications: From February 2002 to October 2020, 741 patients benefited from autologous fat transplant preserving ADCs. The first 72 patients with Chemical Dissociation, from 2002 to 2006, and 669 patients, from 2006 to 2020, with Mechanical Dissociation. The donor site was the abdomen. An average of 40 to 50 million mesenchymal stem cells / 100ml of processed lipoaspirate was isolated with the Mechanical method.

Conclusion: Up to now, adipose-derived cells isolation and fat tissue manipulation was mainly be done in the lab or using expensive processing machines and collagenase. The mechanical method has shown to be reproducible is collagenase free and has been used since 2006 in a long-term evaluation.



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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: Atara Novak (Israel) Affiliation: Bonus BioGroup LTD. Authors: NOVAK A; BRONSHTEIN T; KIVITY V; TZUR E; ROZEN N; MERETZKI S

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 up to 5cm long, with success reported mostly for defects under 2.5cm. Considering the larger defects encountered in the clinic, with a mean of 5.5cm, Bonus developed BonoFill, a tissue-engineered bone graft based on adipose-derived mesenchymal stromal cells (MSC) for the treatment of defects above 2.5cm.

Methods, Results & Conclusions: Bonus 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. BonoFill applied in a preclinical study to a 3.2cm defect in a sheep tibia led to a full recovery in 12 weeks (N=7). A phase I/II clinical trial is currently underway testing BonoFill in patients with bone defects ?2.5cm. Twelve patients with defects up to 8.5cm 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 I/II trial in >20 patients requiring a bone transplant in the upper maxilla with near complete success reported to date. Overall, the solid underlying science and innovative technologies developed by Bonus BioGroup position BonoFill as an exciting alternative to bone autografting.

ABSTRACT 48

INTRA ARTICULAR INJECTION OF AUTOLOGOUS MICROFAT AND PLATELET-RICH PLASMA IN THE TREATMENT OF KNEE OSTEOARTHRITIS: A DOUBLE BLIND RANDOMIZED COMPARATIVE STUDY

Presenter: Jeremy Magalon (France) Affiliation: Culture and Cell Therapy Unit INSERM CBT1409 C2VN Research Unit

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Purpose: Compare a single abdominal microfat (MF) injection mixed or not with PRP Low Dose (LD) or High Dose (HD) in order to improve MRI parameters and alleviate pain and enhance functional capacity in knee osteoarthritis (OA).

Methods: Patients with symptomatic grade 2 to 4 knee OA according to the International Cartilage Repair Society MRI classification were selected. They were prospectively assessed at baseline, at 3 and 6 months of follow-up. The primary endpoint was the change in maximum of value of cartilage relaxation time in T2 mapping sequences (T2max) at 3 months. Secondary endpoints were MRI grade severity and joint space assessment, WOMAC score, pain evaluation, knee range of motion and patient's satisfaction. Adverse events were also collected. The complete cell counts and growth factors content of injected products were assessed to analyze their potential relationship with MRI/clinical outcomes.

Results: Three groups of 10 patients received a single injection of 10 cc of a mix (1:1) containing either MF-Saline, MF-PRP LD or MF-PRP HD. T2max did not change significantly over the time for any of the groups. All treatments significantly improved knee functional status and symptoms relief at 3 and 6 months. All patients were responders in the MF/PRP HD at 3 months and significantly higher compared to MF/PRP LD. Half of the injected PRP in the MF/PRP LD group displayed RBCs contamination over 8% which was correlated with an impairment of T2max.

Conclusion: A single intra articular injection of MF with or without PRP is safe and may offer a significant clinical improvement in patients with OA.

A NOVEL METHOD TO PROVIDE 3D MRI IMAGING FOR EVALUATING HUMAN KNEE CARTILAGE: CLINICAL TRAIL OF SVF THERAPY FOR HUMAN KNEE OA

 Presenter: Xin Xiao Zheng (China)

 Affiliation: Wuhan University Zhongnan Hospital

 Authors:
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Background: The clinical applications of SVF therapy for osteoarthritis (OA) have attracted academic and clinical attention. However, data of the effects of SVF therapy on regeneration of degenerated cartilage are limited in the literature.

Material & Method: We designed and conducted a single center, open labeled clinical study, 6 OA patients were enrolled in this study based on their clinical and MRI evaluation (age range 53-69 years; 5 female and 1 male; both knee cartilage defect I-II). The two knees of each patient were randomly assigned to autologous SVF (108 cells) treatment group or non-treatment control group. The patients were evaluated every 4 weeks for safety and efficacy of autologous SVF therapy. We have established a novel protocol to provide 3D MRI imaging for human knee cartilage enabling us to qualitatively and quantitatively evaluate cartilage regeneration in this study.

Results: Safety: 1. There were no complications from liposuction and SVF injection. Efficacy: 1. The decreasing trend of WOMAC and increasing trend of Lyshom scores were evident between 0-12 weeks, and then leveled off gradually at 12-24 weeks.

2. The qualitative evaluation of 3D MRI imaging of knee cartilage revealed that most defects of femur and/or tibia cartilage of SVF treated group illustrated improvement, some showed significant reduction at 12W and 24W weeks in comparison with 0W. In contrast, most the defects of untreated group showed deterioration and some new defects were noted.

3. The quantitative evaluation of 3D MRI imaging of knee cartilage demonstrated that there was significant increase of cartilage value both in defect cartilage area and whole cartilage area of treated group (p<0.05, 0W vs. 12W or 24W). There was significant increase of thickness and area of both femoral and tibia cartilage in vertical sections of the SVF treated Group (p<0.05, 0W vs. 12W or 24W). There was no significant change of cartilage value, thickness and area from untreated group (p>0.5).

Conclusion: 1. SVF therapy for knee OA is safe procedure. 2. SVF therapy facilitates cartilage regeneration at 12W and 24W post treatment in cartilage defect I-II OA patients.

ABSTRACT 50

IMMUNOREGULATORY PROPERTIES OF A HUMAN LYOPHILIZED 3D SCAFFOLD FREE TISSUE ENGINEERED PRODUCT FOR BONE RECONSTRUCTION

Presenter: Carmen Brenner (Belgium) Affiliation: Novadip Biosciences SA Authors: EPISKOPOU H

Introduction: Bone regeneration is a complex process influenced by a variety of factors such as tissue interactions, inflammatory responses, and progenitor cells. NVDX3 is an allogenic product intended for use in bone voids. It is a lyophilized, terminally sterilized powder derived from a 3-D cell product containing extracellular matrix and adipose derived osteodifferentiated cells associated with hydroxyapatite/beta-tricalcium phosphate (HA/bTCP) particles. One of the main concerns in the field of allogenic stem cell technologies is the potential induction of an unwanted immune response, whereas the low immunogenicity and potential immunosuppressive capacities of mesenchymal stem cell (MSC) are properties of major importance for MSC-based therapeutic approaches.

Methods: NVDX3's immunoregulatory properties were evaluated through the investigation of its (i) immunogenic potential and (ii) immunoregulatory function on different populations of immune cells, including stimulated T cells and macrophages. The potential immunogenic and immunosuppressive/immunoregulatory effect of NVDX3 was investigated using in vitro bioassays.

Results: NVDX3 did not induce CD3+, CD4+ and CD8+ T cell proliferation or IFN-g production when added on unstimulated human PBMCs, suggesting the absence of an immunogenic potential. In contrast, a strong immunosuppressive effect was observed in the presence of NVDX3 compounds since their addition on stimulated PBMCs completely inhibited the CD3+, CD4+ and CD8+ T cell proliferation with >98% of inhibition. The immunosuppressive properties of NVDX3 involved also immunomodulation by inhibiting the secretion of pro-inflammatory cytokines on one hand and by promoting the shift of M1 to M2 macrophages polarization on the other. The observed immunosuppression capacity of the product involves prostaglandin E2 (PGE2) and Indoleamine 2, 3-dioxygenase 1 (IDO) pathways.

Conclusion: The primary function of NVDX3 is directed towards bone regeneration by promoting a local balance of factors essential for bone formation. The absence of immunogenicity in combination with the presence of immunomodulatory properties similar to the ones known for mesenchymal stem cells makes NVDX3 a privileged candidate therapeutic.

BONOFILL: A NOVEL TISSUE-ENGINEERED AUTOLOGOUS BONE GRAFT FROM ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS FOR MAXILLOFACIAL BONE TISSUE REGENERATION

 Presenter:
 Dror Ben David (Israel)

 Affiliation:
 Bonus BioGroup LTD.

 Authors:
 BEN-DAVID D; BRONSHTEIN T; ROZEN N; KIVITY V;

 TZUR E; MERETZKI S

Introduction: Bonus BioGroup has developed BonoFill, a novel tissue-engineered, three-dimensional, high-density, autologous bone graft designed to overcome the limitations associated with current maxillofacial bone reconstruction treatments. BonoFill is generated from the patient's adipose-derived mesenchymal stromal cells grown on mineral scaffold particles. Preclinical studies demonstrated the superior osteoconductive and osteoinductive properties of BonoFill, as well as promotion of de novo bone formation and graft integration.

Methods, Results & Conclusions: A phase I/II open label, first-inman clinical study was conducted to evaluate the safety and efficacy of BonoFill as a bone graft for the reconstruction of maxillofacial bone tissue. Twenty-four eligible male and female subjects were enrolled thus far, underwent liposuction and were treated with BonoFill. Clinical follow-up was performed up to six months after BonoFill treatment and included blood work, X-rays, CT scans and biopsies collected from the transplantation site. The autologous BonoFill bone graft was manufactured at a GMP-certified clean room facility, and the study was conducted under the approval of the Israeli Ministry of Health.

The study interim results demonstrate that BonoFill achieved fast, highquality bone tissue regeneration without complications. No incidents of chronic bone infections were recorded as well as no significant changes in blood profile. The patients have reported overall good health during the study. BonoFill treatment resulted in newly-generated bone of high density providing appropriate support for dental implants. In conclusion, BonoFill is a successful novel solution for bone tissue repair in maxillofacial indications showing great promise.

ABSTRACT 52

MECHANICALLY DERIVED TISSUE-STROMAL VASCULAR FRACTION ACTS ANTI-INFLAMMATORY ON CHONDROCYTES IN VITRO

Presenter:Lucienne Vonk (Netherlands)Affiliation:University Medical Center GroningenAuthors:VAN BOXTEL J; VAN DONGEN J; VONK LA; STEVENS HP

Background: Enzymatically isolated stromal vascular fraction as a treatment for osteoarthritis has already shown to be effective. Yet, the use of enzymes for clinical purpose is highly regulated in many countries. Mechanical preparation of SVF results in a tissue-like SVF (tSVF) containing intact cell-cell connections including extracellular matrix and is therefore less regulated. The purpose of this study was to investigate the immunomodulatory and pro-regenerative effect of tSVF on inflammatory chondrocytes in vitro.

Materials & Methods: tSVF was mechanically derived using the Fractionation of Adipose Tissue (FAT) procedure. Characterization of tSVF was performed e.g. cellular composition based on CD marker expression, colony forming unit and differentiation capacity after enzymatic dissociation (from heron referred to as tSVF-derived cells). Different co-cultures of tSVF-derived cells and inflammatory chondrocytes were analysed based on production of sulphated glycoaminoglycans and anti-inflammatory response of chondrocytes.

Results: Characterization of tSVF-derived cells mainly contained ASCs, endothelial cells, leukocytes and supra-adventitial cells. tSVF-derived cells were able to form colonies and differentiate into multiple cell lineages. Co-cultures with inflammatory chondrocytes resulted in a significant increase of the total number of chondrocytes as compared with cultures of chondrocytes alone (p<0.05). IL-1 β and COX2 gene expression was upregulated in TNF α -treated chondrocytes. After treatment with (conditioned medium of) tSVF-derived cells, IL-1 β and COX2 gene expression was significantly reduced (p<0.01).

Conclusion: Mechanically derived tSVF stimulates chondrocyte proliferation while preserving the function of chondrocytes. Moreover, tSVF suppresses chondrocyte inflammation in vitro. This proregenerative and anti-inflammatory effect shows the potential of tSVF as a treatment for osteoarthritis.

BONE-MARROW DERIVED MESENCHYMAL STEM/STROMAL CELLS HAVE ENHANCED VASCULOGENIC POTENCY OVER ADIPOSE DERIVED MESENCHYMAL STEM/STROMAL CELLS IN PERFUSED IN VITRO CULTURES

 Presenter:
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 Authors:
 MYKULIAK A; YRJÄNÄINEN A; GEBRAAD A;

 VUORENPÄÄ H

Insufficient vascularization is a major obstacle for clinical application of tissue engineered transplants. In addition, formation of perfusable vasculature is important for physiologically relevant tissue modelling. Of particular interest is the microvasculature that is crucial for oxygen and nutrient delivery. microvascular networks in 3D can be formed in vitro through the co-culture of endothelial cells (ECs) with supporting pericytic cells. Mesenchymal stem/stromal cells (MSCs) derived from bone marrow (BMSCs) and adipose tissue (ASCs) are an attractive choice for pericytes due to their natural perivascular localization and ability to support formation of mature and stable microvessels. Here, our aim was to explore the vasculogenic potential of ASCs and BMSCs in a perfusable microfluidic device. BMSCs and ASCs were co-cultured with ECs in a fibrin hydrogel in a microfluidic chip. We compared the capacity of BMSCs and ASCs to induce the formation of mature microvascular networks by ECs and to differentiate into pericytes. We studied the effect of MSCs on vessel characteristics such as area, diameter, length, and perfusability. We assessed MSCs pericytic differentiation in terms of pericyte area and pericyte coverage by immunohistochemical staining and quantitative analysis. Furthermore, we evaluated the expression of main vasculogenesis related genes. We demonstrated that using MSCs of different origin resulted in vascular networks with distinct phenotypes. Both types of MSCs supported formation of mature and interconnected microvascular networks. However, BMSCs induced formation of fully perfusable microvasculature with larger vessel area and vessel length compared to ASCs. Co-culture with ASCs resulted in only partially perfusable microvascular networks. Immunostainings revealed that BMSCs had greater potential to differentiate towards pericytes than ASCs. The gene expression analysis revealed significant differences in the expression of endothelial-specific and pericyte-specific genes, as well as genes involved in vasculature maturation and remodeling. Overall, our study provides valuable knowledge on the properties of BMSCs and ASCs as vasculature supporting cells and highlights their distinct directing role in the regulation of microvascular phenotype.

ABSTRACT 54

ISOLATION OF HUMAN ADIPOSE-DERIVED STROMAL CELLS USING SUCTION-ASSISTED OR ULTRASOUND-ASSISTED LIPOASPIRATION AND THEIR THERAPEUTIC POTENTIAL IN CARTILAGE TISSUE ENGINEERING

Presenter: Jian Wang (China) Affiliation: Suzhou Hospital of Anhui Medical University Authors: CAI CH; ZHOU XU

Objective: Harvesting adipose derived stromal cells (ADSCs) for tissue engineering is frequently done through liposuction. We investigated whether differences in formation of tissue engineering cartilage by co-culture microtia chondrocytes and ADSCs obtained by suction-assisted lipoaspiration (SAL)or ultrasound-assisted lipoaspiration(UAL).

Methods: hADSCs obtained by SAL or UAL and microtia chondrocytes were isolated in vitro. In vitro parameters of cell osteogenic differentiation, Chondrogenic differentiation and adipogenic differentiation were performed. There are 3 groups: Co-culture 1, microtia chondrocytes and hADSCs obtained by SAL at a mixing ratio of 1:1 were seeded on the PGA/PLA scaffolds; Co-culture 2, microtia chondrocytes and hADSCs obtained by UAL at a mixing ratio of 1:1 were seeded on the PGA/PLA scaffolds; MC group, microtia chondrocytes were seeded on the PGA/PLA scaffolds. After in vitro culture for 4 weeks and subcutaneous implantation for 8 weeks, the constructs were harvested for gross observation, histology and immunohistochemisty, average wet weights, glycosaminoglycan (GAG) quantification, biomechanical evaluation.

Results: UAL and SAL Yield ADSCs With Equal Osteogenic, Adipogenic and Chondrogenic Differentiation Capacity. In vitro culture for 4 weeks, Histological assays showed no significant difference by HE,COL II,safranin O,Toluidine blue. Samples showed loosened structures and undegraded scaffolds were still observed. In vivo culture for 8 weeks, samples in both MC and co-culture groups formed mature cartilagelike tissues. But some places were still un-matured in MC group. Wet weight,GAG content and Young's moduli of engineered cartilage showed much higher at 8w in vivo than at 4w in vitro. The engineered cartilage in co-culture 1 and co-culture 2 group were significantly higher than MC groups at 4 w in vitro and 8 w in vivo. Co-culture 1 and co-culture 2 group revealed no significant difference.

Conclusion: There are no differences in formation of tissue engineering cartilage by culture microtia chondrocytes and ADSCs obtained by suction-assisted lipoaspiration or ultrasound-assisted lipoaspiration.

AN ALLOGENIC TISSUE-ENGINEERED PRODUCT FOR BONE REGENERATION ENABLING INDUCTION OF ENDOCHONDRAL OSSIFICATION AND INHIBITION OF OSTEOCLASIA

Presenter: Hara Episkopou (Belgium) Affiliation: Novadip Biosciences SA Authors: THEYS N; THIRION G

Introduction: NVDX3 is a promising new therapeutic approach for bone reconstruction. It is a lyophilized, terminally sterilized powder derived from a 3-D cell product containing extracellular matrix and non-viable osteodifferentiated cells.

Methods: NVDX3 product composition was addressed through the investigation of its biomolecular content. The expression levels of 98 osteo-/chondrogenic genes, 15 osteogenic protein factors and 376 miRNA were measured in samples diverted during tissue maturation process. The osteogenic and anti-resorptive properties were evaluated in vitro and in vivo. A pharmacodynamic study was conducted on Nude rats (n=66) with a femoral critical-size bone-defect (CSBD). Qualitative and quantitative immuno-histochemical analysis for tissue composition was performed 30- and 90-days post-implantation.

Results: 12 genes showed a consistent expression profile with a constantly strong induction of genes involved in hypertrophic chondrocytes formation (COL10A1, MMP13). Eight cell-derived osteogenic protein components (IGF-1, OPG, VEGF, Leptin/OB, CTNNB1/B-catenin, Osteopontin, DKK-1, Aggrecan) and 4 miRNA molecules, that have been correlated with enhancement of the osteogenic and/or chondrogenic activity, were also detected in all NVDX3-TBs. Based on data derived from the in vitro bioassays addressing the potency of the products, NVDX3 induced the expression of osteo- and chondrogenic markers in hBMSCs, with the highest levels of induction to be observed for factors involved in endochondral ossification. In addition, the product showed a strong in vitro inhibitory effect on both osteoclast formation and viability. Qualitative and quantitative histological (histomorphometry for Bone area, Bone area/Tissue area, Cartilage area and Cartilage area/Tissue area) and immunohistochemical (RUNX2, OCN, ACAN, SOX9, COLX) on paraffinembedded tissues from NVDX3 treated rat showed that NVDX3 induced a clear increase in endochondral ossification after 30 days and bone tissue after 90 days.

Conclusion: NVDX3 leads to bone formation by promoting a local balance of factors essential for osteogenesis, through promotion of endochondral ossification and strong anti-resorptive properties.

ABSTRACT 56

ADIPOSE MESENCHYMAL STEM CELLS TREATMENT LIMITS CHRONIC RADIATION CYSTITIS

Presenter: Clement Brossard (France) Affiliation: IRSN Authors: LEFRANC AC; DOS SANTOS M; DEMARQUAY C; BUARD V; TARLET G; SQUIBAN C; LINARD C; MATHIEU N; SIMON JM; MILLIAT F; CHAPEL A

Introduction: This study investigates, whether adipose Mesenchymal Stem Cells (adMSCs) reverse chronic radiation cystitis (CRC). CRC is a consecutive pathology of pelvic irradiation characterized by chronic inflammation of the bladder progressing to fibrosis with symptoms such as pain and bleeding. In a preclinical rat model, the different mechanisms of action of adMSCs on CRC have been studied, including bladder function, structure and bleeding.

Methods: CRC was induced by localized irradiation of the whole bladder with two beams, guided by tomography. The irradiation dose of 40 Gray in rats was chosen because it generates a CRC close to the one observed in humans. During the initiation phase of the CRC, four months after irradiation, an intravenous injection of 5 million of adMSCs was administered every two weeks (3 injections in total). This protocol has been used in the treatment of radiation rectitis and permits to maximize the effect of adMSCs. Physiological, histological and molecular monitoring was performed over 12 months after irradiation.

Results: Previous studies have shown the onset of CRC as early as 6 months after irradiation, with bleeding, chronic inflammation, fibrosis and urothelium disorganization increasing with time.

The injection of adMSC delayed the beginning of bleeding up of CRC to 50 at 350 days after irradiation. These results correlated with the reduced vascular lesions up to 12 months post-irradiation measured in cystoscopy.

Fibrosis was investigated, but the time of 12 months seems too short to induce fibrosis. Longer studies are needed to study this parameter and the effect of the adMSC treatment.

Urothelium lesions are characterized by hyperplasia as indicator of the integrity of the urothelium) and by uroplakin III as a marker of the impermeability of the urothelium. We observed a decrease in hyperplasia in rats treated with adMSC compared to control at 12 months post-irradiation nevertheless not correlated to a decrease of uroplakin III labelling.

Conclusion: Treatment with adMSCs delayed bleeding, decreased vascular damage and increased integrity of the urothelium without restoration of impermeability. An analysis is in progress focused on the effect of adMSCs on inflammation, fibrosis and urothelium regeneration.

ALLOGENEIC ADIPOSE DERIVED STEM CELLS MITIGATE ACUTE RADIATION SYNDROME

Presenter: Somaiah Chinnapaka (USA) Affiliation: University of Pittsburgh Authors: YANG S; SAMADI Y; EPPERLY M; HOU W; GREENBERGER J; EJAZ A; RUBIN P

Background: Acute radiation syndrome (ARS) is the radiation toxicity which damage hematopoietic, gastrointestinal, and nervous system having life-threatening consequences. ARS can be caused as a result of accidents such as nuclear explosions, terrorist attacks, and industrial/medical accidents. Current therapy approaches are mainly hematopoietic stem cell transplantation, granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte stimulating factor (G-CSF) having either a limited application for mass application or insufficient efficacy. There is an urgent need to add effective therapies to the national stockpile, which can be applied to the mass population with minimal supervision. Based on the regenerative properties of ASCs, we hypothesized that allogeneic ASCs could be a potential candidate for the mitigation of ARS, and due to ease of harvesting and propagation in cell culture can be produced at large scale for the national stockpile.

Study design: To test our hypothesis, we used 9.25 Gy total body irradiated (TBI)C57BL/6 mice for the ARS model and studied the effect of allogeneic FVB ASCs on the survival of TBI mice. Next, we determined the fate of the transplanted ASCs and tracked intraperitoneally injected GFP-positive transplanted ASCs in C57BL/6host bone marrow. We performed invitro transwell studies to analyze the migration of ASCs towards irradiated bone marrow in a more controlled setting. To delineate the mitigation mechanism, Luminex analysis was performed to identify the pro-survival factors released by ASCs upon interaction with irradiated bone marrow.

Results: Our results demonstrate that a single dose of 5×106allogeneic ASCs mitigate TBI induced ARS. Intraperitoneally injected migrated to irradiated bone marrow. Transwell migration experiment confirmed the strong migration capability of ASCs toward irradiated cells. The survival of irradiated cells was positively correlated to the number of ASCs migrating across the transwell membranes.

Conclusion: Our findings suggest that allogeneic ASC therapy is as effective as autologous ASC therapy to mitigate ARS. Further studies to optimize the time and dose of the therapy will be a big step forward to add this universally applicable ARS mitigator to national stockpile.

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CLINICAL TRIAL EVALUATING THE EFFICACY OF MESENCHYMAL STROMAL CELL INJECTIONS FOR THE TREATMENT OF CHRONIC PELVIC COMPLICATIONS INDUCED BY RADIATION THERAPY

Presenter: Alain Chapel (France) Affiliation: IRSN

Authors: SEMONT A; LINARD C; MATHIEU N; DEMARQUAY C; SQUIBAN C; VOSWINKEL J; ROUARD H; LATAILLADE JJ; MARTINAUD C; BENDERITTER M; GORIN NC; SIMON JM; MOTHY M

The late adverse effects of pelvic radiotherapy concern 5 to 10% of patients. However, a clear medical consensus concerning the clinical management of such healthy tissue sequelae does not exist. Our group has demonstrated in preclinical animal models that systemic mesenchymal stromal stem cells (MSCs) injection is a promising approach for the medical management of gastrointestinal disorder after irradiation.

In a phase 1 clinical trial, we have shown that the clinical status of four first patients suffering from severe pelvic side effects (Epinal accident) was improved following MSC injection (figure). Two patients revealed a substantiated clinical response for pain and hemorrhage after MSC therapy. The frequency of painful diarrhea diminished from 6/d to 3/d after the first and 2/d after the 2nd MSC injection in one patient. A beginning fistulization process could be stopped in one patient resulting in a stable remission for more than 3 years of followup. A modulation of the lymphocyte subsets towards a regulatory pattern and diminution of activated T cells accompanies the clinical response. MSC therapy was effective on pain, diarrhea, hemorrhage, inflammation, fibrosis and limited fistulization. No toxicity was observed.

We are now starting a clinical research protocol for patients with post-radiation abdominal and pelvic complications who have not seen their symptoms improve after conventional treatments (NCT02814864, PRISME). It involves the participation of 6 radiotherapy services for the recruitment of 12 patients. They will all be treated and followed up in the hematology department of Saint Antoine Hospital. The cells will be prepared in two production centers. Treatment is a suspension of allogeneic MSCs. Eligible patients must have a grade greater than 2 for rectorrhagia or hematuria at inclusion and absence of active cancer. Each patient receives 3 injections of MSCs at 7-day intervals. Patients will be followed up over a 12-month period. The main objective is a decrease of one grade on the LENT SOMA scale for rectorrhagia or hematuria. The secondary objective is to reduce the frequency of diarrhea; analgesic consumption, pain and improved quality of life.

ALLOGENEIC ADIPOSE TISSUE-DERIVED MATRIX MITIGATE RADIATION-INDUCED FIBROSIS (RIF)

Presenter: Asim Ejaz (USA) Affiliation: University of Pittsburgh Authors: CHINNAPAKA S; KATHERINE Y; EPPERLY M; WEN U; GREENBERGER J; RUBIN P

Introduction: Radiation-induced fibrosis (RIF) is a complex tissue response characterized by massive deposition of extracellular matrix (ECM) and excessive fibroblast proliferation resulting in loss of tissue function and quality of life. In this study, we tested the efficacy of allograft adipose tissue-derived matrix (AATM) to mitigate RIF.

Methods: We used 40 Gy hind limb irradiated C57BL/6 mice as a skin fibrosis model and studied the effect of AATM on the mitigation of fibrosis. PBS was injected in control mice. The degree of limb excursion, skin epithelium thickness, and deposition of collagens was measured as a reporter of fibrosis. Molecular changes in treated and control skins were measured at gene and protein levels using real-time PCR and Luminex assay respectively. The presence of hepatocyte growth factor (HGF) as a possible contributor to mitigation was measured in AATM using HGF- ELISA assay. In vitro transwell studies were performed to analyze the effect of direct co-culture of irradiated cells with AATM on pro-fibrotic genes expression.

Results: Our results demonstrate that a single dose of 200 μ l of AATM mitigates fibrosis. The mitigation efficiency of AATM was comparable to autologous ASCs. A single dose of AATM injection on day 14 at the irradiated site decreased by day 40: epithelial thickness, collagen deposition, and significantly improved limb excursion compared with irradiated controls. Real-time PCR analysis reveals the down-regulation in the expression of pro-fibrotic genes TGF- β , CTGF, Col1, Col2, and TNF-alpha in AATM treated mice. Similarly, direct co-culture studies revealed downregulation of pro-fibrotic genes in irradiated fibroblast upon co-culture with AATM. ELISA results showed the presence of HGF and noncontact transwell coculture of HGF knockout ASCs above a monolayer of irradiated mouse bone marrow stromal cells failed to downregulate fibrosis-related gene TGF- β expression.

Conclusion: Our findings suggest that allogeneic adipose tissuederived ECM (AATM) is an effective therapeutic option to mitigate fibrosis. Further studies to optimize the time and dose of the therapy will be a significant step forward towards clinical adaptation as a RIF mitigator.

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ADIPOSE-DERIVED REGENERATIVE CELLS AND LIPOTRANSFER IN ALLEVIATING BREAST CANCER-RELATED LYMPHEDEMA: AN OPEN-LABEL PHASE I TRIAL WITH 4-YEARS OF FOLLOW-UP

 Presenter:
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 Authors:
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Patients with breast cancer-related lymphedema (BCRL) have reduced quality of life and arm function. Current treatments are palliative, and treatments improving lymphedema are lacking. Preclinical studies have suggested that adipose-derived regenerative cells (ADRCs) can alleviate lymphedema. We, therefore, aimed to assess whether ADRCs can alleviate lymphedema in clinical reality with long-term follow-up. We treated 10 patients with BCRL using ADRCs and a scar-releasing lipotransfer to the axillary region, and all patients were followed 1, 3, 6, 12, and 48 months after treatment. The primary endpoint was change in arm volume. Secondary endpoints were safety, change in lymphedema symptoms, quality of life, lymphedema-associated cellulitis, and conservative treatment use. There was no significant decrease in BCRL volume after treatment. However, self-reported upper extremity disability and arm heaviness and tension improved. Six patients reduced their use of conservative BCRL treatment. Five patients felt that their BCRL had improved substantially, and four of these would redo the treatment. We did not observe any cases of locoregional breast cancer recurrence. In this phase I study with 4 years of follow-up, axillary delivered ADRCs and lipotransfer were safe and feasible and improved BCRL symptoms and upper extremity function. Randomized controlled trials are needed to confirm the results of this study.

DIFFERENCES OF EMBEDDING ADIPOSE-DERIVED STROMAL CELLS IN NATURAL AND SYNTHETIC SCAFFOLDS FOR DERMAL AND SUBCUTANEOUS DELIVERY

Presenter: Frederik Mamsen (Denmark) Affiliation: StemMedical Authors: MUNTHE-FOG L; KRING MK; DUSCHER D; TAUDORF M; KATZ AJ; KØLLE SF

Background: In recent years, adipose-derived stromal cells (ASCs) have been heavily studied for soft tissue regeneration, augmentation, and dermal wound healing.

Methods: In this review, we investigated the trends in injectable scaffolds for ASC delivery in the dermis, and injectable or implantable scaffolds for ASC delivery in the subcutis. A total of 547 articles were screened across three databases; of these, 22 studies were found to be eligible and were included. The scaffolds were subdivided and analyzed based on their tissue placement (dermis or subcutis), delivery method (injected or implanted), and by the origin of the materials (natural, synthetic, and combinatory).

Results: ASCs embedded in scaffolds generally showed improved viability. Neovascularization in the transplanted tissue was greater when undifferentiated ASCs were embedded in a combinatory scaffold or if differentiated ASCs were embedded in a natural scaffold. ASCs embedded in natural materials underwent more adipogenic differentiation than ASCs embedded in synthetic scaffolds, indicating an etiologically unknown difference that has yet to be described. Increased mechanical strength of the scaffold material correlated with improved outcome measurements in the investigated studies. Wound healing studies reported reduced healing time in all except one article due to contraction of the control wounds.

Conclusions: In future clinical trials, we recommend embedding ASCs in injectable and implantable scaffolds for enhanced protection, retained viability, and improved therapeutic effects.

Trial registration: This review was registered with PROSPERO: ID=CRD42020171534.

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SKIN-DERIVED EXTRACELLULAR MATRIX HYDROGELS LOADED WITH ADIPOSE TISSUE-DERIVED STROMAL CELL-SECRETED FACTORS AS TREATMENT FOR ENHANCING WOUND REGENERATION AND VIABILITY OF SKIN FLAPS: A RAT MODEL

 Presenter:
 Cristina Camargo (Netherlands)

 Affiliation:
 University and Medical Center Groningen

 Authors:
 VAN DONGEN JA; CAMARGO CP; VAN DER LEI B; HARMSEN MC; VRIEND L

Background: Insufficient vascularization is a recurring cause of impaired pedicled skin flap healing. The administration of adipose tissue-derived (ASC) secretome is a novel approach to augment vascularization. Yet, the secretome comprised soluble factors that require a sustained release vehicle to increase residence time.

Objectives: We hypothesized that administration of a hydrogel derived from decellularized extracellular matrix (ECM) of porcine skin with bound trophic factors from ASCs enhance skin flap viability and wound repair in a rat model.

Methods: Porcine skin was decellularized and pepsin-digested to form a hydrogel at 37°C. Conditioned medium (CMe) of ASC was collected, concentrated twentyfold and mixed with the hydrogel. Sixty Wistar rats were included. A dorsal skin flap (caudal based) of 3 x 10 cm was elevated for topical application of: DMEM medium (group I), a pre-hydrogel with or without ASC CMe (group II and III) or ASC CMe (group IV). After 7-, 14- and 28-days, skin flaps were harvested for wound healing assessment and immunohistochemical analysis.

Results: Decellularized skin ECM hydrogel contained negligible amounts of DNA (11.56 \pm 0.63 ng/mg), was noncytotoxic and well-tolerated in rats. Irrespective of ASC secretome, ECM hydrogel application resulted macroscopically and microscopically in similar dermal wound healing in terms of proliferation, immune response and matrix remodeling as the control group. However, ASC CMe alone increased vessel density after seven days.

Conclusion: Porcine skin derived ECM hydrogels loaded with ASC secretome are non-cytotoxic but demand optimization to significantly augment wound healing of skin flaps.

ABSTRACT 62 Images:





THE EFFECT OF STROMAL VASCULAR FRACTION CELLS MIXED WITH DIFFERENT TYPES OF HYALURONIC ACID FILLERS IN IMMUNODEFICIENT MICE

Presenter: Heetae Koo (SouthKorea) Affiliation: Un Seoul National University Hospital Authors: JIN US

Introduction: Stromal vascular fraction (SVF) is a cellular extract containing heterogenous cell population.

In clinical fields, SVF is usually delivered by direct injection of SVF cell suspension or in mixture with fat graft. Hyaluronic acid (HA) filler is widely used for soft tissue augmentation, and there has been attempts to use HA fillers as scaffolds for adipose derived stem cells.

Herein, we designed a study to compare the durability of HA filler and distribution newly formed tissue from SVF cells according to different filler rheology.

Method: Stromal vascular fraction cells were isolated from liposuctioned human adipose tissue of a single-donor, using an automated SVF isolation device system (Cellunit; CGBio Inc., Seongnam, Gyeonggi-do).

5 different types of HA fillers (Juvéderm Voluma, Restylane skinboosters, Restylane perlane, Giselleligne universal, Giselleligne signature 2) were selected as scaffolds for SVF cells. For the test group, SVF cell suspension 40mmL was dispersed in 160mmL of each HA fillers using Luer-Lock syringe, to generate total 200mmL mixture of human SVF cells and HA filler.

Using an automated cell counter (Luna-FLTM; Logos Biosystems, Anyang), total amount of injected SVF cell was 7.5×10^{5} in each mixture .

For the control group, no SVF cells were mixed and 200mmL of corresponding HA filler was prepared.

35 mice were separated into 5 groups depending on HA filler types and the injections were made at 2 sites under general anesthesia : the left back composing the test group and the right back composing the control group.

Results/Conclusion: A demonstrable nodular swelling was formed in all 35 mice, and no acute complication was observed until 2 weeks following the injection.

We are planning to compare the maximum width and height of formed nodule, and measure the exact volume using ultrasonography at week 8 after the injection.

Autopsy will be followed, and histologic examination will reveal distribution and amount of remaining HA particles in dermis. Immunohistochemical study will allow us to identify what tissue is formed when SVF is injected with HA fillers.

ABSTRACT 64

3D PRINTED MICRONIZED FAT-LADEN COLLAGEN CONSTRUCTS FOR TREATMENT OF CHRONIC WOUNDS

Presenter: Nathan Katz (USA) Affiliation: Jointechlabs Inc Authors: SCHMITT T.; KISHORE V.

Chronic skin wounds such as diabetic ulcers and pressure ulcers have high prevalence in patients with poor circulation, neuropathy, and infection. A regenerative approach using a combination of adipose stem cells (ASCs) and fat-derived stromal vascular fraction (SVF) can be an effective strategy for healing of chronic wounds via regulation of growth factors and cytokines, targeted cell response, and new blood vessel formation. In this realm, we have developed a breakthrough device MiniTC capable of processing patient-derived lipoaspirates to produce micronized fat graft and purified SVF in a single closed loop system. The micronized fat graft consists of a rich fraction of ASCs and endothelial cell progenitors preserved in their native niche without excess oil and debris. To the best of our knowledge, the current study is the first attempt to combine micronized fat with a polymeric ink to 3D print fat-laden crosslinkable constructs with high print fidelity and stability. 3D bioprinting will allow for encapsulation of precise ratios of micronized fat into anatomically complex structures that can be applied to heterogeneous wound sites of varying depth and complexity for complete and expedited healing of chronic wounds.

The results of this feasibility study demonstrate that 3D printed micronized-fat laden collagen constructs hold promise for use in wound healing applications. Since the cell viability is limited up to one week, reapplication of the micronized fat graft on a weekly basis would be an effective strategy to achieve continued and efficient healing of chronic wounds. This is a highly feasible approach since micronized fat from patient-derived adipose tissue can be cryopreserved without loss in cell viability. Future studies will assess the neovascularization potential of 3D printed micronized fat-laden constructs in vitro and in vivo using a mouse model.



ABSTRACT 64 Images:

EXTRACELLULAR MATRIX-DERIVED HYDROGELS TO AUGMENT DERMAL WOUND HEALING: A SYSTEMATIC REVIEW

Presenter: Martin Harsem (Netherlands) Affiliation: University and Medical Center Groningen Authors: SINKUNJAS V; VRIEND L; VAN DER LEI B; VAN DONGEN JA; CAMARGO CP

Background: Chronic, non-healing, dermal wounds form a worldwide medical problem with limited and inadequate treatment options and high societal burden and costs. With the advent of regenerative therapies exploiting extracellular matrix (ECM) components, its efficacy to augment wound healing is to be explored. This systematic review was performed to assess and compare the current therapeutic efficacy of ECM hydrogels on dermal wound healing.

Methods: The electronic databases of (Embase, Medline Ovid, Cochrane Central) were searched for in vivo and clinical studies on the therapeutic effect of ECM-composed hydrogels on dermal wound healing (13th of April 2021). Two reviewers selected studies independently. Studies were assessed based on ECM content, ECM hydrogel composition, additives and wound healing outcomes such as wound size, angiogenesis and complications.

Results: Of the 2102 publications, nine rodent-based studies were included while clinical studies were not published at the time of the search. Procedures to decellularize tissue or cultured cells and subsequently generate hydrogels were highly variable and in demand of standardization. ECM hydrogels with or without additives reduced wound size and also seem to enhance angiogenesis. Serious complications were not reported.

Conclusion: To date, preclinical studies preclude to draw firm conclusions on the efficacy and working mechanism of ECM-derived hydrogels on dermal wound healing. The use of ECM hydrogels can be considered safe. Standardization of decellularization protocols and implementation of quality and cytotoxicity controls will enable obtaining a generic and comparable ECM product.



ABSTRACT 66

STROMAL VASCULAR FRACTION-ENRICHED FAT GRAFTING AS TREATMENT OF ADHERENT SCARS: STUDY DESIGN OF A PROSPECTIVE COHORT STUDY

Presenter: Linda Vriend (Netherlands) Affiliation: University and Medical Center Groningen Authors: VAN DONGEN JA; PIJPE A; NIEUWENHUIS MK; JONGEN SJ; HARMSEN MC; VAN ZUIJLEN PM; VAN DER LEI B

Background: Autologous fat grafting has been used in the last decades to treat adherent scars. The observed regenerative and scarreducing properties have been mainly ascribed to the tissue-derived Stromal Vascular Fraction (tSVF), containing adipose-derived stromal cells (ASCs). ASCs increase angiogenesis, can induce mitosis in resident tissue cells and are able to remodel collagen. We hypothesized that tSVF potentiates fat grafting-based treatment of adherent scars. Therefore, this study aims to investigate the effect of tSVF-enriched fat grafting on scar pliability over a 12-month period.

Methods & Design: A clinical multicenter prospective cohort trial will be conducted in Dutch Burn Centers Beverwijk and Groningen. Patients (>18 years) with adherent scars caused by burns, necrotic fasciitis or degloving injury that are eligible for fat grafting will receive tSVF-enriched fat grafts underneath their scars. In total, 46 patients will be included. The primary outcome is the change in scar pliability between pre- and 12 months post-grafting. Secondary outcomes are scar color and pigmentation measured by the DSM II Colormeter, scar quality assessed by the patient and observer scales of the Patient and Observer Scar Assessment Scale (POSAS) and histological analysis of tSVF and scar biopsies. This study has been approved by the national Central Committee for Clinical Research (CCMO).

Discussion: This study will contribute to elucidating the scar reducing properties of tSVF-enriched fat grafting in a clinical setting and to optimizing surgical treatment of patients with adherent scars.

Trial status: Dutch Trial Register: NL 8461, registered on 16 March 2020.

Keywords: scars, Stromal Vascular Fraction, Adipose-derived stromal cells, ASC, burn scars, fat grafting

ABSTRACT 66 Images:

Figure 1. Study Design





ABSTRACT 67

DIRECT COMPARISON OF BIOLOGICAL-BASED THERAPIES ON HUMAN FOLLICLE DERMAL PAPILLA CELL FUNCTION

Presenter: John Cole (USA) Affiliation: PHTC Authors: COLE MA

Abstract: Background: Biological-based therapeutics are becoming the standard of care in regenerative medicine; however, little work has been done to assess their comparative efficacy with respect to hair growth.

Objectives: This work evaluates the effect of activated platelet-rich plasma (aPRP), platelet lysate (hPL), decellularized amnion, adipose-derived stem cell-conditioned media (ASC-CM), and exosomes from human bone marrow-derived mesenchymal stem cells, on human follicle dermal papilla cell (HFDPC) migration and proliferation.

Methods: HFDPCs were cultured in the presence of select biotherapies. A scratch assay was used to evaluate cell migration with respect to aPRP, hPL, and amnion concentration. An alamarBlue fluorescence assay was used to quantify proliferation. Scratch-inducted production of fibroblast growth factor-7 (FGF-7) by the HFDPCs was analyzed by ELISA.

Results: HFDPC migration demonstrated an inverse relationship with treatment concentration that was maximized at 2.5 vol% hPL. Conversely, proliferation and FGF-7 production had a direct relationship with treatment concentration, wherein each achieved an optimal result at 10 vol% amnion. Nevertheless, a direct comparison of all biotherapies at 10 vol% indicated that HFDPC proliferation and FGF-7 production are maximized in the presence of ASC-CM.

Conclusion: ASC-CM has the greatest efficacy of tested treatments with respect to HFDPC proliferation and FGF-7 production.

ABSTRACT 67 Images:



ABSTRACT 68

AUTOLOGOUS FAT GRAFTING AS TREATMENT OF POSTHERPETIC NEURALGIA - RESULTS OF A DOUBLE-BLINDED PLACEBO-CONTROLLED RCT

Presenter: Martin Sollie (Denmark) Affiliation: Odense University Hospital Authors: THOMSEN JB; SØRENSEN JA

Background: Postherpetic Neuralgia (PHN) is a common debilitating chronic pain syndrome occurring after an outbreak of Shingles/Herpes Zoster (HZ). The most common definition of PHN is pain lasting for over three months after the resolution of the shingles rash. Dermal pain is the predominant symptom, and no effective treatment exists today. Autologous fat grafting (AFG) has previously shown promise in treating other chronic pain syndromes. We, therefore, hypothesized that it could help reduce and possibly cure PHN.

We have previously published a pilot study investigating the effect of AGF on PHN. In order to test the validity and reproducibility of these results, this study aimed to compare AFG to a placebo/sham treatment as a means to treat PHN.

Methods: We included 46 patients suffering from PHN. All participants received treatment under general anesthesia. The area of the fat harvest was prepared by installing a ringer-adrenalin solution. All participants had liposuction performed using the BodyJet System (body-jet; Human med AG, Schwerin, Germany, http://www.humanmed. com) with an attached 3.5 mm cannula. The lipoaspirate was then set to decant for fifteen minutes and sedimented. After liposuction, participants were randomized to either the intervention or the control group. The preparation of fat consisted solely of liquefication through a three-way Luer-lock connecter between two syringes. The intervention group received a fat graft to the subdermal area of pain with an amount of 1/2 cc per cm2. The control group received a placebo treatment of the same amount of saline solution. The primary outcome was the degree of average and maximum pain (NRS). The secondary outcomes were Quality of Life (SF-36) and quality and degree of neuropathic pain (NPSI). Follow-up was at three and six months.

Discussion: This is the first randomized study comparing the effect of AFG to a placebo treatment when treating PHN. If successful, this could mark a paradigm shift in the management of these patients as a shift from treating to curing could be made possible. Results will be available primo September 2021.

Trial registration: This trial was, prior to initiation, registered at ClinicalTrials.gov. Identifier: NCT04099706

ABSTRACT 68 Image:



ABSTRACT 69

REVIEW OF ADIPOSE GRAFTING AND PLATELET RICH PLASMA FOR VOLUMIZATION AND SCAR RELEASE

Presenter: Sherry Collawn (USA) Affiliation: UAB Authors: JAFFE TJ; TEKUMALLA ST

Introduction: Adipose grafting with platelet rich plasma (PRP) is being used for volume and skin improvement in the face. It is also used in the treatment of densely adherent scars. For volume replacement in the face and buttocks patients have had successful improvement. Impressively, patients with retracted scarring have had relief of pain with adipose/PRP grafting.

Methods: This retrospective chart review covers a total of 20 fat graft/ PRP procedures performed on the face and body from May 2020 through June 2021. Of these, 13 faces were grafted with fat/PRP at a ratio of 0.8 fat/0.2 PRP. Five of these cases were facelifts with fat/PRP facial grafting for rejuvenation. One was for cosmetic grafting on the nasal dorsum. Three were for burn scars face. One was for a forehead scar. One was for HIV facial atrophy. One was for keloid excision sites face. Retracted burn scars on the neck were injected in 2 patients. Two cosmetic buttock fat grafting cases were for a retracted scar and for an excised keloid site. Fat was generally harvested from the abdomen, thighs, or flanks using toomey syringes or an enclosed power-assisted system with 3.7 or 3.0mm cannulas. Injection in the face,neck, and small scars was with the 0.9mm Tulip single port injection cannula.

Results: The average amount of fat/PRP grafted in the face was 20 ml. The patient in Figure 1 shows the excellent improvement in perioral fat grafting 3 months after face and necklift, browlift, with injection 20 ml fat/PRP injected in the face. In this series there was a complication of a submental hematoma in a facelift patient on post op lovenox and aspirin. There were no cases of infection or embolization.

Conclusions: Fat grafting with PRP is a safe and reliable method for volumization and scar release.

ABSTRACT 69 Images:



Figure 1. The patient is shown pre op in 1A. In 1B she is shown three months after facelift, neck lift and browlift with 20ml periorbital and perioral fat/PRP grafting.

TRANSDERMAL NANOFAT DELIVERY ACCELERATES SKIN RESURFACING RECOVERY FOLLOWING CO2 LASER AND MINIMALLY INVASIVE PERCUTANEOUS COLLAGEN INDUCTION TREATMENT

Presenter: Brannon Claytor (USA) **Affiliation:** Claytor/Noone Plastic Surgery **Authors:**

Purpose: Limitations of facial rejuvenation are often centered around recovery time. Deeper tissue remodeling delivers more superior results. However, prolonged down time and increased post procedure pain can limit patient acceptance and therefor result in lest definitive improvements.

By utilizing nanofat as a rescue following aggressive combined CO2 laser treatment and minimally invasive percutaneous collagen induction performed coterminously, we hypothesize improved aesthetic results with reduced down time and less post procedure discomfort.

Methods: 20 patients underwent facial rejuvenation with treatment areas included peri oral region and cheeks. Treatments included CO2 laser, immediately followed by microneedling to a depth of 2.5 mm. Fat was harvested and converted to nanofat by filtration through nanomesh filters. The nanofat applied directly on the skin during the microneedling and for 20 minutes after the completion of microneedling. Endpoint of microneedling was pinpoint bleeding. 1 patient had tissue biopsy of areas treated with CO2 and microneedling alone and with CO2, microneedling and nanofat.

Results: Patients were evaluated for timing of full recovery and post procedure pain. All patients were fully recovered by 6 days post procedure. Pain scale evaluation revealed 0 out of 10 for post procedure pain the first evening and at second day post procedure. Tissue biopsies revealed substantial tissue damage at 4 days post procedure at the dermal epidermal layer when no nanaofat was incorporated in the procedure. This is contrasted with near completed tissue remodeling at 4 days when nanofat is incorporated in the treatment at the time of microneedling.

Conclusions: Combining skin surface remodeling with CO2 and percutaneous collagen induction microneedling is an aggressive treatment combination that maybe considered too aggressive for simultaneous treatment. However, by incorporating nanofat delivery through microneedling induced access to the reticular dermis, the skin recovery is accelerated and pain receptors are rendered quiescent. Tissue biopsy demonstrates accelerated tissue remodeling with incorporation of nanofat at the time of microneedling compared to no nanofat utilization. Further studies are needed to explore these findings.

ABSTRACT 71

SYNGENEIC FAT GRAFTING ATTENUATES HYPERSENSITIVITY IN A LEWIS RAT NERVE CRUSH INJURY MODEL

Presenter: Benjamin Scott (USA) Affiliation: Massachusetts General Hospital Authors: MCCORMACK MC; BEJAR MK; AUSTEN WG

Background: Stimulus evoked allodynia and hyperalgesia after sciatic nerve crush injury has been demonstrated in rats. Regenerative properties and anti-inflammatory effects of grafted adipose tissue have been described in the literature. In recent years, autologous fat grafting has been used clinically as a method to alleviate neuropathic pain after nerve injury, despite a paucity of evidence demonstrating the benefits of fat grafting after nerve injury. We hypothesized that syngeneic fat grafting would decrease hypersensitivity following sciatic nerve crush injury in a rat model.

Methods: Male Lewis rats (n=10, 300-350g) underwent Von Frey sensitivity testing prior to right hindlimb sciatic nerve crush injury. Group 1 (n=5) underwent syngeneic fat grafting at four weeks post-injury, once hypersensitivity in the affected limb was evident. Group 2 (n=5) did not receive fat grafting and served as a control. Von Frey testing was performed weekly post-crush injury until the end of the study (8 weeks). At the end of the study, the bilateral hindlimb gastrocnemius muscles were harvested to assess muscle mass retention as a measure of functional recovery.

Results: All rats had the same baseline Von Frey sensitivity (60 grams of force). At four weeks post-crush injury, both Group 1 (8 ± 5 g) and Group 2 (5 ± 3 g) were hypersensitive, with no statistically significant difference between groups (p=0.24). 10 days post-fat grafting, hypersensitivity was significantly improved in Group 1 rats (44 ± 22 g vs. 7 ± 3 g, p=0.005). Sensitivity returned to baseline levels at both 17 and 24 days post-fat grafting in Group 1 rats. Group 2 rats showed improvement at 17 days (38 ± 30 g) and 24 days (51 ± 20 g) but did not return to baseline sensitivity by the end of the study. There was no statistically significant difference in gastrocnemius weight.

Conclusion: This data suggests syngeneic fat grafting at the site of injury attenuates hypersensitivity after nerve crush injury in rats. Further study is necessary to elucidate the mechanism of improvement and may warrant investigation in large animals and/or a well-designed clinical trial.

Late Breaking Abstracts

ABSTRACT A

AUTOLOGOUS FAT GRAFTING AS TREATMENT OF POST-MASTECTOMY PAIN SYNDROME: A RANDOMIZED CONTROLLED TRIAL

Presenter: Martin Sollie, MD (Denmark) **Affiliation:** Research Unit for Plastic Surgery, Odense University Hospital, DK

Authors: Sollie M, Toyserkani N, Bille C, Thomsen JB, Sørensen JA

Background: Post-mastectomy pain syndrome is a common and disabling side effect of breast cancer treatment. Medical treatment seems to be insufficient for a considerable proportion of patients. Fat grafting has shown promise in relieving pain from PMPS, but no randomized clinical trial comparing fat grafting to a sham operation or an interventional control group has been performed to date.

Objective: The aim of our study was to compare the effect of fat grafting compared to a sham/placebo operation for treating post-mastectomy pain syndrome.

Methods: We conducted a single-center double-blinded randomized clinical trial with two arms between October 2017 - September 2020. We assessed forty-five patients suffering from PMPS for inclusion. The intervention group received scar-releasing rigottomy and fat grafting to the area of pain. The control group received scar-releasing rigottomy and a placebo of saline solution. The fat graft was prepared using decantation and sedimentation. The injection was performed using a 3.5 mm cannula. The primary outcome was the degree of pain measured using the numerical rating scale (NRS). The secondary outcomes were the degree and quality of neuropathic pain (Neuropathic Pain Symptom Inventory) and Quality of Life (Short Form-36). Follow-up was six months.

Results: Thirty-five participants completed follow-up: eighteen participants in the intervention group and seventeen in the control group. We detected no statistically significant changes in average and maximum pain or neuropathic pain. Regarding the quality of life, the control group reported a statistically significant improvement in emotional problems parameters, whereas the intervention group reported a deterioration. We observed no serious adverse effects.

Conclusions: In conclusion, we found no evidence to support that fat grafting is superior to placebo when treating post-mastectomy pain syndrome. Our results do not support the routine use of simple autologous fat grafting for treating post-mastectomy pain syndrome.

ABSTRACT B

ENHANCED POTENCY OF A CELL-FREE STEM CELL THERAPY FOR TREATMENT OF ISCHEMIC STROKE BY DEPLETION OF MCP-1

Presenter: Yansheng Du

Affiliation: Indiana University School of Medicine **Authors:** Xin Yi, Huiying Gu, Brian H Johnstone, Michael Coleman, Keith L March, Yansheng Du

Adipose mesenchymal stem cells support repair when administered to damaged tissues, most likely through secreted trophic factors. Collaborating with Theratome, we tested whether a 100X concentrated. cell-free fraction of conditioned medium derived from human adult adipose mesenchymal stem cells (T-101) is safe and neuroprotective when administered IV once up to 2d and 7d after exposure to ischemia and reperfusion in a MCAO mouse model. T101 was infused through the jugular vein of C57BL/6J mice subjected to MCAO injury (10/ group). The results demonstrated that IV injection of T-101 at 0.2-2 mg/ kg immediately after blood reperfusion resulted in a dose-dependent reduction in the brain infarct volume and swelling, and increased longterm survival (infarct volume (%): 34±4.6 vs.19±4.4-2.3±1.3, p<0.05). To elucidate the exact mechanism of action, we first determined the concentrations of individual therapeutic factors and discovered that an efficacious dose of T-101 contains between 100- to 1000-fold lower levels than typically administered for single protein factors. The lower levels of factors working in concert provides for an increased safety factor by minimizing off-target effects noted at higher doses of single agents. Additionally, we discovered that T-101 composition contains not only beneficial neurotrophic, regenerative, and angiogenic factors but also potentially detrimental inflammatory factors, such as monocyte chemoattractant protein-1 (MCP-1or CCL2). Selective depletion of MCP-1 from T-101 (mT-101) markedly increased the neuroprotective potency induced by mT-101 at 0.2 mg/kg in the MCAO model (infarct volume (%): 19±4.4 vs. 8.9±2.9, p<0.001). Based on the known function of MCP-1 and the biodistribution of T-101 after IV injection, we believe that enhanced efficacy following selective depletion most likely resulted from reduced inflammatory stimulus in the peripheral immune system such as spleen, leading to reduced inflammation in brain. Interestingly, our recent data demonstrate neuroprotective exosomes isolated from T-101 (exT-101) do not contain MCP-1. Thus, it is necessary to compare the neuroprotective efficacy among T-101, mT-101 and exT-101 in the MCAO model. If our hypothesis is confirmed in on-going testing, T-101-induced neuroprotection could be significantly potentiated by specifically modulating the peripheral immune system through reducing activation of the MCP-1/CCR2 pathway, resulting in enhanced potency for treatment of stroke

ABSTRACT C

EVALUATION OF THE SAFETY AND EFFICACY OF AN AUTOLOGOUS ADIPOSE SVF TREATMENT OF KNEE OSTEOARTHRITIS

Presenter: Robert Harman, DVM, MPVM (USA) Affiliation: Personalized Stem Cells, Inc. Authors: Rogers C, Harman R, Sheinkop M, David T, Hanson P, Sampson S, Aufiero D, Desai R, Malanga G, Bowen J, Amback M

Introduction: The objective of this FDA-approved Phase 1/2a clinical study was to evaluate the safety of an intraarticular injection of an investigational biologic product (IBP), PSC-01, the patient's own adipose-derived SVF extracted from a lipoaspirate sample, to treat osteoarthritis in a single knee. The secondary objective was to collect data on efficacy of the PSC-01.

Materials and Methods: This clinical study was designed as a single-arm safety study. Twenty-nine patients completed the final study visit. The population was male/female, 18-80 years of age with Kellgren-Lawrence (K-L) grade 2, 3, or 4 in one knee and pain duration for a minimum of 3 months and failing conservative therapy. The lipoaspirate was shipped in a temperature-controlled container and was processed in a cGMP facility by enzymatic digestion, washing, and separation into the SVF and cryopreserved. QC samples were evaluated for cell count, cell viability, sterility, endotoxin, and flow cytometry. The average delivered dose was 4.0 1.8 x 106 viable nucleated cells. PSC-01 was thawed and directly injected at the Investigator site under ultrasound guidance.

Safety Evaluation: Safety was assessed by questionnaire, history, examination, laboratory assessment, self-reported assessment, and post-procedure observations.

Efficacy Evaluation: The Knee Injury and Osteoarthritis Outcome Score (KOOS) is a knee-specific instrument that assesses knee symptoms. The KOOS was assessed at screening, treatment, follow-up visit, and at the final visit (Day 84) and 12-months post-study.

Safety Results: Safety assessment showed that lipoaspiration had a minimal safety impact with all AEs reported as grade 1 or 2 (of 4), no SAE's, and all resolving with minimal intervention. The evaluation of IBP injection showed minimal safety impact with all AEs reported as grade 1 or 2 with no SAEs.

Efficacy Results: The KOOS showed a strong improvement from baseline to study end.

Conclusions: This clinical study has demonstrated a solid safety and efficacy profile that supports the Sponsor's progression to a Phase 2 randomized clinical trial.

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Notes	

Notes





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