Overview Actual Research Activities

Summary

My research activity covers four main areas:

(i) development of an artificial placenta,
(ii) dual ex-vivo closed-loop placenta perfusion to assess the question how the placenta contributes to the cytokine exposure of the fetus in case of chorioamnionitis,
(iii) optimizing nutrition and growth of very low birth weight (VLBW) infants in order to improve neurodevelopmental outcome and minimize the risk for the developmental onset of adult diseases, mainly metabolic, cardiovascular and neurologic ones (DOHaD) as well as
(iv) developing and implementing efficient strategies that help premature babies with their postnatal adaptation, but minimize the risk for side effects and iatrogenic complications.

Detailed discussion

(I) Artificial placenta

The ultimate goal of this research activity is to develop a medical device that can be connected to the circulating blood and that provides additional gas exchange. The target population for this device are term and preterm infants suffering from end-stage respiratory failure with immanent death or severe respiratory disease that requires very aggressive ventilation with a high risk of severe permanent lung damage. The device shall have a high efficiency with a small priming volume, shall work in ambient air, shall be connected to the natural umbilical vessels, shall be pump less and biocompatible and shall have a modular concept so that it can be easily adjusted to various body sizes.

We have established an impressive network of researchers and are proud to collaborate with three professors (Dr. Selvaganapathy, Dr. Brash, Dr. Brook) at the Faculty of Bio-Engineering at McMaster. I have also been cross-appointed to this faculty in 2010, a status which allows me to accept and supervise Masters students from the BME program - as is the case with Asma Manaan (since Jan 2012). Our network also includes Dr. Anthony Chan and his co-worker Dr. Les Berry from McMaster’s Children’s Hospital, as well as Prof. Wagner from the Institute for Polymer Technology at the Free University in Berlin and A. Pfaff from the Pfaff Company in Freiburg (both Germany).

So far we have been able to create a prototype that has been extensively tested and optimized in-vitro and also first animal experiments in newborn piglets have been quite successful in normalizing the arterial oxygen saturation to normal levels. We believe that we have provided proof of principle that this concept works. In April 2013 we have received the approval of our CHRP program grant (545.000 Can$ from 2013 - 2016). We have filed a US patent to protect the intellectual property of our group.

We are currently searching for industrial partners to apply for different funding agencies, a process which is facilitated with an existing partnership of a suitable Canadian company. An exciting actual sub-project is to solve the coating problem, i.e. to develop a “coating” procedure for the inner surface of the oxygenator as well as to create a safe, wide bore access through the umbilical vessels in order to assure sufficient flow through the device.
Collaborations with institutions at McMaster (Faculty of Bio-Engineering) and at the international level (Pfaff GmbH, Freiburg, Germany) have been successfully made to solve this issue. The work has also resulted in one peer-reviewed publication, three peer-reviewed conference papers, one manuscript accepted by a high-impact peer reviewed journal as well as five oral or poster presentations at national and international conferences. We have also been invited to give a lecture in May 2013 at the 9th International Conference on Pediatric Mechanical Circulatory Support Systems & Pediatric Cardiopulmonary Perfusion in Hershey, PA, USA.

This research will hopefully result in an improved survival rate of newborn infants with the most severe form of lung disease.

**Fig 2:** Schematic illustration of the artificial placenta (upper panel) and image of the prototype (lower panel)

**(II)** **Dual closed-loop ex-vivo perfusion of the placenta**

From my previous work place we have long-standing experience with this exciting model. The ultimate goal of our work is to understand how the placenta contributes to the cytokine exposure of the fetus in pregnancies complicated by chorioamnionitis. Chorioamnionitis is an infection of the uterus usually caused by bacteria that can lead to severe inflammation of the mother. The fetus can subsequently become compromised because inflammatory cytokines are either transferred via the placenta or because they induce themselves placental cytokine production and release. Little is known about this
mechanism, but there is convincing evidence that a systemic inflammatory response (FIRS) is detrimental for the developing brain and negatively impacts later neurodevelopmental outcome. In this regard it does not play a role whether inflammation in the infant is induced by a postnatal sepsis or by chorioamnionitis. The long-term aim of our research is to either block transplacental transfer or suppress placental production and release of cytokines into the fetal circulation.

During the last three years we have established the perfusion model at McMaster and we have also created some modifications of the perfusion chamber. We were able to demonstrate viability of the placenta even after 6 hours of perfusion.

In our lab, a masters thesis is actually being performed investigating the response of the placenta to LPS stimulation. LPS (i.e. lipopolysaccharid) is a breakdown product of gram-negative bacteria and as such a strong inducer of the inflammatory cascade. In our experiments we have found that LPS is indeed capable to induce a strong TNF-a response in the placenta. However, we surprisingly detected a significant TNF-a release in control experiments that are supposed to run LPS-free. We have identified bovine serum albumin (BSA), one of the component of the perfusion buffer solution, as the source of contamination. In the meantime we are now able to establish LPS-free perfusions by altering components. However, we have also learned that there is a significant memory effect of the tubes and of the perfusion chamber once they have been flushed with the LPS contaminated buffer. While these findings are important for us to run our experiments with properly defined conditions, they imply on the other hand that most of the published placental perfusion studies using the established BSA source have been run under mild LPS stimulation. We are currently preparing a manuscript summarizing these findings. In order to characterize the experimental setup more in detail we are also actually studying the relationship between different LPS levels and inflammatory response.

Even though our lab is in close proximity to the L&D facilities and we are closely collaborating with Dr. deFrance from MFM/OB, we are still experiencing a shortage in placenta supply at McMaster; this is mainly due to a number of competing studies that need to have access to cord blood, but not to the placenta itself. We have started to address this issue with the research groups involved in this field and will hopefully establish an algorithm that can satisfy the needs of all groups.

In summary, we are expecting that this research will contribute to develop strategies to protect the developing fetus against these substances.
(III) Optimizing nutrition and growth of very low birth weight (VLBW) infants in order to improve neurodevelopmental outcome and minimize the risk for the developmental onset of adult diseases, mainly metabolic, cardiovascular and neurologic ones (DOHaD) (four studies)

(a) It is well established that breast milk provides the best basis for nutrition of preterm babies due to it containing many “good” substances like antibodies, growth factors, etc. However, the amount of macronutrients like proteins, calories, etc. is often not sufficient and therefore, needs to be fortified. Current standard fortification does not correct or take into account the individual variation for the composition of breast milk. We, therefore, developed a concept to individually fortify breast milk on the basis of the measured macronutrient content (IFO study). We have managed to establish the infrastructure and have completed the first pilot trial that shows that this concept is feasible in a clinical environment. Our initial data indicates that it is safe and will improve postnatal growth in these babies. To perform this pilot study, we developed a new method to determine lactose in breast milk using one of our new mass spectrometers. The results of this study have recently been published in an international peer-reviewed journal. In addition, we have established micro-methods to use as little breast milk as possible for lab analysis since it is a very valuable substance for preemies. We are now able to measure all macronutrients using a milk volume of 1.4mL instead of needing 30 to 100mL which was used in prior methods. We have further evaluated commercially available milk analyzers and could show that these devices which have been developed for use in the dairy industry need revisions of their algorithm to obtain valid results for human milk. This study has lead to three publications over the summer with two more to be submitted soon. We also started a double-blind randomized controlled clinical trial in November 2012 for which we have now received funding ($472,000) from the Canadian Institute of Health Research (CIHR). We were ranked #1 of 56 applications in this competition. Also, in the meantime the manuscript of the pilot trial has been accepted by Journal of Pediatrics for publication.
The IFO study goes in line with two other studies in which we investigate further aspects of postnatal growth of preterm infants:

(b) the “PEAPOD study” aims to establish longitudinal body composition reference data for stable preterm infants using bedside methods, such as the PEA POD, a device using air displacement plethysmography. Currently, weight, length and head circumference are used as surrogate parameters to indicate nutritional status and somatic growth of the infant and clinical decisions of nutritional adjustment are usually made on these parameters. However, body composition data with detailed measurements of fat and lean mass is critical as it provides a better indication of infant nutritional status compared to simple anthropometric data alone. There is now growing evidence that an unfavourable body composition will affect adult health and predispose to metabolic, neurological and cardiovascular disease. We therefore feel the need to improve the infants’ neurodevelopment and outcomes – with special respect to understand better metabolic programming which will have an upcoming role in adjusting nutrition to achieve healthy growth patterns in extremely preterm infants. In order to do this, detailed longitudinal reference data of fat and muscle mass is required. We have composed a multi-disciplinary team, including physicians, research fellows, a study coordinator and a Master’s student who are responsible for the organization of this project.

So far we were able to measure postnatal body composition in more than 60 preterm infants on more than 180 occasions down to 30 weeks of gestation. Due to a recent technical development that we have introduced, evaluated and validated we will now be able to include also infants on intravenous support. This will allow us to expand the age range towards less mature infants (target 27 weeks of gestation) as well as perform measurements in preterm infants earlier after birth (target day 1 of life). We are not aware that any research group has done such early measurements before.

Fig. 4: The PEAPOD device (left paneel) and a diagram depicting the data about body composition (lean mass and fat mass) of the first 60 infants included in the study (right paneel)
(c) The GroWTH study aims to establish longitudinal weight data during the first 14 and 21 postnatal days in healthy preterm infants from 31 to 36 weeks of gestation. “Healthy” has been defined by a panel of exclusion criteria such that these infants had only minimal or no intensive care support and thus represent the healthiest 10% of all infants of a given gestational age. The postnatal weight trajectory of these subjects will be plotted into the intrauterine growth charts; and this “new postnatal” percentile will be considered as the “most golden” standard that indicates where preterm infants most probably will have to adjust to after completed postnatal adaptation. These infants shall therefore be taken as the role model of healthy postnatal growth. So far, we have included more than 570 preterm infants in this study, and a total of more than 5500 preterm infants have been screened so far for eligibility. In order to recruit a significant number of subjects for this study we have managed to establish collaborations with other centres: St Joe’s in Hamilton, St. Mike’s in Toronto, Children’s Hospital University of Heidelberg as well Children’s Hospital University of Greifswald. Preliminary results indicate that the infants adjust on average to -0.74 z-scores across all gestational ages and that the “new” postnatal trajectory can be reliably predicted just from gestational age and birth weight (r² of 0.95). In a next step we will validate the model and will also try to expand it to gestational ages below 31 weeks of gestation, ideally down to 24 weeks. With the result of this study we will be able to give clinicians for the first time an answer to the question of to which intrauterine percentile postnatal growth an individual infant should be adjusted to.

Fig 5: Adjustment of body weight to a new postnatal percentile (left paneel) and accuracy of the model (right paneel) to predict the new percentile (error of only +/- 0.03 z-scores)

(d) In the “Lipid Study” we aim to better understand the impact and contribution of parenteral lipid administration on postnatal growth. The two other macronutrients, carbohydrates and proteins, have been extensively studied by the scientific community in the past and major developments have been made and led to advanced commercially available i.v.-solutions. Similar research is missing for lipid emulsions and currently less fat is tolerated by the i.v.-route compared to enteral intake. We
are currently comparing profiles of fatty acids between different commercially available products (Canada and other countries) and we also investigate fatty acid profiles that are achieved under different nutritional regimes (parenteral vs. enteral). We aim to achieve a better tolerance of i.v. lipids emulsion.

*Figure 5. Percent composition of fatty acids in various lipid emulsion products and preterm breast milk*

(IV) Developing and implementing efficient strategies that help premature babies with their postnatal adaptation, but minimize the risk for side effects and iatrogenic complications

This research activity targets to implement and evaluate new clinical strategies that are efficient in supporting preterm infants with their postnatal adaptation, but have the potential to create less iatrogenic harm. We currently investigate non-invasive ventilation strategies like nasal CPAP, nasal intermittent ventilation (NIPPV), nasal high-frequency oscillatory ventilation (nHFOV) as well as non-invasive application of inhaled nitric oxide (iNO) and surfactant (MISURF). Also, the attempt to restrict the use of umbilical catheters falls in this category. This area is quite new for North America, but is much more advanced in Europe and Australia/New Zealand.

The rationale of evaluating these approaches is that there is now strong evidence that these techniques have a strong potential to improve short and long term outcomes of extremely premature infants. Naturally, such approaches require a different level of experience and mindset amongst the NICU staff and the transition towards it will be a longer lasting process. However, the Canadian neonatologists (CNN) have recognized that this might become the future way to go. We have especially identified the perinatal centre in Cologne (GER) as one of the leading centres for gentle resuscitation and care (GentleR) in the world. I have been asked by the EPIQ group of CNN to spearhead this process and start an exchange with McMaster NICU and Cologne. The goal is that the McMaster NICU shall become the CNN training centre so that all perinatal centres across Canada can come and learn from us. We have
therefore created a working group consisting of RT’s, RN’s, NP’s and physicians that will also go to Cologne divided as three delegations. It is of interest to note that this exchange process has started two weeks ago and I have been over in Cologne to facilitate the start of our first delegation.

Apart from this long-term project we are currently performing three clinical studies that investigate new procedures associated with ventilation.

(V) In addition to these studies, we established multiple methods for analyzing new substances of interest. For example: kynurenine (a potential sepsis marker), vitamin D, cortisol in saliva (a marker for stress in neonates) and oxo-8-DG (marker for oxidative stress) and the identification of circulating endothelial cells to study their release when umbilical catheters are introduced. We have begun local (Dr. M. Larche, Dr. D Snider, Dr. J. Jansen, Institute of Pathology, Dr. Macri, Dr. Rosenthal), national (Dr. Debbie O’Connor, Dr. Sheilah Unger, Hospital for Sick Children, Toronto) and international collaboration (Prof. Dr. A Franz, University of Tuebingen (GER); Prof. Dr. M. Heckmann, University of Greifswald (GER); Prof. Dr. J. Poeschl, University of Heidelberg (GER); Prof. Dr. N. Haiden, University of Vienna (AUT)). We have started two clinical studies to characterize the urinary excretion profile of kynurenine and oxo-8-DG in premature infants. We are very confident that we will be able to report the progress of these studies in more detail in the next report.