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IMGC HYBRID Symposium 2022
October 18-20, 2022

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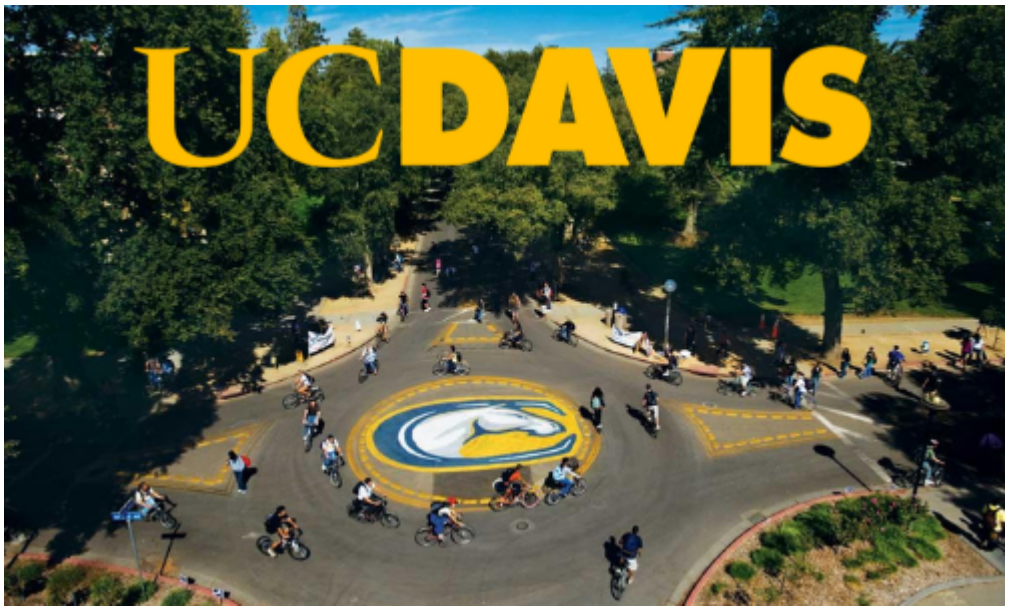

Welcome to the 19th Annual IMGC HYBRID Symposium 2022 at UC Davis, in Davis, California, USA!

IMGC's flagship conference brings together international experts in lactation, milk science, chemistry, microbiology, immunology, nutrition, genomics, anthropology and bioinformatics to discuss and share the outcomes and implications of the latest discoveries in scientific research on lactation and milk for human health.

The Symposium targets hundreds of scientists from all over the world to foster cross-cutting discussion and collaboration.

Presentations will focus on the following **five** session topics during the 2.5 day symposium:

- Hottest topics in milk science: what the world should know
- Applications of milk extracellular vesicles, miRNAs, and nucleotides for human health
- Discovery and novel applications of milk bioactives
- Immunity tackles immune challenges of the 21st century
- Functional discoveries of the microbiome, glycome, and metabolome

Click **here** to learn about the registration fees, our COVID-19 policy and more.

Students may participate **virtually** for **free** (if registered by the early-bird deadline)!

Read abstracts for the oral presentations on "Milk Extracellular Vesicles, miRNAs, and Nucleotides"

Sarah Andres, Ph.D.

OREGON HEALTH & SCIENCE UNIVERSITY

Title: *Many human milk extracellular vesicle proteins are lost in human digestion, do the survivors convey beneficial effects?*

Extracellular vesicles (EVs) are lipid-membrane-encased nanoparticles that carry biological cargo (including proteins) from mom to baby via human milk. Although studies in animal models demonstrate the beneficial effects of human milk EVs on intestinal barrier function, we do not know if these cargoes survive in vivo human digestion. Addressing this knowledge gap will remove critical barriers to using human milk EVs and associated proteins as additives to neonatal nutrition or therapeutics for necrotizing enterocolitis. 1. Test if human milk EVs promote the survival of cargo proteins to the human intestine; 2. Delineate molecular mechanisms of EV-cargo mediated gut barrier protection. EVs were isolated from 3 paired human milk and neonatal intestinal content (digesta) samples using density-gradient ultracentrifugation. Digesta were collected after gastric feeding from naso- or orojejunal sampling tubes. EVs were characterized by nanoparticle tracking analysis, western blot, and electron microscopy. EV protein cargo was profiled by a C18-UPLC paired with Orbitrap mass spectrometry. Effects of candidate proteins on human intestine were examined using neonatal enteroids derived from patient intestinal stem cells. All studies are part of OHSU IRB-approved protocols. Only 4.68% +/- 0.02 (p=0.0012 vs undigested milk) of EV proteins from human milk survive to the human intestine, but nearly half 48.96% +/- 0.02 (p=0.007 vs undigested milk) of the protein diversity is preserved. Protein cargoes within the surviving EVs are involved in extracellular matrix interactions, membrane trafficking, metabolism, and cell survival. These EVs are taken up by intestinal cells where they exert their effects. We demonstrate that the majority of EV protein cargo is lost between the mouth and the intestine, but that nearly half of the protein cargo diversity is preserved. These findings indicate the importance of examining cargo that survives to the intestine

when investigating potential milk-mediated mechanisms of disease prevention.

Shannon Kelleher, Ph.D.

University of Massachusetts Lowell

Title: *Identification of human milk-derived microRNAs associated with low milk supply, reduced infant growth and early breastfeeding cessation*

To identify milk miRNAs associated with low milk supply in women and ascertain molecular pathways in the lactating mammary gland that are responsible. RNA isolated from foremilk collected from women with low (LMS, n=47) and adequate (AMS, n=123) milk supply was sequenced. Longitudinal changes in miRNA candidates were measured and relationships between milk miRNAs and milk production, breastfeeding outcomes, and infant weight gain, were assessed. Infants of mothers with LMS (20.6 ± 11.8 oz/d) had a lower mean weight-for-length z-score (0.05 ± 1.2) at four weeks compared to infants of mothers with AMS (28.1 ± 15.6 oz/d; p = 0.003) whose mean weight-for-length z-score was 0.50 ± 1.1 (p = 0.013). Mothers with LMS were also more likely to have ceased breast feeding at 24 weeks (38.2%), compared to mothers with AMS (17%; p = 0.0003). Five milk miRNAs were associated with suboptimal lactation; let-7a-5p, let-7g-5p and miR-22-3p levels were higher in milk of mothers with LMS compared with mothers with AMS one week after delivery, whereas miR-16-5p and miR-151a-3p levels were lower. Milk volume remained significantly associated with miR-16-5p (R = -0.14, p = 0.0088), miR-22-3p (R = 0.13, p = 0.011), and let-7g-5p (R = 0.12, p = 0.023) levels throughout 16 weeks. KEGG pathway analysis suggested cell cycle, fatty acid biosynthesis, adherens junctions, Hippo signaling and TGFb signaling were probable target pathways, indicated FUT9, ESR1, ELOVL6, SLC30A4, and IGFBP2 were downregulated, while PAPPA, COL25A1, FASN, and FGF2 were upregulated molecular targets in the mammary gland. Specific miRNAs are differentially expressed in milk from women with low milk supply and may identify molecular pathways that impair milk production. We propose specific miRNAs may be used as non-invasive biomarkers for predicting risk for low milk supply and early cessation of breastfeeding.

Spencer Marsh, Ph.D.

Title: *Bovine milk-derived exosomes as a novel injury-targeting drug delivery system*

A novel protocol provides large amounts of highly purified small extracellular vesicles (also called exosomes) from bovine milk (Marsh et al, PMID: 34367882). We sought to examine targeting of these pure, highly concentrated bovine milk-derived extracellular vesicles (mEVs) to injured cells and tissues. Targeting of mEVs to injured cells and tissues was tested in vitro using a scratch assay on human dermal fibroblast (hDFs) and MDCK cell monolayers, and in vivo, using mouse models of skin wounding and cardiac injury - as we have reported in PMID:34246197; PMID:29351451. mEVs were isolated using our published approach, then fluorescently tagged with Cell Tracker Deep Red (CTDR). Labelled mEVs were then applied to cell cultures at 20 ug/mL for 15 minutes post wound; cells were then rinsed, fixed and stained for cell nuclei. Mice were provided 2 ug/kg loaded mEV's by oral gavage before skin surgery and induction of cardiac ischemic reperfusion injury. Mice were sacrificed 4 hours post-surgery, and fixed by perfusion with 4% paraformaldehyde and PBS rinsing, followed by cryosectioning, staining for nuclei and actin FITC-phalloidin. Imaging was performed on a Leica SP8 laser scanning confocal microscope and quantification mEV uptake normalized to cell nuclei using ImageJ. mEV uptake was significantly increased in scratch wounded cultures of both Hdefs and MDCK cells ($p<0.001$), over uninjured control cells. Similarly, injured heart and skin tissues exhibited significantly increased exosomal uptake ($p<0.001$), relative to sham injury controls and skin tissues remote from the injury. Our experiments indicate that injured cells and tissues show increased uptake of milk derived exosomes, with in vitro

data suggesting that this enhanced uptake occurs, at least in part, in a cell autonomous manner. The data also supports that our isolation protocol provides an mEV-based drug delivery system that may preferentially targets injured or diseased tissues.

Marie Stampe Ostenfeld, Ph.D.

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Title: *Industrial large-scale isolation and characterization of milk extracellular vesicles for utilization in infant nutrition*

Milk extracellular vesicles (MEV) are gaining increasing attention due to their cargo of bioactive components, which potentially prime infant health development through recipient cell uptake. The biogenesis of MEVs and milk fat globules (MFGs) follows distinct routes. Thus, profiling of their content and understanding potential differential roles is warranted. Commercial infant formulas are largely depleted for MEVs as compared to human milk, making it timely to explore outcomes of adding bovine MEVs to infant formulas. Bovine MEVs were isolated in a dairy pilot plant production setup using skim milk subjected to acidification for casein precipitation followed by sequential filtration steps. Sub-fractions relatively enriched in MFGs, MEVs, small MEVs/proteins were obtained and analyzed by transmission electron microscopy (TEM) and for whey protein, fat, and miRNAs content. The industrial whey protein concentrate (WPC)-A -MEV fraction was further analyzed for purity of MEV vs. MFGM content using a relative quantification-based mass spectrometry method of tetraspanins (CD9, CD63, and CD81), butyrophilin, lactadherin, and xanthine oxidase. The fractions were used as emulsifiers in an in vitro infant lipolysis model and piglet model to test lipid bioavailability and impact on brain development. A novel industrial acid WPC-based fraction of MEVs was obtained with high MEV:MFGM purity (based on TEM and CD9:BTN MS ratio). The WPC-A-MEV fraction differed in content (proteins, fat, miRNAs) from the other sub-fractions. Emulsification with WPC-A-MEV elicited a significant increased lipolysis rate in vitro, and increased triglyceride bioavailability and hippocampus maturation compared to soy lecithin in neonatal piglets. A MEV fraction was successfully produced from a novel industrial-scale process. This MEV fraction applied as an emulsifier in an infant formula diet led to improved lipid bioavailability and brain hippocampus maturation. The results indicate preservation of unique bioactive MEV components and a potential for milk EVs in next generation infant formulas.

Fons van de Loo, Ph.D.

Radboudumc
university medical center

Keynote Speaker

There is compelling evidence that bovine milk-derived extracellular vesicles (nanosized lipid bilayer particles) contain an immunoregulatory cargo. To evaluate their potential therapeutic properties for rheumatoid arthritis we tested the oral application of bovine milk-derived extracellular vesicles mEVs) in two arthritis models. In both models, mEVs delayed arthritis onset and reduced cartilage loss and bone marrow edema. Evidence is emerging that mEVs can pass the intestinal tract, reach the circulation, and by this route may directly target cartilage and bone. We recently showed that mEVs exert a protective effect on human osteoarthritic cartilage explants by reducing proteoglycan release and expression of MMP1 and ADAMTS5, two cartilage-destructive enzymes. On bone cell precursors, mEVS accelerated osteoblastogenesis towards osteocytes and mineralization. Additionally, milk EVs and steered the osteoclast differentiation towards the formation of small osteoclasts with less bone resorbing activity. Milk EV treatment indeed increased the number of osteoclasts and osteocytes but the overall effect was less bone loss in both obesity-or ovariectomy-induced osteoporosis in mice. It must be emphasized that the site of action remains to be determined in our in vivo studies. During rheumatoid arthritis the intestinal barrier function is compromised and this makes the intestinal

Title: *Emerging prospects of bovine milk-derived extracellular vesicles for arthritis therapy*

epithelium a prime target for mEVs in arthritis. The most consistent finding on HT29 epithelial cell-line was the induction of interleukin-8 (IL-8) by mEVs. IL-8 is a first response cytokine to environmental changes and a chemoattractant for rapid recruitment of immunosuppressive immune cells such as myeloid derived suppressor cells, dendritic cells, and regulatory T cells (Tregs). Milk EV can induce immune-suppressive Tregs and thereby suppress the osteoclastogenic Th17 cells in arthritis. These observations show the broad therapeutic potential of mEVs to reduce arthritis pathology. To translate this into a success story in humans, a large scale isolation method to obtain pure active mEVs will be pivotal.

Janos Zempleni, Ph.D.

University of Nebraska Lincoln

Title: *Genetically altered milk exosomes facilitate nutrition research and drug delivery*

The objective of this research was to develop genetically altered milk exosomes suitable to interrogate milk-dependent pathways (nutrition research) and optimize the delivery of therapeutic cargo encapsulated in milk exosomes (drug delivery). Nanoparticle size analysis, immunoblotting, transmission electron microscopy and near-infrared imagining analysis were used to demonstrate that bovine mammary alveolar MAC-T cells secrete milk exosomes and are amenable to genetic engineering. Lentiviral protocols were used to express fusion proteins in MAC-T cells that localize to exosomes. Proprietary exosome modifications were used to with the goal to alter exosome uptake by bone-marrow-derived macrophages (BMDMs), uptake by glioblastoma multiforme brain tumor cells [(R132H)GBM], and homing to brain tumors in a mouse model of human GBM. Both exosome modifications UNL1 and UNL2 decreased by 50% the uptake of exosomes in BMDM cultures compared to wild-type exosomes ($P<0.05$, $n=3$). Exosome modification UNL3 increased by 40% the uptake of exosomes in (R132H)GBM cell cultures compared to wild-type exosomes ($P<0.05$, $n=3$). Exosome modification UNL2 increased the homing of exosomes to glioma: exosomes accumulated in tumors but not in healthy brains in a mouse model of human GBM. This proof-of-concept study provides experimental evidence that the genetic engineering of MAC-T cells affords investigators a tool to interrogate milk exosome-dependent pathways in nutrition and identify candidate modifications for improving drug delivery by nanoparticles.

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