Virtual Poster Session: Infection & Inflammation | Friday June 12, 2020 5:30 – 6:30 EDT

https://vimeo.com/showcase/7214276

18| Yifeng Song- Western University; Undergrad

"Cream therapy to prevent autoimmunity in rheumatoid arthritis mouse model"

Rheumatoid arthritis (RA) is a chronic inflammatory disorder preceded for years by a pre-clinical phase (pre-RA) associated with asymptomatic autoimmunity. Circulating anti-citrullinated and anti-homocitrullinated peptide/protein autoantibodies (ACPAs and AHCPAs, respectively) detected in pre-RA are disease-predictive biomarkers. Autoimmunity may be preventable by delivering RA-associated autoantigens into the skin to induce immune tolerance. We explore the efficacy of a transdermal cream treatment containing citrullinated and homocitrullinated peptides in preventing RA autoimmunity in DR4tg mice. DR4tg mice express the strongest human RA genetic risk factor. To model RA, mice were immunized with homocitrullinated peptides to induce autoimmunity. Mice were treated with the transdermal cream containing citrullinated and homocitrullinated peptides before immunization. ACPA/AHCPA levels were screened using ELISA biweekly up to and including the endpoint 137 days later. Mice treated with peptide-containing cream (n=8) had significantly lower AHCPA IgG levels over time than mice treated with peptide-absent control cream (n=8) ($p<0.0001$). No differences in ACPA IgG levels or ratios of pro- (IgG2b) vs. anti-inflammatory (IgG1) AHCPA were observed. T cell responses were measured at the endpoint using 3H-Thymidine incorporation assay but were undetected in most mice. We prevented an RA-specific autoimmune response using a novel skin-based treatment. Using transdermal cream to induce immune tolerance towards autoantigens has potential for innovative RA-specific prevention therapies.

40| Matthew Lawrence- Western University; Masters (In progress or completed)

"3D Printed Polypyrrole Scaffolds for Bone Regeneration"

Background: Areas of large bone loss are healed using autologous bone grafts which have a complication rate of 10–40% during harvesting. Due to issues with traditional bone grafts, the use of polymer scaffolds as a bone graft alternative is proposed. Polypyrrole is a biocompatible electroactive polymer that can hold and release drug molecules for delivery inside the human body. A change in pH around bone injury and regrowth will act as the stimulus for the polypyrrole scaffold, causing the dopant drug to be released. Anti-inflammatory dopants delivered with a polymer matrix create an efficient environment for bone regeneration after surgery. Western University is one of only facilities that can 3D print polypyrrole polymers using digital light projection, allowing for customized scaffolds.
Methods: Polypyrrole-only scaffolds were placed in solutions between pH 2–11, measuring fluorescein release with UV spectroscopy to characterize drug loading capacities before incorporating co-polymers. Successful resin was a mixture of polypyrrole, poly(ethylene glycol) methyl ether methacrylate (PEGMA) , riboflavin (vitamin B2), silver nitrate and triethanolamine. The two-polymer system employed silver nitrate to cure the pyrrole while the riboflavin and triethanolamine polymerized the PEGMA.
Results: UV spectroscopy showed a significant increase in fluorescein release from polypyrrole at higher pH values, and further increasing with the presence of microstructures. The resin formulation successfully cured using a digital light projection printer with a wavelength of 385 nm.
Discussion: Tests show initial success in the creation of a biocompatible resin mixture that can be used to create 3D printed polypyrrole cell scaffolds for pH sensitive drug release in areas of bone regrowth. Future work includes in-vitro cell culture tests using MC3T3-E1 pre-osteoblasts to ensure a biocompatible scaffold. This collaborative project aligns with the Bone and Joint Institute core values.

95| Brent C Wakefield- Western University; PhD (In progress or completed)

"Pannexin 3 regulates fat deposition and inflammation in male mice"

Obesity is excessive fat accrual and causes inflammation contributing to comorbidities such as insulin resistance and osteoarthritis. Thus, the identification of target genes that lead to drug therapies to combat obesity is warranted. Pannexin 3 (Panx3), a membrane bound channel-forming protein involved in purinergic signalling, is a candidate gene linking body weight and inflammation. We hypothesized that mice lacking Panx3 would have less body weight, fat mass and inflammatory phenotype compared to wildtype (WT) mice in the context of forced exercise (FEX) and a high fat diet (HFD). First, body weight, composition, and metabolism were measured at 12 and 24 weeks of age in a cohort of male WT and Panx3 KO mice. Next, a cohort of male and female WT and Panx3 KO mice litters were equally distributed to either a sedentary (SED) or FEX group from 24 to 30 weeks of age. Body weights were recorded bi-weekly. At 24 and 30 weeks, blood glucose tolerance and body composition were analysed. At sacrifice, insulin, cholesterol, and triglyceride levels were measured in plasma using ELISA. Metabolic organs were used for analysis of inflammatory markers using qPCR. An additional cohort of male WT and Panx3 KO mice were placed on a HFD (60% kcal fat) and body weights were recorded at 20 and 28 weeks. Metabolic organs were analyzed for inflammatory markers. Male Panx3 KO mice weigh less, have lower fat and higher lean mass than WT mice. When WT mice are subjected to FEX, they have reduced fat mass and blunted weight gain, while there is no effect of FEX on body weight and composition in the Panx3 KO mice. On a HFD, Panx3 KO mice gain less body weight and have reduced inflammatory markers in quadriceps and fat tissue. In female mice, there were limited effects of genotype on body composition, however, Panx3 KO mice weigh less. This Research suggests Panx3 regulates body weight and fat accumulation in a sex specific manner, and may be a drug target for the treatment of obesity.

98| Olivia J Lee- University of Guelph; PhD (In progress or completed)

"Standardization of Potency Assessment and Use of Equine Mesenchymal Stromal Cells"

Background/Rationale: Inflammatory joint diseases are significant causes of morbidity in horses. Equine mesenchymal stromal cells (MSCs) hold promise as cell-therapy candidates due to their secretory, non-progenitor functions. MSCs derived from umbilical cord-blood (CB) are of clinical interest due to ease of procurement, multipotency, and immunomodulatory ability. Using mononuclear cell suppression assay (MSA), we demonstrated that equine CB-MSCs are mononuclear cells(MNCs) suppressive in vitro. Due to the inherent heterogeneity of MSCs and varied culture expansion protocols, development of methods to circumvent donor-to-donor heterogeneity, as well as robust and easily deployable methods of potency assessment is beneficial for improving MSCs' predictability in treating inflammatory diseases.
Purpose/Objectives: This study focuses on the development of robust in vitro potency assays and the assessment of potential MSC therapeutic end-products generated from pooled equine MSCs(pMSCs).
Methodology: pMNCs were generated by pooling equine MNCs isolated from peripheral blood of five donors in equal ratios. pMNCs were labelled with carboxyfluorescein succinimidyl ester(CFSE) and stored in liquid nitrogen until use. Similarly, pMSCs were generated by pooling MSCs from multiple equine donors in equal ratios. MSC cultures were assessed with pMNCs in MSA using Bromodeoxyuridine(BrdU) ELISA and CFSE.
Results: Proliferation assessment of MNCs from individual donors revealed varied responses to ConA stimulation. MSA using MNCs from single donors further demonstrated MNCs donor variability. Both individual MSCs and pMSCs were able to suppress pMNCs proliferation.
Significance:MSA based on pMNCs provides a consistent and reproducible equine MSCs potency assay. Utilizing this assay, we have also demonstrated that pMSCs have immune suppressive properties. This knowledge could be used in production monitoring of cellular potency and as release criteria prior to clinical use.
