

Research Portal

Application - Research Tools and Instruments

Identification

Applicant

Family Name: Amsden

First Name: Brian

Middle Names:

Current Position: Professor

Administering Organization

Organization Queen's University

Department/Division Chemical Engineering

Application

Application Title Cell culture suite

Language of the Application English French

Suggested Evaluation Group 1511 Materials and Chemical Engineering

Hours per month to be devoted to the research/activity, or use of equipment or facility 20

Summary of Proposal

Summary

Our research team, consisting of two Chemical engineers (Amsden and Fitzpatrick) and two Mechanical and

Personal information will be stored in the Personal Information Bank for the appropriate program.

PROTECTED B WHEN COMPLETED

Materials Engineers (Ploeg and Rainbow), are requesting funds to purchase a Cytation 1 hybrid cell imaging and multi-mode detection system, a level II biological safety cabinet, and an Ibidi perfusion system. This equipment suite is needed to support research into strategies to create cell instructive scaffolds for engineering soft connective tissues such as tendons and ligaments and the nucleus pulposus of the intervertebral disc, and an in vitro model of biomaterial host responses in complex environments. The Cytation 1 is a unique instrument that combines automated digital microscopy imaging and data analysis and conventional microplate analyte detection. This instrument does not exist at Queen's and would provide critical analytical capacity to our team and to the broader Queen's university research endeavour. A level II biological safety cabinet is requested to replace an aging and failing safety cabinet that is a workhorse in the Amsden lab. A dedicated Ibidi perfusion system is urgently needed to support the development of human 3D tissue models of biomaterial host responses as access to similar perfusion systems is currently limited on Queen's campus. The primary users of these requested instruments will be the graduate students, post-doctoral fellows, and undergraduate researchers in the applicants' research groups. We anticipate between 15 to 20 HQP will be trained using this equipment over the next 5 years. The projects these HQP will be working on are designed to train personnel for the emerging Canadian biomedical and biotechnology industries. Moreover, given the versatility of the requested equipment, it is expected to contribute to attracting new HQP to our research programs at Queen's as well as to cultivate new interdisciplinary research programs and collaborations. To hire HQP for these projects, we will implement EDI best practices to ensure our teams are diverse. and we excel at tackling unique research questions. In addition, we will prioritize effective communication, and promote the value of equity in teamwork. These practices will ensure that we maintain our group diversity and provide opportunities to all qualified candidates.

Second Official Language Translation

Proposed Expenditures

	Year 1 Amount
Equipment or facility	
Purchase or rental	\$143,285
Subtotal	\$143,285
Other (specify)	
	\$0
Subtotal	\$0
TOTAL PROPOSED EXPENDITURES	\$143,285
Total Cash Contribution from Industry (if applicable)	\$0
Total Cash Contribution from University (if applicable)	\$0
Total Cash Contribution from Other Sources (if applicable)	\$0
TOTAL AMOUNT REQUESTED FROM NSERC	\$143,285

Activity Details

Certification Requirements

Does the proposed research involve humans as research participants?

Yes No

Does the proposed research involve animals?

Yes No

Does the proposed research involve human pluripotent stem cells?

Yes No

Does the proposed research involve hazardous substances?

Yes No

Impact Assessment

Will any phase of the proposed research take place outdoors?

Yes No

Research Subject Codes

The research programs to be supported. We are requesting an equipment suite that includes a Cytation 1 hybrid live cell imaging and multi-mode detection system, a level II biological safety cabinet (BSC), and a Fluigent perfusion system. This equipment will directly support the research programs of 5 investigators: **Brian Amsden, Lindsay Fitzpatrick, and Laura Wells** (Chemical Eng.), and **Heidi Ploeg and Roshni Rainbow** (Mechanical and Materials Eng.). These investigators will be working collaboratively in various teams in the biomedical engineering programs described below.

Cell instructive scaffolds for soft connective tissue regeneration. Injury to, and degeneration of, soft connective tissues represent a significant and growing problem with regards to healthcare costs to the population at large and quality of life for those afflicted. The treatment of these tissues is technically challenging as they have a low inherent healing capacity. Moreover, current surgical approaches are associated with high direct costs. The financial burden of orthopaedic care for the aging population has been called one of the greatest Canadian healthcare challenges of the 21st century. Oftentimes afflicted patients require donor tissue to replace damaged or non-functional tissue. With the demand for replacement tissues far exceeding donor supply, tissue engineering and regenerative medicine have emerged as promising treatment approaches. Within this context, we are focussing on target tissues of significant clinical need: the nucleus pulposus of the intervertebral disc, ligaments, and tendons. ***Nucleus pulposus regeneration (Amsden, Rainbow):*** Degeneration of the intervertebral disc is a common source of chronic pain and deterioration of quality of life to people over the age of 50. Current surgical treatments provide only temporary relief in most cases and cannot repair the damaged disc. While degeneration occurs in all regions of the disc, the most common location of degeneration is in the gel-like central region that provides the shock absorbing capacity of the spine, called the nucleus pulposus. We propose to treat this condition by removing the degenerating extracellular matrix and nucleus pulposus cells (NPs) and replacing with NPs generated from the *ex vivo* differentiation of mesenchymal stem cells (MSCs), delivered within an *in situ* gelling vehicle. The Amsden lab has been developing biodegradable, *in situ* setting hydrogels designed to be mechanically resilient and cell delivery supportive and will collaborate with the Rainbow lab to develop dynamically loaded bioreactor conditions to pre-differentiate MSCs to NP cells. ***Scaffolds mimicking the microarchitecture and biomechanics of tendons and ligaments (Amsden, Ploeg):*** The most frequently injured ligament is the anterior cruciate ligament of the knee (ACL). The ACL does not heal when ruptured, and if left untreated, this condition may lead to chondral and meniscal injury, and early osteoarthritis. Therefore, surgical reconstruction of the ACL is often required. Approximately 100,000-200,000 North Americans annually require reconstructive ACL surgery. The most frequently injured tendon is the Achilles tendon (AT). Due to its poor healing capacity, the tissue formed around a ruptured AT when healed is predominantly scar tissue that is mechanically inferior to native tissue. As a result, surgical intervention is often required using predominantly autografts, with an estimated 1.2 to 7 million graft procedures performed annually worldwide. However, the use of autografts suffers from the need to damage one tissue to heal another, donor site morbidity, and ultimately diminished mechanical tissue properties. Ligament and tendon tissue engineering strategies are being examined by Amsden and Ploeg to overcome these problems. Novel, biodegradable polymers are being developed for the creation of biomimetic AT and ACL scaffolds. These scaffolds will possess the mechanical, physical, and biological properties necessary to support the formation of tissue possessing biochemical and mechanical properties comparable to native tissue. In the ACL project, biodegradable microfibrillar polymer scaffolds are being developed that possess a similar microarchitecture as the crimped collagen fibres in ligaments. Given the challenges with obtaining sufficient ACL fibroblasts to populate these scaffolds, we are currently investigating the use of covalently attached decellularized ECM on MSC differentiation towards a ligament fibroblast phenotype. In the AT project, given the recent finding that the AT exhibits auxetic behaviour when stretched, we are creating an auxetic scaffold utilizing an additive manufacturing technique called melt electrowriting (MEW). In MEW a polymer melt is extruded through an electrically charged needle and collected on a moving plate as a micron-sized fibre. The fibre pattern is programmable, thus MEW generates microarchitectures with high resolution and

reproducibility. We have already prepared an auxetic scaffold using our custom-built MEW. These scaffolds will be populated with ACL fibroblasts and AT tenocytes, placed within dynamically loaded bioreactors, their mechanical properties assessed, and the biochemical composition of the tissue formed compared to that of native tissue. The polymer development will be done by the Amsden group, mechanical assessments will be done by the Ploeg group, and the bioreactor work will be done jointly.

In vitro models of biomaterial host responses in complex environments. The success or failure of medical devices is critically dependent on the patient's immune response to the biomaterials used in the device construction, and how the immune response may impact material performance. For biomaterials in current clinical applications, adverse inflammatory and fibrotic host responses often limit the lifespan of an implant, and in some cases can cause premature device failure. Furthermore, clinical translation of many emerging medical technologies depends on the development of materials that integrate with host tissue without eliciting adverse inflammatory and fibrotic responses. However, significant challenges in improving host responses to biomaterials are (1) our incomplete understanding of how biomaterial properties (e.g., chemical, topographical, mechanical, etc.) affect the host response at a cellular and molecular level in complex, tissue-specific environments, and (2) how inherited and acquired patient-dependent factors such as sex, age, race and disease profiles may affect the host response in individual patients. **Identifying novel targets for modulating macrophage-material interactions (Fitzpatrick, Wells, Amsden):** To address the first problem, the Fitzpatrick lab has developed 2D in vitro mouse models of biomaterial host responses that model the acute and chronic phase of foreign body reactions, from tissue damage and protein deposition at the time of implant, to the acute macrophage-material interactions that initiate inflammation, to downstream inflammatory signaling that induces fibroblast recruitment and myofibroblast differentiation. These 2D mouse models of biomaterial host responses are currently used for investigating the role of damage-associated molecular patterns (DAMPs) and Toll-like receptor (TLR) signaling in macrophage response to insulin infusion cannulas and evaluating candidate TLR inhibitors to modulate the host response. Critical aspects in rapidly advancing these models requires the ability to image and phenotype macrophage populations, perform proliferation, migration and chemotaxis assays that would be provided by the Cytation 1. The Fitzpatrick lab will work in collaboration with the Wells and Amsden labs to develop biomaterial-based strategies to modulate the immune response to implanted materials, for example through surface modification with immunomodulatory factors of interest (i.e., agonist or inhibitors of TLR or other host defence signalling pathways). **Modelling human biomaterial host responses in complex environments (Fitzpatrick, Wells, Amsden):** Insulin pump therapy is a promising treatment for patients with Type 1 Diabetes (T1D). However, short wear times (2-3 days) and high failure rates of the insulin infusion system and the risks of unexplained hyperglycaemia are a significant burden for insulin pump users. Although the inflammatory response to the insulin infusion system (IIS) cannulas has been suggested as an underlying contributor to poor reliability of IIS, there is little research on biomaterial host responses in adipose (fat) tissue that consider the high insulin concentrations that result due to local insulin delivery and the adipose and immune cell dysfunction that are associated with T1D. The Fitzpatrick lab is developing a 3D adipose tissue model to study the effect of supraphysiological insulin concentrations, hyperglycemia and the crosstalk between adipocytes (fat cells), macrophages (innate immune cells) and fibroblasts on the inflammatory response to model insulin infusion cannulas. The model tissues will be constructed using a modular tissue engineering approach and maintained in perfusion culture with model cannulas for up to 3 weeks to study key aspects of biomaterial host responses in this tissue-specific environment. As the research program develops, this platform will be used to investigate the impact of identity factors, such as sex, age, race and/or disease profiles on key aspects of biomaterial host responses.

The requested Cytation 1 will be instrumental for these projects by allowing us to simultaneously image and analyse live cell interactions with materials while undertaking gene, and protein assays to determine the efficacy of our strategies. The biological safety cabinet will be routinely used for cell culture to support

this work. The Fluigent perfusion system is required for maintaining long-term 3D tissue cultures.

The equipment requested and the reasons for the configuration chosen. *Cytation 1*: The Cytation 1 is a cell imaging multi-mode microplate reader that combines automated digital microscopy imaging and data analysis, including statistics, and conventional microplate analyte detection. This unique combination provides a cost-effective means of simultaneously obtaining phenotypic cellular information as well as quantitative data on the same cell populations in real time. It can be used for a wide range of applications other than cell imaging and analysis, including cell proliferation studies, protein expression, biomarker quantification, and nucleic acid and protein quantification assays. Importantly, these assays can be done on multiple wells and in multiple well plates in an automated fashion thereby increasing workflow and student time efficiency while minimizing measurement error. BioTek is the only manufacturer of this unique hybrid imaging and analytical system and holds its patent rights. *BSC*: A Class II BSC has been requested to support the cell studies to be undertaken with the Cytation 1. Quotes were obtained from Fisher Scientific and VWR for a Class II BSC. Both quoted BSCs had similar features; however, the Fisher Scientific BSC was the least expensive. *Fluigent Perfusion System*: The Fluigent perfusion system is a high quality, modular, microfluidics pressure controller with two recirculating perfusion loops. It is compatible with cell culture incubators and can support long term experiments because it is a closed system. This pump system was selected for its versatility in the range of flow rates, shear stresses and reservoir volumes it can accommodate, its ability to maintain two independent, recirculating laminar, flow paths, and the long-term culture within an incubator that it can provide. Also, it is a modular system (4 independent pressure controller) and can be operated with or without a computer, adding to its versatility.

The need, urgency, and impact of a delay. Currently there are 10 HQP in the Amsden lab, 2 HQP in the Rainbow lab, 12 HQP in the Ploeg lab, 4 HQP in the Wells lab and 3 HQP in the Fitzpatrick lab working on projects reliant on this equipment. The requested equipment will be essential in moving these research projects forward. The Cytation 1 will replace an obsolete and nonfunctional microplate reader in the Amsden lab providing much needed microplate assay capacity. Moreover, characterizing the temporal cell response to environmental conditions designed to mimic normal physiologic conditions is key to the generation of de novo constructs possessing the desired biochemical composition and mechanical properties of the tissue they are meant to replace, and for assessing the dynamic cell-cell and cell-material interactions in *in vitro* models of host responses. Capturing this information using our currently available equipment can only be accomplished through individual time point sample analysis involving separate staining steps and individual sample imaging followed by image analysis and then separate data analysis. These tedious and time-consuming steps are combined in the Cytation 1 leading to a reduction in the number of samples needed to obtain the same data set, resulting in savings in both HQP operating time and material costs while minimizing the possibility for error. The requested BSC will replace a 15-year-old Thermo-Fisher BSC in the Amsden lab that is no longer supported by Fisher Scientific and for which replacement parts are no longer available. We have already had to replace the blower motor two years ago from a third party whose motor did not match the required specifications. It is not cost-effective or reliable to try to continue to replace components of this BSC as it ages. The Fitzpatrick lab does not have a perfusion system and requires a dedicated pump system to maintain perfusion cultures within the cell culture incubator over extended times. With 3 HQP relying on a perfusion system to maintain viable 3D tissue models, this piece of equipment is urgently needed to advance the development of the human 3D tissue models of biomaterial host responses. Delaying the acquisition of the perfusion system will also significantly impact Fitzpatrick productivity and tenure application.

The availability of similar equipment locally. The Cytation 1 is unique and no such instrument, or its equivalent, currently exists at Queen's University or within the greater Kingston area. More importantly, the automated image processing and analysis feature does not exist at Queen's and no other equipment provides the ability to obtain both cell images and quantitative analysis on the same live cell populations. Other BSCs exist across campus but are in high demand and heavily used as BSCs are standard and

necessary cell culture equipment required by all labs performing cell culture work. Having a BSC dedicated to these projects would allow the HQP to complete projects in a timely fashion with minimal impact to their progress. Similarly, there are other perfusion systems on campus, but these are all heavily used by the labs that perform microfluidics and perfusion culture. Furthermore, the cell source for adipose modules is unpredictable, as it depends on the timing of elective surgeries and the availability of human fat samples. This, coupled with the extended duration of perfusion culture experiments (up to 3-4 weeks for adipose tissue), would make securing access to an existing perfusion system used for mammalian culture impossible. Therefore, a dedicated perfusion system is essential to ensure HQP can complete their experiments without delays.

The accessibility and degree of utilization of the requested equipment. The equipment will be housed in lab space on the fourth floor of the Biosciences Complex. The equipment will be accessible to all researchers within the Amsden, Fitzpatrick, Ploeg, Rainbow and Wells groups while researchers within the Amsden lab will manage the equipment. The equipment will be made widely accessible to other researchers at Queen's and within industry. It is expected that the requested equipment will be in high use, given the number of projects reliant on it. Given the HQP in the research groups, we anticipate average use per week being 20 h of the Cytation 1 and BSC. The perfusion system is anticipated to be in almost constant use, with 3 HQP performing long-term experiments on an on-going basis.

How the proposed equipment will be used for training. The proposed program is highly relevant for training HQP in the field of biomedical engineering, biomaterials, and bioengineering. The projects of the proposed program are inherently interdisciplinary, requiring expertise at the intersection of biomechanics, materials development, cell biology, and surgical application. The primary users of the requested equipment will be the graduate students, post-doctoral fellows, and undergraduate researchers in the applicants' research groups. We anticipate between 25-30 HQP will be trained using this equipment over the next 5 years. The projects these HQP will be working on are designed to train personnel for the emerging Canadian biomedical and biotechnology industries. The applicants have an excellent track record of HQP entering the Canadian biomedical engineering workforce. HQP will receive expert technical training and interdisciplinary experience in polymer biomaterials design, cell-material interactions, biomechanics, tissue engineering and regenerative medicine. This research will also expose undergraduate summer research students to the tissue engineering and regenerative medicine field with the aim of demonstrating to these students the importance and advantages of pursuing graduate studies in this area. Finally, because the requested equipment is versatile and not available elsewhere, it is expected to contribute to attracting new HQP to our research programs at Queen's as well as to cultivate new interdisciplinary research programs and collaborations. The requested equipment would also provide a rich environment for training where trainees from different backgrounds interact with each other. **EDI considerations:** In biomedical engineering, a notable challenge is that only 26% of doctoral degrees have been awarded to women (<https://engineerscanada.ca/> 2020). The applicants implement EDI best practices when hiring team members. As a result, our teams are diverse, and we excel at tackling unique research questions. As leaders, the applicants prioritize effective communication, and promote the value of equity in teamwork. These practices will continue to ensure that our research groups maintain their diversity and provide opportunities to all qualified candidates. At Queen's, one challenge for faculty is to become aware of potential barriers in the lab that affect inclusivity. Such barriers include equipment access and appropriate training on equipment. To remove these barriers, all rooms to equipment have a standard process to provide unbiased access. We have created an online digital lab protocol folder. All incoming graduate students receive training on equipment necessary for their projects from senior HQP and are required to demonstrate competency before being granted access to the equipment. We encourage all HQP to ask questions and ensure that answers are prompt, complete, and polite. To foster a community of equity and inclusion, current and incoming graduate students will participate in EDI Workshops offered through Queen's Expanding Horizons Office.

Budget Justification

This proposal requests funds to purchase a suite of equipment to be used in the development of novel tissue engineering and regenerative medicine strategies and to assess host responses to implanted biomaterials. The specific equipment to be purchased includes a BioTek Cytation 1 combined live cell imaging and analysis multimode plate reader system, a Thermo Scientific Class II biological safety cabinet (BSC) and a Fluigent microfluidic flow control system with 2 recirculation loops. Quotes for the requested Cytation 1 and BSC were received in Canadian dollars, while the Fluigent system was received in USD and converted to Canadian dollars using an exchange rate of 1.2379.

BioTek owns patent protection on the Cytation 1 and it is the only instrument that combines automated digital microscopy and multi-mode analyte detection in a single system that includes image processing and image analysis software for automated analyses for live cell assays. As such, quotes for competing equipment are not available.

Quotes were requested for a Class II BSC from Thermo Fisher and VWR. For comparable specifications and features, the Thermo Scientific BSC was the most cost effective, providing the largest operator space (60-inch opening) for the price. VWR quoted a Labconco 48-inch BSC for \$15,816 and an even more expensive 72-inch BSC.

Three quotes were solicited for a pressure driven pump system capable of supporting extended recirculating perfusion cultures. Both the ElveFlow OB1 flow control system and Fluigent Flow-EZ control system produce four independently controlled flows and can be combined with the recirculating valves to have two independent recirculating flow paths. An added benefit of the Fluigent Flow-EZ system is that it is modular, where the four control modules can be combined and operated as a single unit or operated individually with or without a computer, adding significant versatility to the system. In contrast, the Ibidi perfusion system is a single port pressure pump that can produce up to 4 identical recirculation flow pathways in parallel. The Ibidi system was also slightly more expensive than both the Elveflow system (21,879 €; \$31,512 CAD), and the Fluigent system (\$22,685 USD; \$28,081 CAD), which was most cost-effective option. Therefore, the Fluigent system was selected for its ability to produce two independent recirculation programs, the modular construction and lowest quote.

Item	Quantity	Cost/unit in original currency	Exchange Rate	Total Cost in CAD
BioTek Cytation 1	1	\$99,762.35 CAD		\$99,762
Thermo Scientific Class II 60-inch BSC	1	\$10,716.20 CAD		\$10,716
Fluigent Pressure Control	1	27,599 USD	1.2379	\$28,081
Subtotal:				\$138,560
Institutional tax rate (%):				3.41
Total tax:				\$4,725
Total cost:				\$143,285
Total confirmed from other source(s):				
Total requested from NSERC:				\$143,285

Relationship to other research support

There is no budgetary overlap between this proposal and other research support held or applied for, although the requested equipment will be crucial for the successful completion of the projects below.

Amsden Funding

CONNECT!, NSERC Create Program in Soft Connective Tissue Regeneration/Therapy, NSERC CREATE Program. Dr. Amsden, PI, with Dr. Bryant (Queen's), Drs. Flynn, Gillies, Seguin and Beier (Western), Dr. Simmons (U Toronto), and Drs. Herzog and Shrive (Calgary). \$1,650,000 for 6 years, 25%, 2015-2022, 1-year COVID extension plus additional \$30,000. This is a training grant supporting collaborative research teams developing new methods for treating or regenerating damaged soft connective tissue. This funding is solely for HQP salary, training, and travel support. There is no budgetary or conceptual overlap.

Liquid degradable aliphatic polycarbonate as an injectable drug delivery vehicle, NSERC Idea to Innovation Grant \$125,000, 2019-2021, 1-year COVID extension. This funding supports development of an injectable polymer for non-opioid drug delivery for chronic pain. The funding is for salary and operating budget only. As such, there is no conceptual or budgetary overlap.

Aliphatic polycarbonates: building blocks for new biodegradable biomaterials, NSERC Discovery Grant, \$64,000/yr, 2020-2025, plus additional \$10,000 COVID supplement in 2020. Projects supported by this funding include development of an ocular delivery system to treat optical neuropathy based on photodegradable aliphatic polycarbonates, development of an injectable polymer vehicle for peptide delivery, and generation of tendon and ligament tissue engineering scaffolds based on photo-cross-linkable aliphatic polycarbonates. This funding is for salary and operating budget support only.

Local pro-angiogenic peptide delivery for treating critical limb ischemia, CIHR Project Grant, \$120,488/yr, 2021-2025. This funding supports the development of a peptide delivery strategy to treat critical limb ischemia. This funding is for salary and operating budget support only.

Donald and Joan McGeachy Chair in Biomedical Engineering, \$30,000/yr, 2021-2026. This funding is being used to support HQP salaries only.

Ploeg Funding

Bone Care Science, Queen's University Catalyst Fund, \$25,000 Sept 2021-Sept 2023. The primary goal of the proposed research is to push the frontier of Bone Care Science to a new level by using a unique approach that combines stimulation, big data analysis, and computer modelling for the long-term responses of human trabecular bone. All the budget supports HQP, there is no funding overlap with this RTI grant application.

Investigation of the Mechanics of Dental Implant Primary Stability, Extension NobelBiocare, Switzerland, \$93,800+ in kind, Sept 2019 - Dec 2021. The purpose of this study is to develop a stepwise understanding of the mechanics of dental implant anchorage and fixation using parallel mechanical tests and finite element analyses. The budget supports HQP and supplies, there is no funding overlap with this RTI grant application.

Chair for Women in Engineering, Faculty of Engineering and Applied Science, Queen's University, 2020-2025. \$125,000. There is no funding overlap with this RTI grant application.

Patient-Specific Bone and Joint Health Technologies, Canadian Foundation for Innovation John R Evans Leaders Fund (CFI-JELF) \$500,000 2019-2025. Infrastructure Operative Fund (IOF) \$60,000. R. Rainbow Co-PI. A modular, experimental apparatus to test how muscles, cartilage and bone may promote each other's growth and adaptation is being developed. There is no funding overlap with this RTI grant application.

Cell culture Suite

Amsden, B.G.

Bone Integrity for a Lifetime through Mechanical Loading, NSERC Discovery Grant, \$230,000, and Discovery Accelerator Supplement \$120,000, 2019-2024. The short-term objectives of this program are to quantify and simulate the response of trabecular bone cores and bone implant systems to strain rate, load and hormones with the objective to predict the response of bone and bone implant systems to stimuli. The DAS Program provides substantial and timely resources to researchers who have an established, superior research program that is highly rated in terms of originality and innovation, and who show strong potential to become international leaders within their field. The budget supports HQP, there is no funding overlap with this RTI grant application.

Queen's University Research Initiation Grant, \$70,000 2018-2023. This funding is being used to purchase fundamental lab supplies and support HQP. There is no funding overlap with this RTI grant application.

Fitzpatrick Funding

MyD88-dependent modulation of host response to insulin infusion cannulas, CIHR Project Grant, \$110,925/yr, 2019-2023 plus a \$11,589 COVID-19 Wage Supplement (2020). This funding supports the investigation of MyD88 as a critical modulator of the host response to insulin infusion cannulas. This funding is for salary and operating budget support only. There is no funding overlap with this RTI grant application.

Rapid biomaterial-screening in zebrafish. NSERC Discovery Grant, \$30,000/yr, 2015-2022, including three additional installments of \$30,000 for 2 maternity leaves (2017, 2019) and 1 Early Career Researcher DG grant extension (2021), plus a \$4,800 COVID Supplement in 2020. Projects supported by this funding include development of models for biomaterial screening, and are currently being used to develop the 3D tissue perfusion models proposed in this grant. This funding is for salary and operating budget support only. There is no funding overlap with this RTI grant application.

Rainbow Funding

Moving beyond bone mineral density: Computer models to help diagnose bone fragility Wicked Ideas, Queen's University. Drs. Rainbow and Beland (PIs), \$75,000, 2021-2022. Project supported by this funding involves the adaptation of the Lattice Element Method, a numerical computer simulation scheme, to predict bone fragility and fractures. The funding is for salary and operating budget support. There is no funding overlap with this RTI grant application.

Mechanochemical regulation of muscle on developing cartilage. NSERC Discovery Grant, \$28,000/yr, 2019-2024 plus ECR Discovery Grants Launch Supplement, \$12,000 in 2019. Projects supported by this funding include developing of *in vitro* platforms for studying neighbouring tissue morphogenesis and establishing strategies that integrate developmental mechanisms to engineer functional, load-bearing tissues. The funding is for salary and operating budget support. There is no funding overlap with this RTI grant application.

Queen's University Research Initiation Grant, \$40,000 2018-2024. This funding is being used to purchase fundamental lab supplies and support HQP. There is no funding overlap with this RTI grant application.