W21: LIVE WEBINAR: Innovations in Urogenital Tissue Repair

Workshop Chair: Marianna Alperin, United States

Start	End	Topic	Speakers
		Closer Look at Pelvic Floor Muscles	Marianna Alperin
		Endometrial Mesenchymal Stem/Stromal Cells and its Application in Urogenital Tissue Repair	Caroline Gargett
		Uncovering Elastin Homeostasis in Pelvic Organ Prolapse using Knockout Model	Margot Damaser
		Body-on-a-chip	Linda Griffith

Aims of Workshop

This workshop will focus on novel and innovative research across multiple disciplines to uncover the basic biology that drives tissue repair processes. Showcasing the latest tools in stem cell biology, proteomics, lab on a chip and genetically modified knock out models, this work will enlighten the audience with how cues of basic fundamental biological mechanisms can drive novel treatment designs. The workshop aims to share the understanding, application and feasibility of latest research methods in improving uterine tissue repair.

Learning Objectives

Importance of Basic Science Innovation in driving high quality patient care

Target Audience

Urology, Urogynaecology and Female & Functional Urology, Pure and Applied Science

Advanced/Basic

Basic

Suggested Learning before Workshop Attendance

Gargett, C., Gurung, S., Darzi, S., Werkmeister, J., & Mukherjee, S. (2019). Tissue engineering approaches for treating pelvic organ prolapse using a novel source of stem/stromal cells and new materials. Current Opinion in Urology, 29(4), 450-457. https://doi.org/10.1097/MOU.0000000000000634

Marianna Alperin, M.D., M.S.

A closer look at pelvic floor muscles

Pelvic floor muscles (PFMs), which consist of coccygeus and the individual components of the levator ani complex, are integral for the supportive function of the female pelvic floor. The majority of the human PFM data are derived from imaging studies that demonstrate increased levator hiatal area in women at the end of gestation compared to non-pregnant women. Just like decreased vaginal stiffness, bigger levator hiatus in pregnancy facilitates childbirth, as evident by the association of larger hiatus with a shorter second stage of labor and successful uncomplicated spontaneous vaginal delivery. However, levator hiatus accounts only for the most medial portion of the levator ani complex. Furthermore, despite increase in hiatal area with straining in pregnancy, it remains substantially smaller than the area required for fetal delivery. Finally, levator hiatal area does not correlate with PFMs' strength or endurance. Using the rat model, validated for the studies of the human PFMs, we have demonstrated that pregnancy induces unique adaptations in the PFMs' intrinsic structural components. Under functional conditions of pregnancy, PFM increase their fiber length and consequently their excursion by adding sarcomeres in series in the process termed sarcomerogenesis. In the likely event that similar adaptations take place in the human PFMs, increased excursion afforded by pregnancy-induced sarcomerogenesis could explain increase in levator hiatus with strain observed in pregnant compared to non-pregnant women. Pregnancy also induced increase in total collagen content of the PFM intramuscular ECM and specifically of collagen V isoform; rise in passive tension with strain; and changes in the PFM proteomic signature in the preclinical model. Together, these antepartum changes attenuate myofiber stretch and the extent of sarcomere hyperelongation, the primary cause of mechanical skeletal muscle injury, during parturition. Increased fiber length is protective against such damage since large mechanical deformations are distributed across a greater number of sarcomeres. Furthermore, increase in ECM and higher passive tension with strain observed in the PFMs of pregnant relative to non-pregnant animals, further shield myofibers from excessive mechanical strain during parturition. Understanding functionally relevant changes that occur at a tissue- and cellular levels in the pelvic floor supportive structures, such as PFMs, in response to mechanical and other physiological cues associated with pregnancy and delivery is important for identifying novel preventative and therapeutic strategies for PFM dysfunction and the associated pelvic floor disorders.

Caroline E Gargett
Endometrial Mesenchymal Stem/stromal cells and their application in Urogenital Repair

Human endometrium is a highly regenerative tissue undergoing cyclic growth, differentiation and shedding during a woman's reproductive life. Mesenchymal stem cells (eMSCs) have been identified and characterised as rare, clonogenic cells with high proliferative potential, self-renewal in vitro, differentiation capacity, and tissue reconstitution in vivo. Markers purifying eMSC; co-expression of CD146 and PDGFRβ, or the single marker SUSD2, revealed their pericyte/perivascular identity in both basalis and functionalis endometrium. Endometrial MSC can therefore be easily harvested in an office-based procedure without anaesthetic or from menstrual fluid. Moving toward clinical translation, we purify eMSC by SUSD2 magnetic bead sorting of cell suspensions from endometrial biopsies of premenopausal and estrogen-treated post-menopausal women.
We have developed a culture expansion protocol in serum-free medium that maintains eMSC stemness using a TGFβ-Receptor inhibitor, A83-01. This protocol results in cultures with 90-95% SUSD2+ eMSC, important for quality assurance of autologous cell preparations and clinical translation. To understand the mechanism by which A83-01 maintains the eMSC phenotype, combined Assay of Transposase Accessible Chromatin (ATAC)- and RNA-sequencing revealed that TGFβ receptor inhibition opens chromatin loci of transcription factor binding sites and upregulates gene networks of developmental and stem cell signalling pathways, while closing collagen and extracellular matrix synthetic pathways, characteristic of undifferentiated MSC. In addition, A83-01 prevents eMSC apoptosis and senescence by upregulating anti-oxidant and downregulating senescence genes. Such cultured eMSC have a secretome enriched in pro-angiogenic proteins and a desirable gene profile of upregulated MSC potency, proliferation, immune response genes and induction of anti-fibrotic and anti-apoptotic genes for clinical translation. We have demonstrated potential clinical utility of eMSC in small and large animal pre-clinical models of wound healing and pelvic organ prolapse, showing angiogenic, immunomodulatory and anti-inflammatory properties when delivered on novel non-degradable polyamide, degradable nanofiber and 3D printed meshes. In summary, eMSC are a readily available and accessible source of MSC, easily cultured in A83-01-containing medium, producing a transplantable MSC type with properties that enhance mesh integration and promote tissue repair in urogenital applications.

Margot S. Damaser, Ph.D.
Uncovering elastin homeostasis in pelvic organ prolapse using knockout model

Pelvic floor disorders, including pelvic organ prolapse (POP), stress urinary incontinence, and fecal incontinence, are usually thought of as pathology of the biomechanics of the pelvic floor since they are caused by a reduction in the physical pelvic floor support for the pelvic organs and for closure of the urethral and anal sphincters. However, the structures that provide this support consist of living tissue: extracellular matrix connective tissue as well as muscles innervated by nerves. Therefore, although the endstage disease symptoms result from insufficient biomechanical support, this can and often is caused by a disruption in biochemical signaling triggered by childbirth, genetics, aging or POP. Elastin in particular provides elastic recoil capability to the vagina and pelvic floor and women with POP have reduced elastic recoil and decreased expression of lysyl oxidase-like 1 (LOXL1), an extracellular matrix crosslinking enzyme important for crosslinking elastin fibers. We have used the LOXL1 knockout (KO) mouse model of POP to study the pathophysiology of elastin homeostasis and elastin morphometry as a function of vaginal delivery and prolapse to begin to build a mechanistic understanding of how extracellular matrix dysregulation can lead to the biomechanical insufficiencies resulting in POP. In this talk, I will provide a background on extracellular matrix biology, with a focus on elastin homeostasis mechanisms, and will summarize our work with LOXL1 KO mice and place it in the context of POP and pelvic floor biomechanics.

Linda Griffith
Body-on-a-chip

"Mice are not little people" – a refrain becoming louder as the strengths and weaknesses of animal models of human disease and drug responses become more apparent. At the same time, three emerging approaches are headed toward integration: powerful systems biology analysis of cell-cell and intracellular signaling networks in patient-derived samples; 3D tissue engineered models of human organ systems, often made from stem cells; and micro-fluidic and meso-fluidic devices that enable living systems to be sustained, perturbed and analyzed for weeks in culture. Endometriosis, adenomyosis, and other gynecological disorders are paradigms of chronic inflammatory diseases that can only partially be modeled in animal systems. In this talk, approaches to classify patients on the basis of analysis of cell-cell communication networks will be described as a motivation for building patient avatars that capture salient features of the disease processes. Then, approaches to use synthetic extracellular matrices to build tissue-engineered models of the endometrium and endometriosis lesions, including microvascular networks and immune cell recruitment, will be highlighted, along with the potential to integrate these approaches for developing new drugs to treat endometriosis and adenomyosis.