Fondazione Ri.MED

SCIENTIFIC REPORT 2016

DRUG DISCOVERY

TISSUE ENGINEERING AND BIOMEDICAL DEVICES

REGENERATIVE MEDICINE AND BIOLOGICS

ENG

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Presidenza del Consiglio dei Ministri

Regione Siciliana

Consiglio Nazionale delle Ricerche

University of Pittsburgh

UPMC LIFE CHANGING MEDICINE

The Ri.MED Foundation was founded in 2006 by the Presidency of the Italian Council of Ministers as an international private-public partnership. Our mission is to develop biotechnological and biomedical translational research approaches, disseminate scientific knowledge and train highly qualified professionals in the Life Science sector.

Translational research is focused on the complementary integration of resources and skills of different matrices: basic research, preclinical research and development of new therapies, medical devices and biomarkers, and finally clinical trial. It is thanks to the nature of the founding partners, especially the Italian National Research Council (CNR), the University of Pittsburgh and the University of Pittsburgh Medical Centre (UPMC), that the Foundation has been able over the years to develop truly translational research programs.

Thanks to the strategic partnership with the IRCCS ISMETT, Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione, it has been possible to foster multidisciplinary projects in the field of regenerative medicine and cellular therapies including the creation of a GMP cell factory to support clinical trial. With the coexistence of premises and staff, the goal of the Foundation is to facilitate the know-how between physicians and researchers, from bench to bedside.

The research in Ri.MED follows three main approaches: tissue engineering and more in general bio-engineering, drug discovery and vaccine development, and finally regenerative medicine. The focus of the three approaches is on therapeutic areas such as oncology, neuroscience, cardiovascular and metabolic diseases.

Over the years, the Foundation has pioneered a real culture on alliances that can deliver mutual benefits operating at regional, national and international level with both public and private research institutions.

And it is thanks to the ability to put together interdisciplinary expertise and build a critical mass, with the guide of a strong management leadership, that the Foundation has today a balanced and diversified projects portfolio, sustained by intellectual property and a high level of scientific papers in peer review journals with relevant impact factor.

In this report, it is described a scientific summary report for 2016 with an outlook for 2017. These results have been achieved thanks to the contribution of our research scientists and in general of the Ri.MED people, the real added value of Ri.MED Foundation. The passion of our team is dedicated every day to help improving patients' life, to train new generations of scientists and professionals and to have a socio-economic impact in Sicily and southern Italy.

Alessandro Padova
Director General

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DRUG DISCOVERY

The researchers from the Ri.MED Foundation are committed to understanding the mechanisms that lie behind incurable diseases. Approaches in genomics, proteomics and secretomics have led to the functional validation of new therapeutic targets for neurodegenerative diseases such as, for example, Parkinsons disease or tumours.
These projects are currently in the so-called drug discovery phase.
Thanks to an integrated platform of structural biology, bioinformatics and drug design, development has begun on a collection of hundreds of thousands of synthetic and natural molecules that can be used as a toolkit for the development of new medicines and adjuvants for cellular therapy. The process provides for the optimisation of biologically active molecules through medicinal chemistry and then pre-clinical experimentation, the study of effectiveness through a platform of molecular imaging and the characterisation of the pharmacokinetic and toxicological profile suitable for experimentation on patients. At the same time, development is under way for predictive methods for the monitoring of potential medicines and for the stratification of patients responding to therapy.

Therapeutic targeting of the hypoglycosylated MUC1/CIN85 complex that promotes tumor growth, invasion and metastasis

Project Leader	**Sandra Cascio, PhD**
Brief description	Metastatic spread of cancer is responsible for most cancer deaths. Critical steps in cancer cells leaving a solid tumor are the loss of epithelial polarity and acquisition of migratory and invasive capabilities. MUC1 is a transmembrane glycoprotein over-expressed and abnormally glycosylated in most epithelial cancer cells, which correlates with an aggressive metastatic phenotype.
Results achieved in 2016	We identified CIN85 (Cbl-interacting protein 85 KDa) as a key binding partner of MUC1 in tumor cells. Moreover, we found that co-localization of MUC1 and CIN85 on invadopodia-like structures enhances invasion and migration of cancer cells. MUC1/CIN85 complex is found in early as well as advanced clinical stages of breast, ovarian, colon and prostate cancer among others. Thus, our hypothesis is that preventing the formation or dissociating existing CIN85/MUC1 complexes may result in a less aggressive tumor by decreasing local invasion, and preventing distant metastases. To test this hypothesis, we have identified and tested two novel MUC1 analog drug compounds that at 10 uM significantly reduced the association between MUC1 and CIN85 and drastically decreased the migratory activity of mouse and human epithelial cancer cells.
Goals for 2017	**Aim 1.** To understand the biological significance of MUC1 and CIN85 in ovarian cancer cells. We hypothesize that the interaction between MUC1 and CIN85 is dependent on the glycosylation state of the MUC1 extracellular domain. Aberrant glycosylation of MUC1 in tumors enhances the association between MUC1 and CIN85. We propose to investigate the MUC1/CIN85-dependent signaling pathway with a particular focus on the ability of CIN85 to modulate MUC1 glycosylation by controlling its plasma membrane-Golgi trafficking. Moreover, we plan to identify other molecules involved in invasion and metastasis induced by MUC1/CIN85 complex by performing a microarray analysis of metastasis-associated gene expression profile.

Aim 2. To test *in vivo* efficacy of two drugs that have already shown ability to reduce MUC1/CIN85 complexes *in vitro*. Epithelial tumor cell lines that are equally tumorigenic, but display different metastatic potentials ranging from non-metastatic to highly-metastatic, will be intraperitoneally injected in mice. At various time points, mice will be injected with the drugs and tumor growth and metastasis will be measured. Tumors will also be removed and evaluated for levels of expression of MUC1/CIN85 complexes.

Aim 3. To generate additional compounds and test their anti-migratory activity *in vitro* and *in vivo*. We will select more specific and efficient small molecules that will target the interaction of MUC1 with CIN85 at uM concentration. To better design and select new molecules, we will also study the specific binding sites of MUC1 and CIN85 involved in the interaction. To assess that, we will perform protein/peptide binding assay. Specifically, we will use recombinant protein of CIN85 and MUC1 derived biotinylated peptides. Binding will be detected by using streptavidin-bound beads.

Hypoglycosylated MUC1 serves as a link between inflammation and cancer

Project Leader	**Sandra Cascio, PhD**
Brief description	The link between inflammation and cancer has been well established. MUC1 is a transmembrane glycoprotein expressed in its abnormal hypogycosylated form on human adenocarcinomas as well as in chronic inflammatory conditions. Aberrant glycosylation of MUC1 in cancer cells alters its normal function and affects the behavior of cancer cells. The MUC1 extracellular domain also participates in activation of the NF-kB family members such as phospho-IkBa and phospho-p65.
Results achieved in 2016	Our data showed that the tumor form of MUC1, in association with p65, regulates the transcriptional activity of pro-inflammatory cytokines, including IL-6 and TNF-alpha, by binding to their promoter regions in both mouse and human cancer cells. We explored the role of MUC1/p65-regulated transcription of IL-6 and TNF-alpha in a human MUC1 transgenic mouse model of colitis-associated cancer. Human MUC1 transgenic mice showed a significant increase in intestinal inflammation and tumor incidence compared to WT mice. We found that hypoglycosylated form of MUC1 upregulated the expression of NF-κB-target pro-inflammatory cytokines in intestinal epithelial cells. Tumor form of MUC1 modulated the expression of histone methyltransferase Enhancer of Zeste protein-2 (EzH2) and its interaction with TNF-alpha and IL-6 promoters. Our

data also suggested that the function of EZH2 is independent of its histone methyltransferase activity on lysine 27 of histone 3 (H3K27).
Additional analysis of histone post-translational modifications revealed that a crosstalk of epigenetic modifications regulates IL-6 and TNF-α gene expression.
Next, in order to understand the significance of MUC1/p65-modulated cytokines expression in the tumor microenvironment, we analyzed infiltration of inflammatory cells.
Tumor-associated macrophages (TAMs) are key players in inflammation and cancer and they are a major source of cytokines. Our preliminary results indicated that the presence of human MUC1 induces the recruitment of TAM cells in mouse colonic tissue during colitis-associated carcinoma.

Meetings and Publications

Presentations at international conferences during 2016

- Society for Glycosylation, New Orleans, LA, USA 2016, November 19-21 (Poster Presentation)
- AAI Annual Meeting, Seattle, WA, USA 2016, May 12-17 (Invited Speaker)

Pubblications during 2016

- Intra- and Extra-Cellular Events Related to Altered Glycosylation of MUC1 Promote Chronic Inflammation, Tumor Progression, Invasion, and Metastasis. Cascio S, Finn OJ.Biomolecules. 2016 Oct 13;6(4). pii: E39. Review. (No impact factor available yet)

Other Activities:
Guest editor for a Special Issue on tumor microenvironment and immune cells for Cellular Immunology IF: 2.4

Goals for 2017

Aim 1: To investigate whether aberrant glycosylation of MUC1 is involved in macrophage M1/M2 polarization. Experiments will be performed by co-culturing epithelial cancer cells with macrophages freshly isolated from human blood. We also analyze whether polarized macrophages can affect the glycosylation status of MUC1.

Aim 2: Hypoglycosyalted MUC1 is overexpressed in chronic inflammation including in inflamed colon tissues. In collaboration with Dr. Al Hashash (Department of Gastroenterology, UPMC), I will analyze the expression of EzH2 and other histone modification factors in human samples of Inflammatory Bowel Disease (IBD).
The expression of epigenetic factors will be correlated with hypoglycosylated MUC1. In addition, to discover new molecular target that promotes inflammation in IBD patients, we will conduct microarray analysis on normal and inflamed human colon tissues of IBD patients.

Grants for 2017

1. 2016-present Ovarian Cancer SPORE-RPCI-UPCI Career Enhancement Program (CEP) Award $40.000/year
2. Ovarian Spore CEP award extension (Submission data May 2017). $40.000/year
3. Establish a new collaboration with GlaxoSmithKline company. GSK group will eventually develop and provide new drug compounds to be tested *in vitro* and *in vivo* (See Project 1 illustrated above).
4. In collaboration with the Gastroenterology team, University of Pittsburgh, we have established a new IRB protocol that will allow us to work on human samples. Ongoing experiments will provide preliminary data for a new collaborative NIH grant application to be sent around June 2017 as Principle Investigator
5. Based on new results from Project 1, a new grant will be sent around September, 2017

Planned pubblications in 2017

- A critical role of EzH2 in the MUC1-mediated induction of pro-inflammatory cytokines in colitis-associated tumorigenesis. S Cascio, J Faylo, J Sciurba, J Xue, S Ranganathan, J Lohmueller, P Beatty and O J. Finn. Manuscript will be submitted to Cancer Cell. IF:28
- Molecular aspects of Il-13 up-regulation by CD8+ T cells from SSC patients. S Cascio, A Moss, M Jessup, C Milkareck and P Fuschiotti. Submission to JEM IF:14.7

Generation and validation of a zebrafish model of inherited cerebellar ataxia: the study of CAMTA1

Project Leader	**Chiara Cianciolo Cosentino, PhD**
Brief description	Molecular basis of hereditary ataxia caused by CAMTA1 mutations and potential development of new therapeutic strategies for the treatment of hereditary ataxias
Impact	These research efforts will advance our knowledge on the molecular mechanism leading to the cerebellar neuron degeneration responsible for the ataxic phenotype. The ultimate goal of this research is the development of new therapeutic strategies for ataxia and other neurodegenerative diseases
Results achieved in 2016	• knock-down experiments with camta1a antisense morpholino • Camta1a CRISPR/Cas9 mutant (camta1a-/-) fish generation • Camta1a-/- fish screening, morphological, functional and behavioral analyses • Cerebellar neurons isolation, FACS sorting and transcriptome analyses (in mice)

Goals for 2017

Identification and characterization of camta1 transcriptionally regulated genes

- From FACS results, Identification of camta1 downstream targets
- Identification of molecular alterations caused by camta1 mutation leading to ataxic phenotype

Identification of possible therapeutic strategies

- Calcium imaging *in vivo* in zebrafish cerebellar neurons to detect changes in calcium homeostasis
- Test in zebrafish calcium blockers as possible therapeutic strategy

Modeling macular dystrophies *in vivo* using zebrafish as model organism

Project Leader

Chiara Cianciolo Cosentino, PhD

Brief description

This is a project started in 2015 in collaboration with ROCHE pharmaceuticals. The major goal of the project is to develop new therapies for retinal diseases and in particular for Age Related Macula Degeneration (AMD), a complex, multifactorial disease characterized by the degeneration of photoreceptors and retinal pigment epithelial (RPE) cells.

Impact

Development of new therapies for AMD and retinal diseases, a leading cause of vision loss among people age 50 and older.

Results achieved in 2016

- We searched for zebrafish orthologues of extracellular matrix (ECM) genes associated with different autosomal dominant inherited macular dystrophies: *ctrp5* (Late onset retinal dystrophy), *fibulin3* (Malattia levantinese), *fibulin5* (AMD) and *timp3* (Sorsby fundus dystrophy).
- We performed expression analyses. In parallel, we generated a transgenic line that expresses GFP in the RPE.
- Using this regulatory element we transiently overexpressed our genes of interest in the RPE and analyzed the effects on retinal health

Goals for 2017

- Stable zebrafish lines overexpressing our genes of interest have been generated and will be analyzed for potential effects on retinal morphology
- All the mutations associated with retinal degeneration in human patients have a conserved amino acid in the zebrafish genes. Those corresponding mutations are currently being introduced into expression constructs and will be injected into tg(rpe65a:GFP) zebrafish embryo
- Potential effects on retinal morphology will be analyzed as was already done for the wildtype constructs.

Nups, nephrotic syndrome and zebrafish model

Project Leader	**Chiara Cianciolo Cosentino, PhD**
Brief description	Project started in 2016 in collaboration with a group in the Institut Jacques Monod, Univ. Paris Diderot. The major goal of the project is to study the role of nucleoporins in nephrotic syndrome (NS), using zebrafish as model system.
Impact	Elucidation of unknown molecular mechanisms leading to the development of NS thereby potentially opening new approaches to therapy
Results achieved in 2016	• We searched for zebrafish orthologues of Nup genes potentially involved in NS • We completed expression analyses. In parallel, we performed knock-down experiments with antisense morpholino.
Meetings and Publications	Meetings • 6th International Kidney.CH Symposium, Zurich, Switzerland, 2016 June 01 Publications • Mansouri M, Bellon-Echeverria I, Rizk A, Ehsaei Z, Cianciolo Cosentino C, Silva CS, Xie Y, Boyce FM, Davis MW, Neuhauss SC, Taylor V, Ballmer-Hofer K, Berger I, Berger P. (2016) Highly efficient baculovirus-mediated multigene delivery in primary cells. Nat Commun. 2016 May 4;7:11529. PMID: 27143231. • Chiara Cianciolo Cosentino & Stephan CF Neuhauss. Paradigms for the quantification of behavioral responses in zebrafish. Chapter for the Springer book project "Decoding the Structure and Function of Neural Circuits" (under review).
Goals for 2017	• Morphological analyses of nup morphants • Rescue of the knock down phenotype with full length human nup133 • Functional analyses of the glomerulus and the kidney proximal tubules in nup morphants • Podocyte analyses (TEM)

Role of GABAa receptor and its modulation in pediatric epilepsy

Project Leader

Pierangelo Cifelli MD, PhD

Brief description

Focal cortical dysplasia (FCD) is the most frequent cortical malformation found in pediatric epileptic patients. Seizures in these cortical malformations are thought to occur as a result of altered excitatory/ inhibitory balance.
Thus, an intriguing hypothesis is that an incomplete cellular maturation in FCD tissue and an altered GABAA-R function contributes to epileptogenesis (the processes able to transform a healthy brain in an epileptic one). Inflammatory cytokines as IL-1ß and TNF-a levels are up regulated in FCD brain tissue and contribute to seizure mechanisms in experimental models. In this project, we want to investigate the functional properties of GABAA-Rs and the role played by inflammatory cytokines in surgically resected cortical brain tissues from drug-resistant epileptic pediatric patients.
Our intent is to study the rare FCD patient material under different approaches to establish the proof-of concept evidence that specific inflammatory pathways can be targeted, by means of phytocannabinoids to control seizures or delay/prevent epilepsy development in children.

Impact

The goal of this project is to investigate the mechanisms underlying inflammation-associated hyperexcitability in pediatric epilepsy to verify whether these diseases could be the target of anti-inflammatory drugs and to investigate how phytocannabinoids are able to modulate the GABAergic response under inflammatory condition.

1: This study has been designed to be performed using pediatric epileptic patients (clinical data and surgical tissues) affected by one of the most common form of cortical malformation.
2: Our approaches represent a strong effort towards improving the knowledge of the inflammatory mechanisms in epilepsy. Specifically, i) electrophysiological recordings on pediatric slices are outstanding, given the limited availability of human tissues and the technical difficulties; ii) the use of microtransplantation approach to address whether and how the inflammatory cytokines affect the GABAergic transmission in epilepsy is completely new. Moreover, since the interest in cannabinoids effects on epileptic pediatric patients is increasing, we will clarify their effect directly on specimens obtained from patients. Notably, this study will pave the way to find alternative anti-epileptic drugs with less side effects if compared with classic antiepileptic drugs already present in the market.

Results achieved in 2016

During 2016, the Project Leader had the possibility to improve his knowledge and expertise in the double-voltage clamp technique on xenopus laevii oocytes. Moreover, the collaboration with the department of neurology at the hospital "Policlinico Umberto I" gave him the possibility to perform experiments using samples obtained directly from patients. This interesting approach allow the research group to perform a large number of electrophysiological experiments starting from small amount of tissues. Interestingly, thanks to the collaboration with the "University of Amsterdam" we used human bioptic tissues as age-matched controls.

In the first work published in 2016, thanks to the collaboration with Prof. Maurizio Inghilleri, director of "ALS center" at "Policlinico Umberto I" in Rome, we demonstrated, with a strong translational approach, how the muscles might be a therapeutical target in this disease. Moreover, we demonstrated that the use of the PEA, and endogenous cannabinoid, is able to improve the muscular function of these patients. Specifically, we demonstrated that PEA is able to reduce significantly the desensitization of the muscular acetylcholine (Ach) receptors in ALS patients and that this effect is specific for the adult ε sub-unit containing receptors. For what concern the clinical aspect of this study, the treatment of the patients with PEA induced an improvement of the forced vital capacity (FVC). Our hypothesis is that PEA may potentiate the muscles activity improving their function.

The second work regards a pediatric epileptic syndrome known as Tuberous sclerosis complex (TSC). TSC is a genetic disorder affecting cellular differentiation, proliferation, and migration early in development, resulting in a variety of hamartomatous, lesions that may affect virtually every organ system of the body. A combination of symptoms may include intellectual disability, developmental delay, behavioral problems, skin abnormalities, and lung and kidney disease. However, the most common symptom is the refractory epilepsy caused by highly epileptogenic cortical lesions. In this paper, we investigated whether TSC cortical tissues could retain GABAA and AMPA receptors at early stages of human brain development thus contributing to the generation and recurrence of seizures. Given the limited availability of pediatric human brain specimens, also in this study we used the microtransplantation method of injecting Xenopus oocytes with membranes from TSC cortical tubers and healthy control brain tissues. Moreover, qPCR was performed to investigate the expression of GABAA and AMPA receptor subunits (GABAA α1-5, β3, γ2, δ; GluA1, GluA2) and cation chloride co-transporters NKCC1 and KCC2. The evaluation of nine human cortical brain samples, from 15 gestation weeks to 15years old, showed a progressive shift towards more hyperpolarized GABAA reversal potential (EGABA). This shift was associated with a differential expression of the chloride cotransporters NKCC1 and KCC2. Furthermore, the GluA1/GluA2 mRNA ratio of expression paralleled the development process. On the contrary, in oocytes micro-transplanted with epileptic TSC tuber tissue from seven patients, neither the GABAA reversal potential nor the GluA1/GluA2 expression showed similar developmental changes. Our data indicate for the first time, that in the same

cohort of TSC patients, the pattern of both GABAAR and GluA1/GluA2 functions retains features that are typical of an immature brain. These observations support the potential contribution of altered receptor function to the epileptic disorder of TSC and may suggest novel therapeutic approaches. Furthermore, our findings strengthen the novel hypothesis that other developmental brain diseases can share the same hallmarks of immaturity leading to intractable seizures.

The third paper published in 2016 is a translational study in collaboration with the "Epilepsy Center" at "Policlinico Umberto I" in Rome. In collaboration with the neurologists Dr. Carlo Di Bonaventura and Dr. Annateresa Giallonardo, starting from a clinical observation, we described how the use of a daily small amount of therapeutic cannabis could significantly reduce the number and the severity of seizures and improve the cognitive functions in a sever pharmaco-resistant form of epilepsy (epileptic encephalopathy). This improvement was associated with a high plasma concentration of a cannabinoid, namely cannabidivarine (CBDV). We decided to test in our expression system these molecules, and for the first time we demonstrated that this drug is able to significantly modulate the GABAa receptor function, reducing its desensitization (GABAa run-down). In conclusion, we demonstrated that our patient's electroclinical improvement supports the hypothesis that cannabis could actually represent an effective, well-tolerated antiepileptic drug. Moreover, the experimental data suggest that CBDV may greatly contribute to cannabis anticonvulsant effect through its possible GABAergic action.

Meetings and Publications

Publications

- Alessandra Morano*, Pierangelo Cifelli*, Paolo Nencini, Letizia Antonilli, Jinane Fattouch, Gabriele Ruffolo, Cristina Roseti, Eleonora Aronica, Cristina Limatola, Carlo Di Bonaventura, Eleonora Palma and Anna Teresa Giallonardo. Cannabis in epilepsy: from clinical practice to basic research focusing on the possible role of cannabidivarin. Epilepsia Open. 16 AUG 2016 10:15AM EST | DOI: 10.1002/ epi4.12015 I.F. in calculation

- Ruffolo G, Iyer A, Cifelli P, Roseti C, Mühlebner A, van Scheppingen J, Scholl T, Hainfellner JA, Feucht M, Krsek P, Zamecnik J, Jansen FE, Spliet WG, Limatola C, Aronica E, Palma E. Functional aspects of early brain development are preserved in tuberous sclerosis complex (TSC) epileptogenic lesions. Neurobiology of disease 2016 Nov; 95:93-101. Doi: 10.1016/ I.F.4.86

- Eleonora Palma, Reyes-Ruiz JM, Lopergolo Diego, Roseti Cristina, Bertollini Cristina, Ruffolo Gabriele, Cifelli Pierangelo, Onesti Emanuela, Limatola Cristina, Miledi Ricardo, Inghilleri Maurizio. Acetylcholine receptors from human muscle as pharmacological targets for ALS therapy. Proc Natl Acad Sci U S A. 2016 Feb 29. Pii: 201600251 I.F.9.4

Goals for 2017

For the next year, we will work on the role of GABAa receptors in epileptic syndromes with a special focus on the relationship between gabaergic and endocannabinoid system. We recently started a collaboration with a young and dynamic start-up in Rome (C4T) with a strong expertise in drugs synthesis. After a series of preliminary meetings, we decided to select a number of very promising cannabinoids molecules, in order to test their functional activity on human epileptic samples and on animal models of epilepsy. Moreover, we will test these new drugs focusing on their possible anti-epileptic activity.

Endogenous electrophilic derivatives of omega-3 fatty acids for the treatment of steroid unresponsive chronic inflammatory lung diseases

Project Leader

Chiara Cipollina, PhD

Brief description

Electrophilic derivatives of omega-3 polyunsaturated fatty acids (n-3 PUFAs) are naturally occurring bioactive lipids generated via enzymatic and non-enzymatic oxidation reactions that we have recently discovered. Due to their efficacy in contrasting inflammatory reactions and their endogenous origin, these compounds have attracted growing attention for the development of new therapies for chronic inflammatory disorders. Chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) are progressive lung diseases that fail to respond to steroids and for which no effective therapy exists. This is in part related to the presence of inflammatory events that are not inhibited by corticosteroids, such as activation of the NLRP3 inflammasome. New drugs to cure these chronic conditions are very much required. The proposed study is aimed at testing the hypothesis that (i) inflammasome activation is increased in the lung of IPF and COPD patients and participates to lung inflammation and fibrosis by upregulating IL-1β and IL-18 levels and TGFβ signaling; and (ii) electrophilic n-3 PUFAs contrast inflammasome activation thus providing new therapeutic opportunities to treat these chronic diseases.

Inflammasomes are large multiprotein complexes that control the release of mature IL-1β and IL-18 via caspase-1 proteolytic cleavage. Increasing evidence suggests that inflammasome activation is involved in the progression of several chronic lung diseases, including IPF and COPD. Inflammasomes consist of a pattern-recognition receptor known as NOD-like receptor (NLR), an adaptor molecule (ASC) and caspase-1. A number of NLRs have been discovered, but the best studied still remains NLRP3. The NLRP3 assembles in response to a variety of stimuli including extracellular ATP and microbial products. NLRP3 activation induces caspase-1 auto-processing, which in turn leads to maturation and release of IL-1β and IL-18. Interestingly, both IL-1β and IL-18, associated with neutrophilic inflammation

in COPD, are increased during exacerbation episodes and have a prominent role in cigarette smoke-driven airway inflammation. Furthermore, high levels of IL-1β are associated with increasing GM-CSF production and E-selectin expression, which in turn promote neutrophilia. Also, IL-1β and inflammasome activation are required for bleomycin-induced lung fibrosis and inflammation in IPF animal models thus showing a key role for this pathway in fibrotic events. Interestingly, recent evidence has shown that ATP, which activates the NLRP3 inflammasome via the P2X7 receptor, is increased in lung fluids of COPD and IPF patients. This provides a common pathological mechanism and suggests that targeting the NLRP3 inflammasome may represent a new successful therapeutic strategy to treat these chronic disorders.

Impact

The achievement of the aims of the project will produce entirely novel and clinically relevant data that will serve as foundation for future clinical investigation for the development of new treatments for chronic inflammatory lung diseases that poorly respond to steroids.

Results achieved in 2016

We have discovered that 17-oxo-DHA strongly suppresses LPS-induced release of IL-1β and TNFα in peripheral blood mononuclear cells (PBMCs) from COPD and healthy individuals. Furthermore, 17-oxo-DHA displays additive anti-inflammatory effects with the steroid fluticasone propionate (FP) by acting through different and complementary mechanisms, both transcriptional and post-transcriptional. More specifically, we have discovered that 17-oxo-DHA, unlike FP, strongly inhibits the activation of the NLRP3 inflammasome thus reducing caspase-1-dependent IL-1β release and glucocorticoid receptor degradation.

Meetings and Publications

Publications

Accepted

Cipollina C, Di Vincenzo S, Siena L, Di Sano C, Gjomarkaj M, Pace E. "17-oxo-DHA displays additive anti-inflammatory effects with fluticasone propionate and inhibits the NLRP3 inflammasome." Sci Rep. 2016 Nov 24; 6:37625. IF 5.228.

Submitted

Di Vincenzo S, Heijink IH, Noordhoek JA, Cipollina C, Siena L, Bruno A, Ferraro M1, Postma DS, Gjomarkaj M, Pace E. "SIRT1/FoxO3 axis alteration leads to aberrant immune responses in bronchial epithelial cells". Submitted to European Respiratory Journal, 29/11/2016.

Bruno A, Cipollina C, Di Vincenzo S, Siena L, Dino P, Di Gaudio F, Gjomarkaj M, Pace E. "Ceftaroline modulates the innate immune and host defense responses of immunocompetent cells exposed to cigarette smoke." Submitted to Toxicology *in Vitro*, 06/12/2016.

In preparation

Siena L, Gjomarkaj M, Ferraro M, Bruno A, Cipollina C, Di Vincenzo S, Pace E. "Effect of 17-oxo-DHA alone and in combination with gemcitabine on lung cancer cell growth"

Poster presentations

Cipollina C, Di Vincenzo S, Siena L, Di Sano C, Gjomarkaj M, Pace E. "The electrophilic 17-oxo-DHA enhances the anti-inflammatory efficacy of fluticasone propionate in COPD patients" European Respiratory Journal Sep 2016, 48 (suppl 60) PA919; DOI: 10.1183/13993003.congress-2016.PA919. European Respiratory Society International Congress, London Sep 3-7 2016.

Siena L, Gjomarkaj M, Ferraro M, Bruno A, Cipollina C, Di Vincenzo S, Pace E. "Effect of 17-oxo-DHA alone and in combination with gemcitabine on lung cancer cell growth" European Respiratory Journal Sep 2016, 48 (suppl 60) OA1526; DOI: 10.1183/13993003.congress-2016.OA1526. European Respiratory Society International Congress, London Sep 3-7 2016.

Bruno A, Cipollina C, Di Vincenzo S, Siena L, Dino P, Di Gaudio F, Gjomarkaj M, Pace E. "Immunomodulatory role of ceftaroline in monocytes and macrophages". Biotecnologie - Ricerca di base interdisciplinare traslazionale in ambito biomedico. Palermo, December 15-16 2016.

Oral presentations

Cipollina C, Di Vincenzo S, Siena L, Di Sano C, Gjomarkaj M, Pace E. "17-oxo-DHA displays additive anti-inflammatory effects with fluticasone propionate and inhibits the NLRP3 inflammasome". Biotecnologie – Ricerca di base interdisciplinare traslazionale in ambito biomedico. Palermo, 15-16 December 2016.

Goals for 2017

- Assess the activation of the NLRP3 inflammasome in the lung of IPF and COPD patients in collaboration with ISMETT;
- Evaluate the effects of the electrophilic n-3 PUFAs on inflammasome activation and on markers of disease in ex vivo models;
- Investigate the molecular mechanisms of action of electrophilic n-3 PUFAs with respect to inflammasome activation.

Predicting the effects of microRNA deregulation using a holistic microRNA: gene interaction model

Project Leader

Claudia Coronello , PhD

Breve descrizione

The project is focused on developing a bioinformatics tool able to predict the gene expression changes due to microRNA deregulation. As an alternative to the classical approach, which considers single microRNA-target interactions, we aim to use a holistic approach, that consider the network of all the microRNA-target interactions and how the deregulation of one player (microRNA or mRNA) can influence the expression of any other player in the network. The algorithm search is based on a comprehensive data set of mRNA and microRNA expression profiles not available in public databases.
We optimized an experimental protocol useful to compile such data set. Specifically, we performed RISC protein immunoprecipitation (AGO2 and GW182 RIP experiments), to collect the following samples:

1) IP, the RNA fraction immunoprecipitated with the RISC Proteins
2) FT, the RNA left as flow-through after the IP
3) IN, the input RNA

In performing this experiment, we assure that the RISC protein complexes are totally precipitated in the IP fraction, leaving the FT fraction deprived of them. We performed the above-mentioned experiments in MCF-7 breast cancer cells and all samples were analyzed in duplicate by Agilent microarray technology.

Impact

The novelty of this project is the proposal to move from miRNA target prediction to the prediction of the effects of miRNA deregulation on the actual mRNA expression profile. We want to move from the actual perspective where only single interactions between miRNA and mRNA target are considered and the output of a miRNA target prediction tool is a list of putative targets regulated by a single miRNA. In our new perspective, the miRNA targeting is contextualized in a sample (knowing its mRNA and miRNA expression levels), and the miRNA target prediction tool is designed to predict the effects on mRNA expression due to the change (artificial or caused by disease) of the miRNA expression level.

Results achieved in 2016

First, we analyzed three AGO2 RIP experiments, in wild type MCF-7 cells. We compared the standard approach used to detect the differentially expressed genes in IP vs IN samples, which needs to compare data from several replicated experiments, with a single sample approach. Our new algorithm efficiently detects the differentially expressed genes in one IP vs IN sample

comparison. Moreover, we designed an algorithm to predict the IP vs IN differentially expressed genes, by only considering the IN sample data (no IP experiment needed).
Then, we designed an algorithm to predict the differentially expressed genes of IP samples in two different conditions. We tested it by analyzing one AGO2 RIP experiment on MCF-7 cells after the inhibition of miR-16, one of the most abundant microRNAs in this cell line. This algorithm takes into account the actual gene expression profile of the sample, and efficiently predicts the targets affected by microRNA expression variation.

Meetings and Publications

Publication

- Coronnello C, Tumminello M, Miccichè S, Gene-based and semantic structure of the Gene Ontology as a complex network, Physica A, (2016) 458, pp. 313-328. IF: 1.78

Goals for 2017

- Publish a paper describing the three novel algorithms developed during the 2016.
- We already performed GW182 RIP experiments on MCF-7 cells both wild type and after and the inhibition of miR-16. This data set shows different characteristics with respect to the analogous data obtained with AGO2 RIP experiments, although both proteins are RISC proteins and we checked that they co-immunoprecipitate. We aim to use this data to improve the performance of our algorithms, by adding new features to the analysis.
- Design a user-friendly web-tool to allow research community to use our prediction algorithms.

α -Synuclein binds TOM20 and inhibits mitochondrial protein import in Parkinson's disease

Project Leader

Roberto Di Maio, PhD

Brief description

α-Synuclein accumulation and mitochondrial dysfunction have both been strongly implicated in the pathogenesis of Parkinson's disease (PD), and the two appear to be related. Mitochondrial dysfunction leads to accumulation and oligomerization of α-synuclein, and increased levels of α-synuclein cause mitochondrial impairment, but the basis for this bidirectional interaction remains obscure.

Impact

Our study defines a novel pathogenic mechanism in PD, identifies toxic species of wildtype α-synuclein, and reveals new therapeutic strategies for neuroprotection.

Results achieved in 2016

We now report that certain post-translationally modified species of α-synuclein bind with high-affinity to the TOM20 presequence receptor of the mitochondrial protein import machinery, prevent its interaction with its co-receptor, TOM22, and impair mitochondrial protein import. Therefore, there is deficient mitochondrial respiration, enhanced ROS production and loss of mitochondrial membrane potential. Examination of post-mortem PD tissue reveals an aberrant α-synuclein:TOM20 interaction in nigrostriatal neurons that is associated with loss of imported mitochondrial protein, thereby confirming this pathogenic process in the human disease. Modest knockdown of endogenous α-synuclein was sufficient to maintain mitochondrial protein import in an *in vivo* model of PD, and in *in vitro* systems, overexpression of TOM20 or a mitochondrial targeting signal peptide had beneficial effects and preserved protein import.

Meetings and Publications

Meetings

- Poster and oral presentation at the Gordon Conference 2016
- Poster presentation SfN 2016, San Diego

Publications

- Di Maio R., Barrett P.J., Hoffman E.K., Barrett C., Zharikov A., Borah A., Hu X., McCoy J., Chu C.T., Burton E.A., Hastings T.G. and Greenamyre J.T., (2016) α-Synuclein binds TOM20 and inhibits mitochondrial protein import in Parkinson's disease. Sci Transl Med. 2016 Jun 8;8(342):342ra78. doi: 10.1126/scitranslmed.aaf3634. PMID: 27280685 I.F. 16.264

Goals for 2017

SNpc viral delivery of the Mitochondrial Targeting Sequence (MTS) will be performed in rotenone model of PD in rat to assay the possible prevention of the disease development In case of positive outcomes, small molecules with analogous MTS activity will be developed and tested *in vitro* and *in vivo* models.

A central role for LRRK2 in idiopathic Parkinson disease

Project Leader

Roberto Di Maio, PhD

Brief description

Mutations in LRRK2 cause familial Parkinson disease (PD) and, in some populations, may account for up to 40% of all cases. The LRRK2 gene locus also contains a risk factor for 'idiopathic' PD (iPD); however, the role of LRRK2 in typical iPD is not clear.

While the mechanism(s) by which mutant LRRK2 causes neurodegeneration are not clear, it is believed that disease-causing mutations may be associated with increased kinase activity. Assessment of the kinase activity state of LRRK2 under various conditions has also been problematic and somewhat cumbersome, although there appears to be a growing consensus that autophosphorylation at Ser1292 correlates with activity. Phosphoserine1292 (pS1292) has generally been detected by western blotting rather than immunocytochemistry, which limits anatomical or cellular resolution.

The activity of LRRK2 is also regulated by its interaction with 14-3-3 proteins, whose binding to LRRK2 is associated with reduced activity. The interaction between LRRK2 and 14-3-3 has generally been assessed by co-immunoprecipitation.

Impact

In this context, the current project is designed to further explore and define the role of wild type LRRK2 activity in typical iPD and models thereof. If we are correct and LRRK2 is, in fact, an important player in iPD, the utility of LRRK2-directed therapies, such as kinase inhibitors, will expand to include virtually the entire population of people with PD. As such, this project has compelling practical significance for PD therapeutics.

Results achieved in 2016

We have developed a pair of novel proximity ligation assays with excellent anatomical resolution that can rapidly provide information regarding activation state, cellular localization and physiological regulators of LRRK2.

Assays have been validated using CRISPR/Cas9 engineered LRRK2-/ and LRRK2G2019S/G2019S HEK and SH-SY5Y cells.

The assay is based on (i) S1292 phosphorylation and (ii) dissociation of 14-3-3 from LRRK2. Using this and other assays, we have compelling evidence that (i) LRRK2 is activated in nigrostriatal neurons in iPD; (ii) sublethal concentrations of rotenone activate LRRK2; (iii) α-synuclein overexpression activates LRRK2; (iv) rotenone-induced S129 phosphorylation of α-synuclein is LRRK2-dependent; (v) rotenone-induced inhibition of glucocerebrosidase is LRRK2-dependent. Together, our results suggest that LRRK2 plays a central role in idiopathic PD.

Meetings and Publications

Meetings

- Research Award: PSG Meeting, Portland – OR
- Oral presentation SfN 2016 San Diego

Publications

- NIH-NINDS R-01 Grant submission (February 2017); R. Di Maio – Co-PI. The study will be submitted for publication on March 2017

Goals for 2017

LRRK2 inhibitors developed by Sanofi and Pfizer Pharmaceuticals will be tested in our *in vitro* and *in vivo* models of PD.

Novel reagents and assays indicate a role for NADPH oxidase 2 in Parkinson disease

Project Leader

Roberto Di Maio, PhD

Brief description

Mitochondrial defects and oxidative stress have been strongly implicated in the pathogenesis of Parkinson disease (PD). While it is generally assumed that the oxidative stress and damage seen in PD derive from mitochondria, there is growing evidence that reactive oxygen species (ROS) generated by NADPH oxidase 2 (NOX2) may be important. Indeed, mitochondrial dysfunction and NOX2 are intimately related. While there is some evidence that NOX2 inhibitors may protect dopaminergic neurons against degeneration, these studies have often been hampered by the lack of highly specific inhibitors. Additionally, it has been difficult to assess the activation state of NOX2 under experimental or pathological conditions with a cellular level of resolution. We now report testing of a novel and highly specific NOX2 inhibitor, Nox2ds-tat, and development of a new histological assay for NOX2 activation that is based on association of NOX2 and p47phox, which is required for activation, and detected by proximity ligation (PL).

Results achieved in 2016

To model certain aspects of PD *in vivo* and *in vitro*, we used the mitochondrial complex I toxin, rotenone. In SH-SY5Y cells exposed to sublethal rotenone, there was clear-cut activation of NOX2. Similarly, in rotenone treated rats, there was strong activation of NOX2 in nigrostriatal dopamine neurons. Importantly, the PL assay also detected NOX2 activation in dopamine neurons in brains of patients with PD. Thus, NOX2 activation occurs in vulnerable neurons in PD and models thereof. In cell culture and *in vivo*, rotenone causes first (i) accumulation, then (ii) oligomerization, and later, (iii) fibrillization/aggregation of α-synuclein. Nox2ds-tat is a peptide that blocks association of NOX2 and

p47phox, thereby preventing NOX2 activation. Co-treatment of cultures with rotenone and Nox2ds-tat prevented the rotenone-induced accumulation, oligomerization and aggregation of α-synuclein.
We recently reported that oligomeric α-synuclein binds to the mitochondrial receptor, TOM20, and inhibits import of presequence-containing proteins. Oligomeric α-synuclein also binds to TOM70, a mitochondrial receptor that binds proteins containing an internal mitochondrial targeting signal. To facilitate import of these proteins, TOM70 also interacts with Hsp70 to prevent misfolding/aggregation of proteins to be imported. Rotenone disrupts the normal TOM70:Hsp70 interaction and this is prevented by co-treatment with Nox2ds-tat, presumably because it reduces rotenone-induced oligomerization of α-synuclein.
Together, these results indicate (i) that NOX2 activation occurs in PD, (ii) that NOX2 activity contributes to α-synuclein pathology, and (iii) that NOX2 activity contributes to mitochondrial impairment.

Meetings and Publications

Meeting

- Poster presentation SfN 2016 San Diego

Publication

- NIH-NINDS R-21 Grant submission (June 2017); R. Di Maio – PI. The study will be submitted for publication on July 2017

Goals for 2017

Nox2ds-tat will be assayed for protection against the development of PD in rotenone and α-synuclein overexpression animal models.
Two small molecules with analogue Nox2ds-tat developed in Dr. Pagano's Lab will be also tested *in vitro* and *in vivo*.

Gastric generation and signaling actions of nitro-nitrates: novel lipid intermediates that form nitrosating and electrophilic species

Project Leader

Marco Fazzari, PhD

Brief description

Acidic gastric conditions lead to nitric oxide and nitrite-dependent unsaturated fatty acid nitration, generating a complex mixture of nitrated species, through mechanisms not fully defined. The characterization of new nitro-nitrate lipids, preferentially generated by the nitration of highly reactive conjugated diene-containing fatty acids, explores unique and never described nitrosation and nitration reactions with both free and esterified unsaturated fatty acids.
Our research proposes nitro-nitrate lipids as a new class of signaling species that can induce vasodilation, nitrosation of cysteine-containing proteins and generate

electrophilic nitro conjugated linoleic acid (NO2-CLA), already identified as a pleiotropic signaling mediator. Moreover, the acid catalyzed generation of nitro-nitrate lipids can induce adaptive signaling responses in pro-inflammatory microenvironments, where the reactions of nitrogen oxides with conjugated diene-containing lipids support the generation of nitro-nitrate lipids.

Results achieved in 2016

We have Identified and characterized novel nitro-nitrate lipid intermediates after *in vitro* gastric acidification of synthetic CLA-containing lipid standards. Furthermore, we have studied the decomposition of nitro-nitrate derivatives under physiological conditions.

Meetings and Publications

U.S. patent pending filed 11-2016, titled: Novel reversible nitroxide derivatives of nitroalkenes that mediate nitrosating and alkylating reactions.

(Oral communication) Fazzari, M. Gastric generation of active nitro-nitrates: novel lipid intermediates that form nitrosating and electrophilic species. 10th Annual RI.MED Scientific Symposium, Bridging health and economic development through public-private partnerships, October 17, Palermo, Italy.

Goals for 2017

Our current and near term research objectives will address the biochemistry and pharmacology of newly discovered nitro-nitrate lipid signaling mediators. We will investigate if nitro-nitrate lipids are biologically active and act as nitric oxide and nitrosating species donors, prior to decay to anti-inflammatory nitroalkene products.
Preliminary data shows soluble guanylate cyclase (sGC) activation and cGMP synthesis upon exposure to nitro-nitrate CLA-containing phospholipid. We propose to characterize the nitric oxide release of nitro-nitrate lipids using electron paramagnetic resonance spectroscopy (EPR) and ozone-chemiluminescence analysis. Finally, we will assess if nitro-nitrate lipid derivatives induce vasodilation and modulate gastrointestinal motility.

Gastric formation, intestinal absorption and lipoprotein-dependent systemic distribution of nitro conjugated linoleic acid (NO_2-CLA)

Project Leader

Marco Fazzari, PhD

Brief description

The body of knowledge about the biological and pharmacological actions of nitro fatty acids (NO_2-FA) strongly contrast the limited information on formation, absorption and distribution. [14C] labeling studies showed that urinary metabolite excretion accounts for over 35 % of a nitro-oleic acid (NO_2-OA) oral dose with additional significant tissue accumulation and enterohepatic circulation. Thus, over 80% of absorbed NO_2-OA remains unaccounted for and it is not related to rapid degradation to its main metabolite nitrostearic acid (NO_2-SA). For NO_2-CLA, no data is available on formation yields, absorption and distribution.

Impact

Our research proposes that a relevant pool of NO_2-FA is stored in protein and lipid compartments, such as triglycerides, to regulate NO_2-FA homeostasis and act as a buffer to maintain systemic steady state levels.

Results achieved in 2016

The pharmacokinetic of NO_2-FA has been studied.

Meetings and Publications

Publications

(Abstract/ Poster/ Attendance) Fazzari, M., Khoo, N., Woodcock, R.S., Jorkasky, D.K., Li, L., Schopfer, F.J., Freeman, B.A. Nitro-fatty acid pharmacokinetics in the adipose tissue compartment. 23rd Free Radical Biology and Medicine meeting, November 16-19, San Francisco, U.S.A.

M. Fazzari, N. Khoo, R.S. Woodcock, D.K. Jorkasky, L. Li, F.J. Schopfer, B.A. Freeman. Nitro-fatty acid pharmacokinetics in the adipose tissue compartment. Journal of Lipid Research 2016 - doi:10.1194/jlr.M072058 – (2015 Impact Factor: 4.368).

S.R. Salvatore, D.A. Vitturi, M. Fazzari, D.K. Jorkasky and F.J. Schopfer. Evaluation of 10-nitro oleic acid bio-elimination in rats and humans. Scientific Reports 2017, 7: 39900 - doi: 10.1038/srep39900 – (2015 Impact Factor: 5.228).

Goals for 2017

Focus will be placed on determining the yields of gastric formation of NO_2-CLA, upon dietary interventions (CLA and NO_2-/NO3-), and its incorporation into chylomicrons.

Neuroprotective studies: Nitrite neuroprotection in Parkinson's disease

Project Leader	**Chiara Milanese, PhD**
Brief description	Our work led to the discovery that nitrite treatment slows Parkinson's disease (PD) progression in multiple phylogenetically diverse animal models (from zebrafish to rodents) as well as in cellular models of the disease.
Impact	Dysfunctional mitochondrial complex I propagates oxidative stress in PD and treatments mitigating this defect may therefore limit disease progression. Therapeutic complex I targeting has been successfully achieved in ischemia/reperfusion by using nitrosonium donors such as nitrite to reversibly modify its subunits and protect from oxidative damage after reperfusion. This evidence led to the innovative hypothesis that nitrite could exert protective effects also in pathological conditions where complex I dysfunction occurs in normoxia, such as in PD.
Results achieved in 2016	We examined the underlying mechanism of protection at the molecular level and demonstrated that inorganic nitrite mitigates PD pathology trough a synergistic mechanism that simultaneously improves mitochondrial efficiency and activates the major Nrf2 antioxidant pathway. Moreover, we demonstrated that inorganic nitrite improves bioenergetics defects in primary fibroblast derived from familial PD patients harboring mutations on the LRRK2 gene. Overall, these results indicate that administration of inorganic nitrite represents a potential therapy to limit irreversible complex I impairment and oxidative stress, which are both hallmarks of Parkinson's disease, and thus mitigate neurological dysfunction

Mechanistic studies: Metabolic redesign upon DNA damage accumulation and aging

Project Leader	**Chiara Milanese, PhD**
Brief description	Accumulation of stochastic DNA damage is intimately associated with the development of several human pathologies and with the progressive functional decline of aging. Transcription-coupled repair amends DNA lesions that block transcription, and defects hereof cause tissue-specific accelerated cell death and consequent segmental progeria. In parallel, these same defects trigger an adaptive response in which suppression of growth, and potentiation of antioxidant defenses oppose the noxious effects of aging.

Impact

We describe a molecular process coupling detection of transcription stalling to metabolic rearrangements operated by ATP mediated allosteric mechanisms to potentiate cellular defenses to stress. Overall, we provide evidence for a mechanism governing the adaptive response elicited by sustained block of transcription caused by defective DNA repair.

Results achieved in 2016

At the Department of Molecular Genetics of the Erasmus Medical Center, we took advantage of the internationally leading expertise of Jan J.H. Hoeijmakers in dissecting the DNA repair processes in health and disease. His team generated the largest series of mouse repair mutants, which enabled detailed insight into the etiology of human repair diseases and disclosed a connection between DNA damage, aging and aging related disorders.

In Dr. Marstroberardino's group, we demonstrated that defective DNA repair, causative of accelerated aging, is associated with overall transcriptional decline leading to increased ATP levels, which in turn acts as a sensor molecule to redesign sugar metabolism via allosteric mechanisms and activates antioxidant responses increasing NADPH levels. Overall, these studies identify a novel, fundamental mechanism governing somatic preservation in aging. The paper "DNA damage-induced transcription arrest elicits allosteric redesign of metabolism and activation of longevity pathways" is in preparation and the submission is planned for the beginning of the upcoming year to major international Journal.

Meetings and Publications

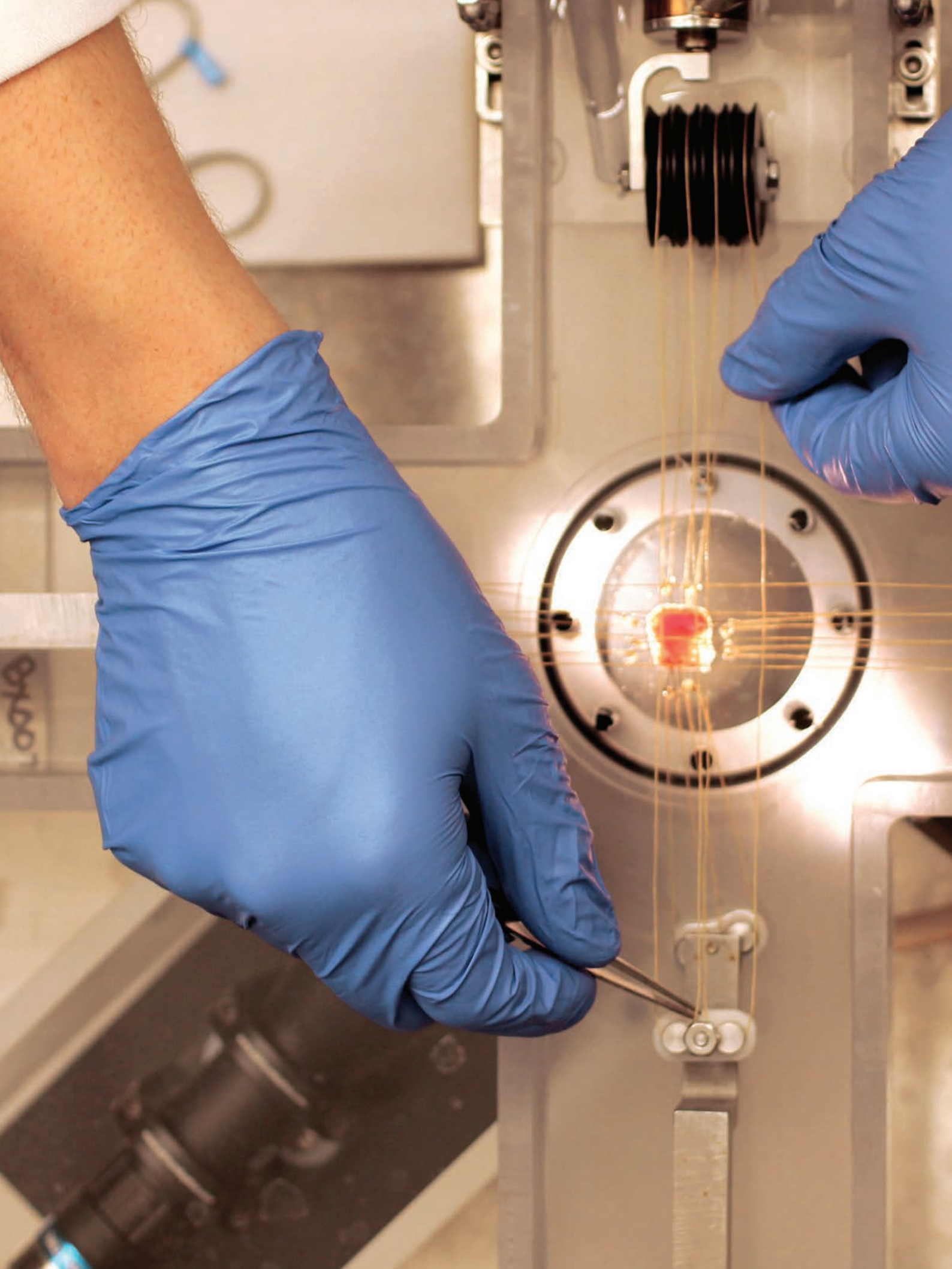
Publications:

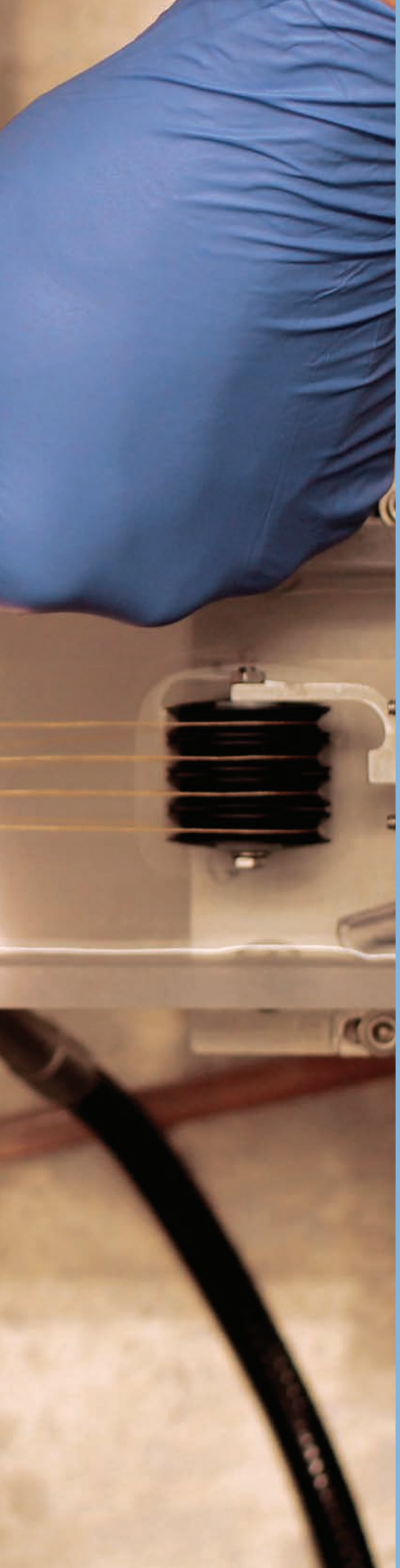
Mesenchymal Inflammation Drives Genotoxic Stress in Hematopoietic Stem Cells and Predicts Disease Evolution in Human Pre-leukemia. Zambetti NA, Ping Z, Chen S, Kenswil KJ, MA Mylona MA, Sanders MA, Hoogenboezem RM, Bindels EM, Adisty MN, Van Strien PM, van der Leije CS, Westers TM, Cremers EM, Milanese C, Mastroberardino PG, van Leeuwen JP, van der Eerden BC, Touw IP, Kuijpers TW, Kanaar R, van de Loosdrecht AA, Vogl T and Raaijmakers MH. 2016. Cell Stem Cell. IF: 22.4

Inefficient DNA Repair Is an Aging-Related Modifier of Parkinson's Disease. Sepe S, Milanese C, Gabriels S, Derks KWJ, Payan-Gomez C, van Ijcken WFJ, Rijksen YMA, Nigg AL, Moreno S, Cerri S, Blandini F, Hoeijmakers JHJ and Mastroberardino PG. 2016. Cell Reports. 15:1866-1875. IF:8.5

Goals for 2017

The goals for the upcoming 9 months are the submission and final publication of these 2 main projects. The first paper is currently under review at ARS and the submission of the manuscript on metabolism will predicted for March 2017.





TISSUE ENGINEERING AND BIOMEDICAL DEVICES

The Bioengineering department of the Ri.MED Foundation is made up of engineers, biologists, chemists and pharmacists who work in close collaboration with doctors and surgeons. The focus of research is the study of bio-material and engineered tissue, their rheological-mechanical characterisation, and the development of relative clinical devices.
Ri.MED is creating a platform based on essential instruments (both physical and computational) and equipment for the carrying out of numerical simulations and experimental tests in order to verify and qualify the structural and fluid-dynamic performance of the clinical solutions developed, in accordance with regulatory requirements for EC certification and FDA approval.
The platform allows for the optimisation of tissue engineered for various applications, in particular in the cardiovascular field, thanks to a better understanding of the effect of fluid-mechanical and structural stimuli on cell differentiation and proliferation. The platform also allows for the use of engineered tissue in the planning, development and pre-clinical validation of organs and new-generation implantable cardiovascular components. The possibility for in-house development and validation of clinical solutions, together with collaboration with the most important clinical centres in the area, facilitates the introduction of patient-specific and population-specific treatment, while at the same time offering new instruments for support in therapeutic planning and in the pre-operative decision process.

Tissue engineering and medical devices for cardiovascular regeneration and repair

Project Leader

Antonio D'Amore, PhD

Brief description

Cardiovascular tissue engineering combines cellular and advanced biomaterials approaches to replace or restore the compromised function of cardiovascular tissue and organs. The Ri.MED' cardiovascular tissue engineering and medical devices division targets three major applications:

- Tissue engineered heart valve (TE-HV);
- Tissue engineered cardiac patch (TE-CP);
- Tissue engineered vascular graft (TE-VG);

TE-HV: to develop effective tissue surrogates and biomedical devices for heart valve replacement and repair.
Specific sub aims:

- to duplicate physiological mechanics;
- endogenous tissue growth/resistance to calcification/low thrombogenicity;
- percutaneous delivery strategies;

TE-CP: to develop effective cardiac restraint devices for post myocardial infarction patients.
Specific sub aims:

- help sustain left ventricle function;
- endogenous tissue growth/reduce scar formation;
- mitigate left ventricle wall thinning;

TE-VG: to develop effective engineered blood vessels for coronary artery bypass graft.
Specific sub aims:

- physiological mechanics;
- endogenous tissue growth/vessel patency/low thrombogenicity;
- reduce intimal hyperplasia.

Impact

These research efforts will advance bioengineering strategies for ischemic heart failure, vascular and valvular diseases which have the potential to be translated to the bedside reducing patients' morbidity and mortality.

Results achieved in 2016

Scientific discoveries/ Innovation

- Designed and validated 5 different electrodes/protocols for TEHVs fabrication including: mitral, tricuspid, aortic, pulmonary, and bicuspid-pathological engineered valves;
- Designed and produced 4 novel medical devices prototypes (see patents section);
- Defined and wrote new code/software for 1) quantitative histology, 2) ePTFE morphological evaluation, 3) blood stagnation time on commercial oxygenators;
- Completed first *in vivo* study on bi-layer cardiac patch providing evidence of several positive effects of the developed device including: mitigated wall thinning, scar formation, improved LV function and angiogenesis;

Grant awarded as principal investigator or co-principal investigator

- 2016-2017) Coulter foundation 2016 $100,000. Assessment of acute response to: polyurethane, stentless, multi-leaflet mitral valve; on porcine model. Co-PI: V. Badhwar, Co-PI: A. D'Amore, Co-PI: W. Wagner, University of Pittsburgh;
- (2016-2017) Clinical & Translational Science Institute (CTSI), University of Pittsburgh; $50,000. Assessment of acute response to: A) self-expanding non-degradable, low-profile percutaneous tricuspid valve; B) "template based", electrospun, biodegradable, tricuspid valve with chordae tendineae; C) electrospun, biodegradable, polyurethane, stentless, multi-leaflet tricuspid valve; on porcine model. Co-PI: V. Badhwar, Co-PI: A. D'Amore, Co-PI: W. Wagner, University of Pittsburgh;
- NIH R01, Dysfunctional muscle remodeling and regeneration in environmental disease, $ 2.6 million for 2016-2021. PIs: F. Ambrosio, A. Barchowski, University of Pittsburgh. Co-Investigators: A. D'Amore (8% efforts), W. Wagner, D. Stolz, University of Pittsburgh;

Funded industrial collaborations

- Industrial collaboration with Livanova (formerly Sorin Biomedica s.p.a). Cardiac surgery division, "Experimental measurement of blood residence time in commercial Sorin oxygenators", $70k for 3-2016 to 10-2016, renewable consultancy service. Co-PI: A. D'Amore, Co-PI: W. Wagner, Co-PI: S. Ye, University of Pittsburgh. Research
- Industrial collaboration with Peca lab, start-up company created by Carnegie Mellon University, "Biomechanical and micro-structural analysis of an expandable vascular conduit composed of ePTFE", structural characterization of Peca laboratory heart valve prosthesis prototypes, the study aims to produce data for class III medical device FDA approval 4-2016 to present, renewable consultancy service. sole PI: A. D'Amore;

Initiated/continued funded research collaborations

- NIH 5R01 AR054940-0. Cellular remodeling of ECM scaffolds. PI: S. Badylak, University of Pittsburgh. Role: A. D'Amore collaborator, ECM-polymeric scaffolds for cardiac patch design and fabrication;

- Armed Forces Institute of Regenerative Medicine, AFIMRII, W81-XWH-13-2-XXXX, $900,000 for 1/2014 - 12/2017.Creating innervated vascularized muscle flaps from elastic, cellularized biocomposites developed in situ for facial muscle reconstruction. PI: W. Wagner, Project leader: A. D'Amore, University of Pittsburgh;
- NIH (NIBIB) 1R21EB016138, Autologous stem cell-based tissue engineered vascular grafts. PI: D. Vorp. University of Pittsburgh. Role: A. D'Amore collaborator, vascular graft fabrication;

Initiated/continued (not financially supported) research collaborations

- Dr C. Migliaresi, Universita' di Trento, artificial chordae tendinae deveopment, role project leader (currently hosting one Master student);
- Drs M. Pilato, G. Raffa, ISMETT, engineered valve functional evaluation, role: project leader, role: project leader;
- Drs S. Pasta, RiMED, engineered valve functional hemodynamic, role: collaborator, (previously shared a Whitaker fellow);
- Drs M. Pilato, G. Raffa, M. Morsolini, ISMETT, cardiac patch grant application, sub-project: "Minimally invasive approaches for epicardial patch placement". Bando GR Ministero della Salute 2016, role: project leader;
- Dr Julie Philippi, University of Pittsburgh, ECM-polymer engineered vascular graft development, role: collaborator;
- Drs V. Brucato, E. La Carrubba, CHAB, Universita' di Palermo, engineered vascular graft development, role: collaborator, (previously shared a Whitaker fellow);
- Dr Teresa Raimondi, Politecnico di Milano, engineered vascular graft and valve leaflets development, role: project leader (currently hosting one Master student);
- Drs A. Bruno, E. Ardizzone, R. Pirrone, CHAB, Universita' di Palermo, software development for automatic detection of blood vessels, role: project leader;

New award/grant applications

- Cardiac patch, Bando GR Ministero della Salute 2016, role: sole PI;
- Dr Joao Soares PI, University of Texas at Austin: NIH-R21 application: "Temporary and Minimally-Invasive Engineered Tissue Epicardial Restrain Patching as Alternative Bridge-to-Transplant Therapy in Pediatric Dilated Cardiomyopathy", role co-PI;
- Dr Leonardo D'Aiuto PI, University of Pittsburgh, NIH Center-grant application: "Pittsburgh Human Tissue Modeling Center for Infectious Diseases (PiHTMID)", role Co-I responsible for the tissue engineering core;
- Dr Giulio Ghersi co-PI, CHAB, Universita' di Palermo, cardiac patch grant application, additional PhD scholarship MIUR, role: co-PI;

Start-up formation

- Completed OneValve business plan;
- Completed OneValve IP landscape analysis with assistance of OTM and Pittsburgh Life Science Greenhouse;
- Completed OneValve market analysis and IP conceptual maps analysis with assistance of OTM and Pittsburgh Life Science Greenhouse;
- Completed potential market analysis for Cardiac Patch - PCT/US2016/051005;

Patents, Meetings and Publications Summary

- 5 US PCT and 1 provisional patent applications, 4 out of these 6 invention as the lead inventor;
- 11 high impact factor (8.97- 1.9) journal research articles publications, 2 out of these 11 articles as first author;
- 5 podium presentations at international meetings, 2 out of these 5 as the presenting author;
- 4 invited talks,1 conference organized as meeting co-chair and 2 as member of the organization and scientific committee.

Patent applications

- Will be converted to US provisional patent within 02/2017. Invention disclosure (Office of technology management internal case number: 03885, filed in 04/2016), topic: biomedical device, title: "An expandable percutaneous venous cannula for use in extracorporeal cardiopulmonary support". Lead innovator/developer: A D'Amore;
- Will be converted to US provisional patent within 02/2017. Invention disclosure (Office of technology management internal case number: 03844, filed in 04/2016), topic: biomedical device, title: "A stentless biopolymer heart valve replacement capable of living tissue regeneration". Lead innovator/developer: A D'Amore;
- US patent application PCT/US2016/019837 with WO (International publication number WO 2016/138416 A1) published in 09/2016, topic: biomedical device, title: "Double component mandrel for electrospun stentless multi-leaflet valve fabrication". Lead innovator/developer: A D'Amore;
- US patent application PCT/US2016/019849 with WO (International publication number WO 2016/138423 A1) published on 09/2016, topic: biomedical device, title: "Retrievable self-expanding non-thrombogenic low-profile percutaneous atrioventricular valve prosthesis";
- Will be converted to PCT within 02/2017. US provisional patent application (US 62/281,422), filed in 01/2016, topic: biomedical device, title: " Trans-atrial access for transcatheter valve repair or replacement";
- US patent application PCT/US2016/051005 filed in 09/2016, topic: biomedical device, title: "Bi-layer polyurethane - extra cellular matrix scaffolds for improved ischemic ventricular wall remodeling". Lead innovator/developer: A D'Amore;

Meetings and Publications

Journal articles

- Bi-layred polyurethane-extracellular matrix cardiac patch improves ischemic ventricular wall remodeling in a rat model. D'Amore, T. Yoshizumi., S. K. Luketich, M. T. Wolf, X. Gu, M. Cammarata, R. Hoff, S.F. Badylak, and W. R. Wagner. Biomaterials 2016 (107), 1–14, 5Y-IF 8.97;
- Large strain stimulation enhances extracellular matrix production and stiffness in an elastomeric scaffold model. D'Amore, J. Soares, J. Stella, W. Zhang, N. Amoroso, J. Mayer. W. Wagner, M. Sacks. Journal of the Mechanical Behavior of Biomedical Materials 2016 (62), 619–635, 5Y-IF 3.15;

- Timing effect of intramyocardial hydrogel injection therapy on left ventricular remodeling after myocardial infarction. T. Yoshizumi, Y. Zhu, H. Jiang, A. D'Amore, H. Sakaguchi, J. Tchao, K. Tobita, and W. R. Wagner. Biomaterials 2016, 83,182–193, 5Y-IF 8.97;
- Abdominal wall reconstruction by a regionally distinct biocomposite of extracellular matrix digest and a biodegradable elastomer. K. Takanari, Y. Hong, R. Hashizume, A. Huber, N. Amoroso, A. D'Amore, S. Badylak, W. Wagner. Journal of Tissue Engineering and Regenerative Medicine;10(9):748-61, IF 4.7;
- Solubilized liver extracellular matrix maintains primary rat hepatocyte phenotype in-vitro. A. Loneker, D.Faulk, G. Hussey, A. D'Amore, S. Badylak. Journal of Biomedical Material Research Part A, 2015 104, (4), 957–965, IF, 3.26;
- Evaluation of the Stromal Vascular Fraction of Adipose Tissue as the Basis for a Stem Cell-Based Tissue Engineered Vascular Graft. J. T. Krawiec, K. Bruce, A. Josowitz, L. Kokai, A. D'Amore, J. Weinbaum, W. Wagner, P. Rubin, D. Vorp. In press on Journal of Vascular Surgery IF 3.77;
- *In Vivo* Functional Evaluation of Tissue Engineered Vascular Grafts Fabricated Using Human Adipose-Derived Stem Cells from High-Cardiovascular Risk Populations. J. Krawiec, H. Liao, A. Josowitz, J. Weinbaum, A. D'Amore, P. Rubin, W. R. Wagner, D. Vorp. Tissue Engineering Part A, 2016 22 (9-10), 765-775, IF 3.89;
- Extracellular Matrix Fiber Microarchitecture is Region-Specific in Bicuspid Aortic Valve-Associated Ascending Aortic Aneurysms. A.Tsamis, J. Phillippi, R. Koch, J. Krawiec, A. D'Amore, S. Watkins, W. Wagner, D. Vorp, T. Gleason. Journal of Thoracic and Cardiovascular Surgery 2016, 151, (6), 1718–1728.e5, IF, 3.51;
- Chronic exposure to arsenic in drinking water promotes NF-κB-mediated myofibroblast dysfunction and matrix remodeling to impair muscle stem cell function. C. Zhang, R. Ferrari, K. Beezhold, K. Stearns-Reider, A. D'Amore, M. Haschak, D. Stolz, P. Robbins, A. Barchowsky, F. Ambrosio. Stem Cells 2016, 34, (3), 732-742 IF 5.90;
- Biomechanical and Microstructural Analysis of an Expandable Vascular Conduit Composed of ePTFE. Loneker, S. K. Luketich, D. Bernstein, A. Kalra, A. Dees, A. D'Amore, D. M. Faulk. In press on Journal of Biomedical Materials Research Part A, IF 2.3;
- Constitutive Modeling of Ascending Thoracic Aortic Aneurysms Using Microstructural Parameters. S. Pasta, J. Phillippi, A. D'Amore, G. Raffa, M. Pilato, S. Watkins, W. Wagner, T. Gleason, D. Vorp. Medical Engineering & Physics 2016, 38 (2), 121-130 IF 1.9;

Podium presentations

- Double components electrospun fibers deposition (DCD): heart valves fabrication with controlled mechanics, micro-structure and Anatomy. D'Amore, G. Raffa, S. Olia, S. K. Luketich, A. Mazzola, F. D'Accardi, X. Gu, M. Pilato, M. V. Kameneva, V. Badhwar, W. Wagner. LIAC Vascular Research Society Annual meeting, September 14-16, Ustica, Italy;
- Double components electrospun fibers deposition (DCD): heart valves fabrication with controlled mechanics, micro-structure and Anatomy. D'Amore, G. Raffa, S.

Olia, S. K. Luketich, A. Mazzola, F. D'Accardi, X. Gu, M. Pilato, M. V. Kameneva, V. Badhwar, W. Wagner .Società Italiana di Chirurgia Cardiaca Bi-annual meeting, November 25-27, 2016, Rome, Italy;

- Design of Thermoresponsive Hydrogels and Biodegradable, Thermoplastic Elastomers for Interventions in Cardiac Wall Remodeling Following Myocardial Infarction. Y. Zhu, X Gu, A. D'Amore, Y Matsumura, W. Wagner. Society for Biomaterials Conference (SFB) April 5-8 2017 Minneapolis, Minnesota;
- Towards Elimination of the *In Vitro* Dynamic Culture Period of SVF Cell-Seeded TEVGs. K. Saleh, D. Haskett, , L. Kokai, J. Weinbaum, A.D'Amore, W.Wagner, P. Rubin, D. Vorp.Biomedical Engineering Society (BMES) Annual Meeting, October 5-8, 2016 in Minneapolis, Minnesota;
- Towards a "Same-Day" Autologous Tissue-Engineered Vascular Graft: Seeding and Implantation of an Elastomeric Scaffold with the Stromal Vascular Fraction. D. Haskett, K. Saleh, J. Krawiec, J. Weinbaum, A.D'Amore, W.Wagner, L. Kokai, P. Rubin, D. Vorp. 15th Biennial Meeting of the International Society for Applied Cardiovascular Biology (ISACB) September 7-10, 2016, Banff, Alberta, Canada;

Invited talks

- (11/2016) Invited speaker, "How to improve control over biomaterials structure-function to design better performing tissue surrogates". Center for Life Nano Science, Istituto Italiano di Tecnologia, Rome, Italy;
- (10/2016) Invited speaker, "Image based structural analysis and quantitative histology for tissue engineering applications". Bridgeside point II, Pittsburgh, USA
- (10/2016) Invited speaker, "Double component electrospinning deposition a novel processing method for heart valve engineering". Tenth annual Ri.MED Foundation scientific symposium, Palermo, Italy;
- (09/2016) Invited speaker, #EuFactor 2016 event organized by the European parliament to promote the interest of college students for research and innovation. Selected as example of Italian young researcher success stories: "Testimonial Stem, ricerca scienti¬fica e innovazione tecnologica", Palermo, Italy;
- (09/2016) Meeting co-chair, scientific and organizing committee, LIAC Vascular research 2016 meeting, Ustica Italy;
- (09/2016) Session chair, "session I, molecular and supramolecular structure" LIAC Vascular research 2016 meeting, Ustica Italy;
- (09/2016) Scientific committee and organizing committee, Italian chapter of the European society of biomechanics, thematic symposium titled: "Frontier biomechanical challenges in cardiovascular physiopathology", Palermo Italy

Goals for 2017

Scientific discoveries/ Innovation

- Complete Coulter grant - implantation and evaluation of engineered mitral valve on 10 juvenile pigs;
- Complete CTSI grant - implantation and evaluation of engineered tricuspid valve on 21 juvenile pigs;

- Complete "Revolutionizing Metallic Biomaterials" (acting as collaborator) grant - implantation and evaluation of biodegradable stent for engineered aortic valve on 10 juvenile pigs, protect related IP;
- Define a surgical strategy for minimally invasive deployment of the patch in collaboration with ISMETT, Drs Pilato, Raffa, Morsolini and Turrisi;
- Validated a novel fabrication protocol for bio-mimetic vascular graft;
- Expand to 3D applications the software/IP. Invention disclosure (Office of technology management internal case number: 02193), copyrighted software, filed in 04/2010, topic: material characterization, title: A method to characterize the complete fiber network topology of planar fibrous tissues and scaffolds. Lead innovator/developer: A D'Amore;

Journal articles

- Submit manuscript: A. D'Amore, G. Raffa, S. Olia, S. K. Luketich, A. Mazzola, F. D'Accardi, X. Gu, M. Pilato, M. V. Kameneva, V. Badhwar, W. Wagner . Double components electrospun fibers deposition (DCD): heart valves fabrication with controlled mechanics, micro-structure and anatomy. To be submitted/in preparation to Biomaterials;
- Submit manuscript concluding the seminal study: NIH 5R01 HL68816- 8, Biomechanical optimization of tissue engineered heart valves. PI: M. Sacks University of Texas at Austin, W. Wagner University of Pittsburgh, J. Mayer Children Hospital, Harvard University. Role: A. D'Amore collaborator and coordinator, leaflet fabrication and explants assessment;
- Submit manuscript on stretch bioreactor study follow up: A. D'Amore, J. Stella, R. Hoff, N. Amoroso, M. Sacks, W. Wagner. Controlling micro-structure to enhance de novo extracellular matrix deposition in elastomeric scaffolds for cardiac tissue regeneration. 3rd TERMIS World Congress in Vienna September 5th – 8th 2012. published on Journal of Tissue Engineering and Regenerative Medicine, Vol 6,110, IF 3.278

Patent applications

- Protect engineered chordae tendinae IP;
- Protect biomimetic vascular graft IP;
- Protect IP/existing software for antibodies topological mapping and ePTFE analysis;

Start-up

- OneValve formation;

Grant applications

- Apply for PO-FESR 1.1.3 grant;
- Apply for EU-ERC-starting grant,
- Topics: (1) to perform a large animal study duplicating the completed work on rats infarction model, (2) to assess OneValve IPs on chronic models;

High throughput bioreactor technology for composite tissues

Project Leader

Riccardo Gottardi, PhD

Brief description

Organoid models are promising systems for studying the pathogenesis of diseases as well as for drug screening and predictive toxicology. However, *in vitro* tissue models are today often limited to single tissue types, not capturing the actual complexity present in human systems, where multiple tissues are adjacent and closely linked. The few organoids models that have been so far proposed to capture the composite nature of human physiology are mostly limited to the juxtaposition of different cell layers, which mimics of a number of tissue-tissue barriers, e.g., the pulmonary-capillary epithelium-endothelium interface, but fail to replicate the more complex 3D systems with multiple layers and in which the extracellular matrix plays a key role. To overcome this limitation, we have recently developed the first prototype of a unique, medium to high throughput bioreactor that can host biphasic tissue units, both engineered and native, and that could complement existing engineered tissue interface models. The biphasic microphysiological system bioreactor (MPS) basic unit has two separate fluidic compartments and the biphasic constructs are placed in between them. With this design, each component of the biphasic construct is exposed to its tissue-specific medium, while remaining in direct contact with the adjacent construct component. We have started our testing of the MPS potential by studying the osteochondral (OC) unit, made of tightly bound cartilage and bone, as a paradigmatic example of connected tissues in which the extracellular matrix (ECM), the 3D environment, and tissue-tissue communication play a key role. Furthermore, our MPS offers the opportunity of directly comparing engineered constructs with the corresponding native, composite tissue units. This is a unique feature of our device and it will be crucial to validate the effective biosimilarity of the physiological responses of engineered constructs with the native tissue units they are mimicking.

Our long-term goal is to develop new capabilities in studying biology, medicine, pharmacology, physiology, and related toxicology of skeletal tissues by combining tissue engineered organotypic microphysiological cultures with human stem cells and non-invasive, real-time analytical techniques. In order to allow optical access for real-time optical monitoring, the dimensions of the constructs must be reduced; hence we are also developing and validating a multi-well system with each well with planar dimensions inferior to those of a single well in a 96-well plate (bioreactor 2.0). The height of each well would be tailored to contain a construct of about 10µl of volume (radius~1.8mm, depth~0.96mm). Each microwell would be sealed by a removable base and a removable lid, equipped with O-rings. A small circular glass cover slip will be placed between each microwell and the lid to allow clear optical access to the microwell. Each microwell is equipped with

two separate fluidic inlets and outlets for provision of specific medium. Wells can be connected in arrays (in parallel or in series) in a similar manner as for the previously described bioreactor to for up to a 96 well system, or they could each be connected by microfluidic channels to external media reservoirs and collection bags. This bioreactor will allow the creation of an organoid system that may be likened to a "living histological section" that may be studied non-invasively by optical microscopy as it responds to physiological and toxic stimuli.

Impact

A human stem cell based MPS accelerates drug research and development by reducing the number of compounds that reach Phase I and II clinical trials while more precisely predicting outcomes of those that are clinically tested, which will also dramatically reduce the cost of drug development. These MPS will also serve as the basis for personalized medicine, in that they can be derived from a patient's own stem cells to replicate the disease *in vitro* and to test the effects of a drug on reducing or reversing the course of the disease. Real-time monitoring of temporal-spatial responses by cells within an MPS to drugs and toxicants aids in identifying mechanisms and options for intervention much more efficiently than current, destructive analytic techniques.

Results achieved in 2016

- Improved and tested bioreactor design for better handling and increased performance (bioreactor 1.2)
- Tested up to 2 weeks of OC tissue viable culture in bioreactor 1.2
- Modelled fluid dynamics in bioreactor 1.2
- Experimental validation of fluid dynamics in bioreactor 1.2
- Modelled fluid dynamics in bioreactor 2.0

Goals for 2017

- Tested up to 4 weeks of OC tissue viable culture in bioreactor 1.2
- Comparison of response to TNF-α of native vs. engineered OC tissue I bioreactor 1.2
- Experimental validation of fluid dynamics in bioreactor 2.0
- Generation of osteochondral constructs in bioreactor 2.0
- Generation of micromass culture in bioreactor 2.0

Effects of microgravity on osteochondral tissues

Project Leader	**Riccardo Gottardi, PhD**
Brief description	We aim at testing the effects of microgravity on bone loss in osteochondral tissues using a 3D microphysiological system (MPS). The MPS will be an invaluable tool to test drugs and develop therapies against bone and cartilage damage in space as well as on the ground. First, we will develop a veritable *in vitro* engineered model of bone for high throughput screening and test its response to bisphosphonates. Then, we will implement the engineered bone model with cartilage in my established osteochondral MPS (OC MPS) to study the effects of microgravity in space and on the ground (simulated microgravity) and the protective action bisphosphonates.
Impact	If successful, this project will (1) confirm the potential protective role of bisphosphonates against bone resorption in microgravity, (2) assess the effects on microgravity and bisphosphonates on cartilage, (3) establish MPS platforms as essential systems for drug development and predictive toxicology of musculoskeletal tissues, both on the ground and in space.
Results achieved in 2016	• Securing CASIS funding • Securing subcontract agreement with Vanderbilt University for their microfluidic controller • Training of research technician • Osteoblastic differentiation test on two candidate osseous scaffolds • Testing in 2D of the assays to be used for the 3D culture model
Goals for 2017	• Testing of osteoblasts/osteoclasts crosstalk in 2D and optimization of cellular ratios to mimic native bone metabolism in response to a biochemical stress signal • Seeding in a 3D construct comprising cartilage and comparison to native tissues in response to the same stress signal for up to 3 weeks • Optimization of the microfluidic platform in collaboration with Vanderbilt University to make it as close as possible to be "flight ready"

Chondroprotection by menstrual cycle hormones: an osteochondral microtissue approach

Project Leader

Riccardo Gottardi, PhD

Brief description

Osteoarthritis (OA) is the progressive damage to articular cartilage, the soft tissue covering the bones of joint. During OA the bone connected to cartilage is also affected, as bone and cartilage continuously influence each other. After menopause, women experience osteoarthritis twice as much as men the same age. Multiple studies suggest that this higher risk of OA is associated with the loss of sex hormones post-menopause. However, current hormone replacement therapies (HRT) only induce improvements in bone health but do not in cartilage. This might depend on the difference between the natural menstrual cycle and HRT. The former is characterized by concentrations of estrogen and progesterone that vary significantly over time and that repeat cyclically every 28 days, whereas the latter is generally a continuous administration of an estrogen (or estrogen and progesterone) concentration that does not vary over time. We aim at identifying which hormone concentration or combination of hormone concentrations can be protective of cartilage against osteoarthritis, taking advantage of the innovative, patent pending bioreactor system we developed to study cartilage and bone together, considered as a single osteochondral (OC) unit.

Impact

If successful, this project will (1) offer new guidelines for HRT regimes that could be protective of cartilage against OA and will (2) identify the mechanisms affecting the onset of OA that are influenced by sex hormone that (3) could be a target to develop treatment for OA for both men and women.

Results achieved in 2016

- Assessed response to cyclic hormonal concentrations mimicking the menstrual cycle of native OC tissues

Meetings and Publications

Meetings

*presenter

- R. Gottardi*, L. Iannetti, G. D'Urso, P. Zunino, P.G. Alexander, R.S. Tuan. A High Throughput Osteochondral Bioreactor. Tissue Engineering and Regenerative Medicine International Society – Americas Annual Meeting, San Diego, CA, December 2016.
- R. Gottardi*, G. Conoscenti, P.G. Alexander, P.A. Manner, V. La Carrubba, V. Brucato, R.S. Tuan. A Continuous Pore Size Gradient PLLA Scaffold For Osteochondral Regeneration. Tissue Engineering and Regenerative Medicine International Society – Americas Annual Meeting, San Diego, CA, December 2016.
- S.K. Patel, A. Greene, S. Rothstein, Y. Zou, S. Choi, A. Glowacki, R. Gottardi, C.

Sfeir, S.R. Little, L. Rohan. Application of USP 4 Dissolution Apparatus to Assess Dissolution of Microparticles for Periodontal Disease. American Association of Pharmaceutical Scientists 2016, Denver, CO, November 2016.

- R. Gottardi*, G. Conoscenti, P.G. Alexander, P.A. Manner, V. La Carrubba, V. Brucato, R.S. Tuan. A Continuous Pore Size Gradient PLLA Scaffold For Osteochondral Regeneration. Biomedical Engineering Society Annual Meeting, Minneapolis, MN, October 2016.
- R. Gottardi*, H. Lin, L. Iannetti, G. D'Urso, P. Zunino, T.P. Lozito, P.G. Alexander, P.A. Manner, E. Sefton, T.K. Woodruff, R.S. Tuan. Validation Of An Osteochondral Bioreactor Applied To Study The Protective Role Of Sex Hormones. Biomedical Engineering Society Annual Meeting, Minneapolis, MN, October 2016.
- R. Gottardi*, A. Pirosa, P.G. Alexander, P.A. Manner, D. Puppi, F. Chiellini, R.S. Tuan. An *In Vitro* Chondro-Osteo-Vascular Triphasic Model Of The Osteochondral Complex. Biomedical Engineering Society Annual Meeting, Minneapolis, MN, October 2016.
- K. Overholt, A. Pirosa, R. Gottardi, R.S. Tuan. Engineering The Bone-Cartilage Interface: An Osteochondral Microphysiological System. Biomedical Engineering Society Annual Meeting, Minneapolis, MN, October 2016.
- I.S. Sondh, D.A. Nichols, E. Bayer, R. Gottardi, S.R. Little. Development of a bioreactor aimed at designing spatial and temporal drug delivery profiles for bone regeneration protocols. Biomedical Engineering Society Annual Meeting, Minneapolis, MN, October 2016.
- I.S. Sondh, D.A. Nichols, E.A. Bayer. R. Gottardi, S.R. Little. Development of a bioreactor aimed at designing spatial and temporal drug delivery profiles for bone regeneration protocols. 2016 Summer Research Symposium, Duquesne University.
- R. Gottardi*, A. Pirosa, G. Conoscenti, H. Lin, G. D'Urso, L. Iannetti, P. Zunino, T.P. Lozito, P.G. Alexander, V. La Carrubba, V. Brucato, P.A. Manner, R.S. Tuan. An Osteochondral Microphysiological System to Study Cartilage-Bone Interaction in Native Tissues and Engineered Model Constructs. AAOS/ORS Tackling Joint Disease by Understanding Crosstalk between Cartilage and Bone Research Symposium, Rosemont, IL, April 2016.
- R. Gottardi*, H. Lin, T.P. Lozito, P.G. Alexander, K.C. Clark, E.A. Sefton, T.K. Woodruff, R.S. Tuan. An Osteochondral Microphysiological System to Study the Pathogenesis of Osteoarthritis and the Effect of Hormonal Exposure. Regenerative Medicine Workshop at Hilton Head 2016, Hilton Head Island, SC, March 2016.
- R. Gottardi*, A. Pirosa, P.G. Alexander, P.A. Manner, D. Puppi, F. Chiellini, R.S. Tuan. An *in vitro* Chondro-Osteo-Vascular Triphasic Model of the Osteochondral Complex for Studying Osteochondral Biology and for Drug Screening. Orthopaedic Research Society Annual Meeting, Orlando, FL, March 2016.
- R. Gottardi*, G. Conoscenti, P.G. Alexander, P.A. Manner, V. La Carrubba, V. Brucato, R.S. Tuan. A PLLA Scaffold with Continuous Gradient Pore Size for Osteo-

chondral Regeneration Validated in a Microphysiological Tissue System Bioreactor. Orthopaedic Research Society Annual Meeting, Orlando, FL, March 2016.

- R. Gottardi*, H. Lin, G. D'Urso, L. Iannetti, P. Zunino, T.P. Lozito, P.G. Alexander, P.A. Manner, E.A. Sefton, T.K. Woodruff, R.S. Tuan. Validation of an Osteochondral Microphysiological System Applied to Study the Protective Role of Sex Hormones. Orthopaedic Research Society Annual Meeting, Orlando, FL, March 2016.

Publications

- Anatomical region-specific enhancement of 3-dimensional chondrogenic differentiation of human mesenchymal stem cells by soluble meniscus extracellular matrix. B.B. Rothrauff, K. Shimomura, R. Gottardi, P.G Alexander, R.S. Tuan. Acta Biomaterialia. Epub ahead of print doi:10.1016/j.actbio.2016.11.046. Impact Factor: 6.025
- Rapidly dissociated autologous meniscus tissue to enhance meniscus healing: an *in vitro* study. P.-o. Numpaisal, B.B. Rothrauff, R. Gottardi, C.-L. Chien, R.S. Tuan. Connective Tissue Research. Epub ahead of print doi: 10.1080/03008207.2016.1245727. Impact Factor: 1.411 (Ranking 11th in Orthopaedics)
- Supramolecular organization of collagen fibrils in healthy and osteoarthritic human knee- and hip joint cartilage. R. Gottardi*, U. Hansen*, R. Raiteri, M. Loparic, M. Düggelin, D. Mathys, N.F. Friederich, P. Bruckner, and M. Stolz PLoS ONE. 11(10): e0163552. doi:10.1371/journal.pone.0163552 *equal contribution. Impact Factor: 3.234
- Distributed and lumped parameter models for the characterization of high throughput bioreactors. L. Iannetti, G. D'Urso, G. Conoscenti, E. Cutrì, R.S. Tuan, M.T. Raimondi, R. Gottardi, P. Zunino PLoS ONE. 2016, 11(9): e0162774. doi:10.1371/journal.pone.0162774. Impact Factor: 3.234
- Cell and Biomimetic Scaffold Based Approaches for Cartilage Regeneration. A.X. Sun, P.-o. Numpaisal, R. Gottardi, H. Shen, G. Yang, R.S. Tuan. Operative Techniques in Orthopaedics. 2016, 26(3): 135–146. Impact Factor: not available
- Anisotropic Viscoelastic Biomechanical Mapping of Knee Meniscus Cartilage. L. Coluccino, C. Peres, R. Gottardi, P. Bianchini, A. Athanassiou, A. Diaspro, L. Ceseracciu. Journal of Applied Biomaterials & Functional Materials. Epub ahead of print doi:10.5301/jabfm.5000319. Impact Factor: 0.934

Goals for 2017

- Use gene array to identify potential targets activated by hormonal sequences *in vitro*
- Start preliminary animal test in minipigs on hormonal protection against induced post-traumatic OA
- Assess response of native human tissues to weekly hormonal exposures, in particular in relation to the candidate target identified previously

Hemodynamic and biomarkers for clinical risk stratification of ascending thoracic aortic aneurysm with bicuspid aortic valve

Project Leader

Salvatore Pasta, PhD

Brief description

This project addresses the challenging topic of developing a computer clinical decision support system (CDSS) for the risk stratification of patients with bicuspid aortic valve (BAV) at high risk for the formation of aortic dilatation (ATAA), and ultimately death. The central hypothesis is that the hemodynamic as derived by computational modeling may play a key role in the development of ATAAs.

Impact

This technology (a software solution) for the management of patients with ATAAs can reduce the tangible economic burden imposed on the healthcare system by improving resource allocation (costs for complex clinical procedure) and overall outcomes by refining surveillance imaging regimens (costs for the monitoring of the patient through radiologic imaging).

Results achieved in 2016

Scientific project progress:

- In collaboration with ISMETT, we are currently enrolling patients in this project for the development of computational models and epigenetic profiling. At present, we have enrolled near n.60 patients, and this enrollment phase is expected to end by mid-2017.

Collaboration:

- We commissioned the development of the visualization tool of the CDSS software to the biotech company Orobix (http://www.orobix.com/).
- Scientific collaboration started with the Ecole Polytechnic of Lausanne, POLIMI and University of Sheffield on the right heart failure in patients referred to left ventricular assist device implantation (see publication output).

Grant submission:

- As principal investigator, a grant proposal was submitted to the Marfan Foundation (https://www.marfan.org/) under the 2016 Faculty Grant program. The project was not funded.
- As a co-principal investigator, a grant was submitted to the call Ricerca Finalizzata 2016 of the Italian Ministry of Health in collaboration with Diego Bellavia from IRCCS ISMETT.
- As cooperation partner, a joint research activity has been created and a grant proposal was submitted to the Volkswagen Foundation with Dr. Marino from Leibniz Universität Hannover.

Resources available:

- Thanks to the budget GR-2011-02348129, a clinical data manager was hired to supervise and coordinate the project activities and management.

- Thanks to the collaboration with Dr. D'Amore, a PhD student from the University of Pittsburgh was supervised here in Palermo for a period of 10 months.
- Several students from UniPA were involved for the routine works of scientific projects and in other studies in collaboration with ISMETT.

Other achievements:

- A symposium titled ""Frontier Biomechanical Challenges in Cardiovascular Physiopathology"" was organized by myself and Prof. Zingales in collaboration with UniPA, CHAB, ISMETT and RiMED.
- Serving as Guest Editor for a Special Issue in Medical Engineering & Physics. (impact factor 1.6)

Meetings and Publications

Meetings

- Predicting Right Heart Failure in Patients with Pulmonary Hypertension. Scardulla F, S P, Bellavia D, Scardulla C. Virtual Physiological Human Conference (VPH 2016). Amsterdarm, NL 2016
- Modelling Right Heart Failure in Patients with Pulmonary Hypertension. Scardulla F, Mercadante P, S P, Bellavia D, Scardulla C. Italian Society of Industrial and Applied Mathematics (SIMAI). Milano 2016.
- Modelling Right Heart Failure in Patients with Pulmonary Hypertension. Scardulla F, Mercadante P, S P, Bellavia D, Scardulla C Gruppo Nazionale Bioingegneria Napoli 2016.
- Flow-mediated Mechanism of Aneurysm Formation in Bicuspid Aortic Valve. Raffa GM, Pasta S. XXVIII Congresso della Società Italiana di Chirurgia Cardiaca (SICCH), Rome 2016.
- Feasibility of Thoracic Aorta Computational Modeling in clinical practice. Gentile G, Pasta S. RSNA Radiology Society of North America. Chicago, IL 2016.
- Accuracy of Standard Echocardiography, Cardiac Magnetic Resonance and Derived Lumped Parameter Model for Prediction of Invasive Hemodynamics in Patients with Precapillary Pulmonary Hypertension: a Pilot Study. Bellavia D, Romano G, Pasta S, Gentile G, Scardulla F, Frangi A, et al. 27th Annual of American Society of Echocardiography (ASE). Seattle, WA 2016

Publications:

- Evaluation of ventricular wall stress and cardiac function in patients with dilated cardiomyopathy. Scardulla F, Rinaudo A, Pasta S, Scardulla C. Proceedings of The Institution Of Mechanical Engineers Part H, Journal of Engineering In Medicine. 2016; 230:71-4. (impact factor 1.0)
- Patients with bicuspid aortic valve are likely to receive an aortic valve prosthesis during prophylactic resection of their ascending aortic aneurysm. Raffa GM, Wu B, Pasta S, Morsolini M, Bellavia D, Romano G, et al. International Journal of Cardiology. 2016; 206:97-100. (impact factor 4.6)
- Early distal remodeling after elephant trunk repair of thoraco-abdominal aortic

aneurysms. Raffa GM, Pasta S, Gentile G, Scardulla F, Wu B, D'Ancona G, et al. Journal of Biomechanics. 2016; 49:2398-404. (impact factor 2.9)

- Pasta S, Scardulla F, Rinaudo A, Raffa GM, D'Ancona G, Pilato M, et al. An *In Vitro* Phantom Study on the Role of the Bird-Beak Configuration in Endograft Infolding in the Aortic Arch. Journal of Endovascular Therapy. 2016;23:172-81. (impact factor 3.1)
- Constitutive modeling of ascending thoracic aortic aneurysms using micro-structural parameters. Pasta S, Phillippi JA, Tsamis A, D'Amore A, Raffa GM, Pilato M, et al. Medical Engineering & Physics. 2016; 38:121-30. (impact factor 1.6)
- Computational fluid dynamics of the ascending aorta before the onset of type A aortic dissection. Malvindi PG, Pasta S, Raffa GM, Livesey S. European Journal of Cardio-Thoracic Surgery. 2016. (impact factor 2.8)
- In Silico Shear and Intramural Stresses are Linked to Aortic Valve Morphology in Dilated Ascending Aorta. Pasta S, Gentile G, Raffa GM, Bellavia D, Chiarello G, Liotta R, et al. European Journal of Vascular and Endovascular Surgery. (in press). (impact factor 2.9)
- Three-dimensional Parametric Modeling of Bicuspid Aortopathy and Comparison with Computational Flow Predictions. Pasta S, Gentile G, Raffa G, Scardulla F, Bellavia D, Luca A, et al. Artificial Organs. (in press). (impact factor 1.9)

Goals for 2017

- Submit a patent on the CDSS for the diagnosis and management of patients with ATAAs and on the rotary artificial lung pump for patients with respiratory insufficiency as a bridge to transplant.
- Work on the development of a startup to assist clinicians in the diagnosis and monitoring of cardiovascular pathologies such as ATAAs by the means of computational modeling.
- Development of biomedical devices in particular a minimally invasive intra-aortic balloon pump and wearable sensors.
- Apply for funding including call from Horizon 2020 (ie, SC1-PM-16-2017) and PON.
- Improve the scientific collaboration network with the Ecole Polytechnic of Lausanne, POLIMI and University of Sheffield, MINES Saint Etienne
- Strengthen the research team in terms of collaboration with students from UniPA, POLITO and University of Pittsburgh and CHAB

REGENERATIVE MEDICINE AND BIOLOGICS

The laboratories for regenerative medicine and research & development of biological therapies are focusing on the development of new cellular therapies for terminal organ diseases and post-transplant complications, as well as on the validation of new vaccine strategies for infectious diseases. The laboratory is strategically located at the IRCCS ISMETT and the team is made up of researchers and technical personnel specialised in the activity of research and development (*in vitro* and *in vivo* studies, first-in-man studies) and in the manipulation of biological samples of human origin. The personnel have been trained to operate according to rules of Good Manufacturing Practice (GMP) and Good Clinical Practice (GCP) for the designing and carrying out of pre-clinical/clinical experimentation and the production of advanced therapies. Projects in the pre-clinical development stage are aimed at the development of cellular products for the repairing and/or regeneration of tissue and the development of organotypic cultures to be used both for regenerative objectives and as models for pharmacological screening. Another important point of focus for research is the study and development of cellular therapies for the prevention of disease recurrence and the treatment of post-transplant infections. The new generation of vaccines, made up of re-combined proteins, is aimed at the treatment of the more important hospital-acquired infections of varying aetiology.

NK cell adoptive immunotherapy for the prevention of post-Transplant HCV re-infection and HCC recurrence

Project Leader

Ester Badami, PhD

Brief description

The aim of our project is the prevention of HCC and/or HCV recurrence after liver transplantation by the infusion of allogeneic NK cells isolated from the liver perfusate of DBD (Donor after brain death) immediately after liver transplantation. From the liver perfusate collected at the time of liver procurement from the donor, we can isolate high numbers of viable NK cells (0.5-2x109). We have optimized a protocol to activate NK cells such that both anti-viral and antitumor function is greatly increased compared to conventional IL2 activation. Intervention at the time of live transplantation, when the major reservoir of HCC and /or HCV has been removed, might favor tumor and/or infection eradication, respectively.

Results achieved in 2016

Patent:
"Immunoterapia NK-mediata e usi di essa";
Inventors: Ester Badami
Patent number: 102016000121081
Status: Pending

Grants:
Project Cell Therapies (ex Bioreactor) – a collaborative R&D project between ISMETT, UPMC, and the Bioreactor Group at the University of Pittsburgh
Status: approved

Call AIFA 2016 – Study Protocol – SIMVOLTx
Proposal code: TRS-2016-00001081
Status: Pending

Award:
Roster, PO FESR grant, Expert in Life Science ET4

Meetings and Publications

Meetings:

- "EATRIS product platforms combined meeting" – Barcelona Jan 2016
- "The International Liver Congress 2016" The European Association for the Study of the Liver EASL – Annual Conference Barcelona Apr 2016

- May 2016 "7th FIRST Meeting" - da Forum of Italian Researchers on Mesemchymal and Stromal Stem Cells" FIRST Annual Meeting – Milan
- Oct 2016 "NK 2016" - 16th Annual meeting of the society of Natural immunity - Taormina

Publications

Papers:

"Proportion of lymphocytes isolated from liver perfusate after harvesting procedures in donors after brain death"
Pagano D, Badami E, Conaldi PG, Di Francesco F, Vizzini GB, Casu A, Demetris A, Luca A, Gruttadauria S.
Status: ready for submission to American journal of transplantation - Brief report; Impact Factor 5.669

"HCV Replication in Gastrointestinal Mucosa: Potential Extra-Hepatic Viral Reservoir and Possible Role in HCV Infection Recurrence after Liver Transplantation"
Russelli G, Pizzillo P, Iannolo G, Barbera F, Tuzzolino F, Liotta R, Traina M, Vizzini G, Gridelli B, Badami E, Conaldi PG.
Status: ready for submission to PLOS Pathogens Impact Factor; 7.003

"Naturally arising antigen-specific Foxp3+CD4+CD25+ regulatory T cells fail to protect from spontaneous autoimmunity"
Badami E, Cexus O, Dyson J, Labrousse D, Londei M and Quaratino S.
Status: ready for submission to Nature immunology; Impact Factor 23.358

Goals for 2017

- *In vivo* proof-of-concept of NK cell anti-viral response after IFN-α activation
  Via CRO, 7th Wave Lab, St Louis, USA
- *In vivo* proof-of-concept of NK cell anti-tumor response after IFN-α activation
  at the IZS, Palermo
- FIRST STEP: OPBA and ISS protocol approval
  Underpin the soluble factors mediating IFNα-NK cell anti-tumor and anti-viral function
  Characterize the MIRNome signature of IFNα-NK cells

Papers for 2017

- IFN-mediated NK cell activation strongly enhances anti-viral immune response
  Badami E, Barbera F, Vella S, Gallo A, Coronnello C, Gaetani M, Carreca AP, Russelli G, Carcione C, Galvagno D, Pizzillo P, Spada M, Gridelli, B, Meuleman P, Conaldi PG (in preparation)
- The omics of IFNα-activated NK cells in tumor response
  Badami E, Barbera F, Vella S, Gallo A, Coronnello C, Gaetani M, Carreca AP, Russelli G, Carcione C, Galvagno D, Pizzillo P, Spada M, Gridelli, B, Meuleman P, Conaldi PG (in preparation).

Analyzing the secretory activity of multipotent fetal dermal cells (MFDCs) and adult dermal cells (ADCs): *in vitro* cell biological functions related to wound healing

Project Leader

Cinzia Chinnici, PhD

Brief description

Human fetal skin cells have been used to treat patients with chronic leg ulcers, as well as pediatric burns, resulting in a safe and efficacious approach. Interestingly, it was observed no trace of fetal cells in the patient tissue six months after therapy, supporting the hypothesis of a paracrine mechanism of healing. Growth factors and cytokines seem in an exclusive position as important modulators of human skin wound healing. However, their role is mainly speculative and need to be elucidated. We showed in a previous study that isolated fetal dermal cells are a population of mesenchymal-like cells, and have characteristics making them potential candidate for cell therapy applications (i.e., low immunogenicity, highly proliferating, ability to differentiate in different cell types, stable immunophenotype, stable karyotype, resistance to cryopreservation). In addition, our recent data showed that their conditioned medium (CM) contains high concentrations of soluble factors with proangiogenic effect.

Impact

Up to 50% of chronic wounds, particularly those with more than 1-year duration, remain unresponsive to advanced treatment approaches. Engineered skins are sophisticated but have several limitations, including very high cost and the lack of a functional vascular plexus. Angiogenesis of newly formed tissue is a critical aspect for a correct wound healing. Impaired local production of growth factors and cytokines has been associated with the onset of chronic wounds. Treatment of these wounds with fetal dermal cells and/or their CM is a low cost approach, and may have the potential to fully restore skin functions due to the secretory capacity of fetal dermal cells. Practical advantages of CM-based therapy over cell therapy includes the limitation of the potential risks associated with cell transplantation, which may pose the risk of a potential tumorigenicity of cells.

Results achieved in 2016

Multipotent fetal dermal cells (MFDCs) release a variety of growth factors and cytokines in their CM. Highly concentrated factors (≥ 3 ng/ml) include SDF-1 alpha, VEGF-A, and MCP-1, whose role in wound healing is related to angiogenesis, remodeling and cell recruitment. These values are promising when considering literature reports on MSC-CM values indicating the low concentration of factors as a major obstacle to CM-based therapy. Interestingly, the CM of MFDCs is active in inducing *in vitro* cellular responses related to wound healing, such as angiogenesis, and cell migration. *In vitro* functional assays showed CM-induced cellular responses such as capillary-like formation of human umbilical vein endothelial cells (HUVECs) and migration of target fibroblasts. We next observed that exosomes isolated

from the CM of fetal and adult dermal cells also promoted capillary-like formation and migration of cells *in vitro*. Preconditioning of target cells with exosomes was necessary in order to induce a cellular response, thus suggesting exosome internalization as a mechanism involved in exosome-mediated responses.

Meetings and Publications

- Gaetani M, Chinnici CM et al. "Unbiased and quantitative proteomics reveals highly increased angiogenesis induction by the secretome of mesenchymal stromal cells isolated from fetal rather than adult skin". Journal of Tissue Engineering and Regenerative Medicine J Tissue Eng Regen Med. 2017 Jan 19. doi: 10.1002/term.2417 (IF 4.7)

- Chinnici CM et al. Exosomes from human multipotent fetal dermal cells and their biological functions related to wound healing *in vitro* (in preparation).

Goals for 2017

Aim 1: to proceed with a further characterization of cell secreted factors by different approaches (i. e., analyzing expression of wound healing-related genes, presence of selected micro RNAs (miRNAs), exosomes characterization by western blot, nanosight, phospho-antibody array).
Aim 2: writing a protocol for the *in vivo* testing of MFDCs, ADCs and their CM in a model of angiogenesis and/or chronic wound.

Multipotent cells isolated from human fetal Liver and their use in liver regenerative medicine

Project Leader

Cinzia Chinnici, PhD

Brief description

We would like to test safety and efficacy of FLPCs in a mouse model of fulminant liver failure induced by i.p. (intraperitoneal) injection of a mixture of D-galactosamine plus bacterial lipopolysaccharides (GalN/LPS). The theoretical advantages of fetal liver progenitor cells (FLPCs) transplantation over hepatocyte transplantation in liver regenerative medicine are multiple: FLPCs are easily expandable *in vitro* and resistant to cryopreservation, making them ideal candidates for establishment of a cell bank (our preliminary results). Advantages of FLPC transplantation over extrahepatic MSCs transplantation may include the fact that being of hepatic origin, these cells are already committed toward the liver tissue, and may work more efficiently in stimulating the liver repair process, either because can differentiate into hepatocytes, or can release more efficiently hepato-protective soluble factors. For this purpose, treatment with FLPCs or their CM will be compared with a control treatment with placenta-MSCs or their CM.

Impact

Because of the shortage of organs, cell therapy has emerged as a potential treatment alternative to orthotropic liver transplantation (OLT). However, hepatocyte

transplantation poses some significant problems, including limited supply of donor organs, loss of hepatocyte viability following cryopreservation, and lack of an *in vitro* expansion of the cells. Clinical studies have shown that the injection of mesenchymal stem cells (MSCs), from the bone marrow, umbilical cord, and placenta for acute and chronic liver disease is a safe and efficacious treatment. Growing evidences suggest that the beneficial effects of transplanted stem/progenitor cells on liver regeneration may be due to a paracrine action of the cells. Therefore, the first step of the study was to isolate a cell population from the human fetal liver that would meet certain requirements: isolable with high yield, expandable *in vitro*, resistant to cryopreservation, and with a potential to restore liver function mainly due to presence of factors with liver regenerative properties in their conditioned medium (CM).

Results achieved in 2016

Wrote the protocol for the animal study to be submitted to the Organismo Preposto al Benessere degli Animali (OPBA) for Ministry of Health approval.

Meetings and Publications

- Chinnici CM et al. Multipotent cells isolated from human fetal liver release growth factors and cytokines with a potential role in liver tissue repair (in preparation).
- Chinnici CM et al. Low cell-density cultured human fetal hepatocytes show *in vitro* evidences for epithelial to mesenchymal transition. Ready to be submitted.

Goals for 2017

Aim 1. Submit the animal study to OPBA
Aim 2. *In vivo* experiments set-up

Hsp10/EPF as a potential immuno-modulator during virus-mediated Type 1 Diabetes

Project Leader

Simona Corrao, PhD

Brief description

The increased incidence of type 1 diabetes (T1D) seems to be related not only to a genetic susceptibility but also to the involvement of environmental factors and association with prior viral infections, such as cytomegalovirus (CMV), Epstein Barr virus (EBV) and others. The molecular mimicry between viral antigens and β-cell proteins (i.e. insulin, GAD65) is one of the potential mechanism to influence immune responses leading to autoimmunity and T1D. Since autoantibodies against Hsp10 have been found in the sera from acute pancreatitis and fulminant T1D patients, a series of in silico analyses were carried out in order to search similarities between Hsp10 and several proteins involved in T1D and immune responses. Moreover, Hsp10, also known as Early Pregnancy Factor (EPF) for its involvement in the mother's immune tolerance to the fetus during pregnancy, exhibits immune suppressive roles.
As mesenchymal stem cells (MSCs) are known to be active in immune modulation,

human MSCs (hMSCs) from different donors were treated or not with the pro-inflammatory cytokines, TNFα and IFNγ, alone and in combination, since the possible influence of tissue inflammation during infections could determine local changes in Hsp10 expression.

Impact

The question to be answered is whether Hsp10 is directly related with the virus-mediated T1D initiation. The hypotheses are: 1) Hsp10 can work as an effector antigen, with possible sequence similarities with hCMV and EBV epitopes working as antigens; 2) Hsp10 could modulate the immune response to viral infections and thus have a role in the physiopathology of T1D.

Results achieved in 2016

1) In silico study showed that Hsp10 has striking similarities with CMV proteins UL57 and pp65, even if the Hsp10 sequence is different from the UL57 sequence involved in the molecular mimicry (674-PYAVAFQPLLAYAY-687). The similarities with EBV proteins were found in different portion of Gp42 sequence, BOLF1, EBNA3C and BFRF3 proteins. No significant similarities were found with gB, gH and gL complex, BLRF2, and BZLF1. For these first analyses about the homologies between Hsp10 and the main viruses associated to the onset of T1D, it is not possible to totally exclude a molecular mimicry exerted also by Hsp10.
2) Donor-deceased pancreases are often serologically positive to CMV, EBV or both. Hsp10 was assessed by immunofluorescence (IF) on human pancreases from donors who were negative or infected and did not develop T1D. Interestingly, Hsp10 not only showed a marked expression in pancreas from non-diabetic infected donors, compared to those from not-infected ones, but also it showed a correspondence with insulin in terms of both localization and intensity. This may suggest a complex role of Hsp10 not only in virus-related immune response but also in modulating β cell functions, such as insulin trafficking and release.
3) qPCR analysis showed that Hsp10 expression is up-regulated after 18h and 48h of treatment with 10ng/ml TNFα, but strikingly not with IFNγ (10 ng/ml). Their effects seem to be reciprocally influenced (no variation in terms of expression) when they are mixed together (cytomix). This may suggest a potential local immuno-modulatory effect favoring viral survival.analyses showed changes in the expression levels of Hsp10 after inflammatory stimuli, with a constant increased expression induced by TNFa and variable expression induced by IFNg treatment.

Other activities

Involvement in human pancreatic islet isolation at the DRI's cGMP Core Facility group, led by Dr. Elina Linetsky. The training provides all the necessary skills required to perform islet isolation from human pancreases using the Ricordi's chamber, and to be independent in setting up a suitable laboratory (perfusion, isolation and purification hoods) under the related Standard Operating Procedure (SOP).
Acquired skill to use a new fully automated islet cell counter (ICC3; Biorep, Miami, FL) to quantitate the mass, size-distribution, and purity of isolated human pancreatic islet cell

preparations, in order to compare it with the current standard manual procedure.
Preliminary retrospective internal study for analyzing and calculating the North American Islet Donor Score (NAIDS) according to the description by Wang et al (2016). The calculated NAIDS was then compared to the yield of islet obtained and the enzyme used for the isolation. The NAIDS could become a predictive score for the success of islet isolation.

Meetings and Publications

Meetings
50th Miami 2017 Winter Symposium - Diabetes: Today's Research – Tomorrow's Therapies (22—25 January 2017| Hyatt Regency Miami, USA

Goals for 2017

1) Further information and analyses with more donors are needed to understand the role of Hsp10 in T1D, such as relationship between inflammatory pathways and viral infections.
The extracellular/immunomodulatory role of Hsp10 expressed by MSCs could be carried out isolating hMSCs from tissues (e.g. bone marrow) from different donors, and inducing them to overexpress Hsp10 for developing new "engineered" MSCs population to be used as cell-based immunosuppressive long-term therapy during human pancreatic islet transplantation.
Moreover, the expression of Hsp10 in human islets will be evaluated by immunofluorescence in other human pancreas, and its possible role in islet function could be evaluated in specimens derived from immunodeficient mice transplanted in the kidney capsule with human pancreatic islets.
2) Improvement of skills acquired in human pancreatic islet isolation (both for research and transplant) and in accepting or refuse pancreases offered for research and/or transplant. Moreover, the retrospective study on donors' features, NAIDS values, and the outcome of the isolations, will be used for comparison and finding possible correlations.

Study of the globin function in zebrafish heart regeneration and development

Project Leader

Paola Corti, PhD

Brief description

Heart regeneration is a natural process in zebrafish occurring upon ventricular resection or cryoinjury of the cardiac muscle. The infarcted site is replaced by new tissue by the cardiomyocyte proliferation. Through biomolecular approaches, we plan to understand the regenerative mechanisms with respect to the globins function and the effect of nitrite in order to understand the pathways that regulate cardiac regeneration.

Impact

Myocardial infarction is one of the leading causes of mortality in developed countries. Human heart loses its ability to regenerate the cardiac tissue early on during embryonic development. To determine the molecular players involved in the

natural cardiac regeneration occurring in zebrafish can ultimately render available new genetic therapies to induce regeneration in humans.

Results achieved in 2016

We have analyzed the effects of nitrite on the regenerating heart under hypoxia and found that the initial inflammatory response is influenced by nitrite exposure. We recorded a strong decrease in the amount of red blood cells (RBCs) accumulated in the injury and an increase in the number of neutrophils and leukocytes in the damaged portion of the ventricle. Moreover, at the beginning of the proliferation program nitrite treatment significantly increased the number of proliferating cardiomyocytes.

We reported the discovery of a new red blood cells (RBCs) globin Globin X (Xgb) of ancient origin found in fish. This newly discovered globin exhibit a six-coordinate geometry and a nitrite reduction rate up to 200-fold faster than human hemoglobin. We established Xgb dependent nitrite reductase activity in fish RBCs to increase nitric oxide (NO) bioavailability *in vitro* and to inhibit potently platelet activation.

We characterized the zebrafish neuroglobin, cytoglobin 1 and cytoglobin 2 and discovered that: neuroglobin has comparable biochemical properties to those of human neuroglobin; cytoglobin 2 (but not 1) is comparable to human Cytoglobin; cytoglobin 1 is unexpectedly penta-coordinate and it is a fast nitrite reductase. We found it for the first time in the fish red blood cells.

We generated the first generation of Globin X, Cytoglobin 1 and Cytoglobin 2 mutants using the CRISPr/Cas9 technology for gene editing.

Meetings and Publications

Meetings

Invited lectureships:

Function of six-coordinate globins. O2BiP Conference, Hamburg (Germany), September 11, 2016.

Publications:

Corti P, Ieraci M, Tejero J (2016). Characterization of zebrafish neuroglobin and cytoglobins 1 and 2: zebrafish cytoglobins provide insights into the transition from six-coordinate to five-coordinate globins. Nitric Oxide 53:22-34. PMID: 26721561. (IF=3.670)

Corti C, Xue J, Tejero J, Wajih N, Sun M, Stolz DB, Tsang M, Kim-Shapiro DB, Gladwin MT (2016). Globin X is a six-coordinate globin that reduces nitrite to nitric oxide in the fish red blood cells. PNAS 113(30):8538-43. PMID: 27407144. (IF=9.423)

Goals for 2017

To establish the zebrafish globins knock out mutant lines and start assays to determine the globins functions *in vivo* during development and heart regeneration.

To characterize the effect of nitrite on the early immune system response in the process of heart regeneration, particularly the nitrite effects on neutrophils and leukocytes.

To analyze the gene expression changes in response to nitrite exposure in a model of bloodless zebrafish. This mutant develops in the absence of RBCs and in comparison with a wild type zebrafish, it will allow to identify genes specifically involved in the response of RBCs to nitrite. These genes will then be analyzed in the heart regeneration model.

Development of novel vaccines against infectious diseases

Project Leader

Bruno Douradinha, PhD

Brief description

To date, no vaccine is available against human immunodeficiency virus (HIV), despite the multiple efforts put on it during the last decades. A vaccine against this viral pathogen must not only elicit both cellular and humoral arms of the immune system, but also induce a mucosal immunity. In fact, sexually transmitted HIV uses mucosal ports of entry, and a mucosal immune response would both prevent a new infection and its further spreading to uninfected individuals. Genetically engineered *Saccharomyces cerevisiae* strains expressing HIV antigens have shown promising pre-clinical results, as their can stimulate a T cell response. However, most *S. cerevisiae* strains tend to induce a poor mucosal immune response, even if administered orally. In this work, we propose the use of probiotic S. *cerevisiae* strains which have been genetically manipulated to express on their surface the HIV Gag antigen. Probiotic S. *cerevisiae* strains are known to naturally induce an immune response in the colon and to be resistant to the gastrointestinal harsh environments, such as acidic gastric juice and bile salts. Also, since the probiotic yeasts can be easily produced and stored for future consumption in lyophilized form, vaccine vectors derived from them would be ideal to be used in developing countries where infrastructures lack the capacity of store and handle products which need to be refrigerated, like most of the vaccines. Furthermore, since probiotic *S. cerevisiae* strains have been used in humans and with veterinary purposes, without indication of adverse effects, they also received genwwwerally regarded as safe (GRAS) status by Food and Agriculture Administration (FDA). We developed an independent research project, aiming to genetically engineer probiotic *Saccharomyces cerevisiae* yeasts and use them as antigen carriers to induce cellular, humoral and mucosal immunity. We are building a collection of probiotic S. cerevisiae yeasts and novel genetic tools to transform them. We are designing DNA sequences containing S. cerevisiae promoters, transcription terminators and membrane proteins, together with selected HIV antigen sequences.

Impact

So far, no vaccine is available against HIV. After multiple, unsuccessful attempts, the most promising strategy to date is RV144, a prime/boost regimen consisting in a canarypox virus encoding env/gag/pro genes and the recombinant Envelope

protein subunit gp120. Clinical trials in Thailand showed a 60% efficacy after 12 months which gradually decreased to 31,2% after 42 months. Thus, novel approaches are still needed for an HIV vaccine. The use of genetically engineered *Saccharomyces cerevisiae* strains expressing HIV antigen gp160 induced a potent cellular immune response in mice. However, a response against HIV must elicit both cellular and humoral arms of the immune system and a mucosal immune response, since this virus can be transmitted sexually through mucosal surfaces. While laboratorial *S. cerevisiae* strains confer a weak mucosal response, their probiotic counterparts induce a potent sIgA secretion in the colon and have immunomodulatory properties. Interestingly, different probiotic S. cerevisiae strains were shown to induce diverse types of immune response.

Results achieved in 2016

Probiotic *S. cerevisiae* strains were transformed with the bicistronic plasmid pCEV-G1-Km (pCEV) in its simple form or with the HIV gag gene sequence optimized for expression in the cell wall of *S. cerevisiae* using the AGA1p/AGA2p system (Gag). We successfully expressed HIV Gag protein in the yeast surface and observed that genetic modification did not impair neither phagocytosis by human dendritic cells from healthy donors *in vitro* nor resistance to simulated gastrointestinal stresses. Based on the cell surface markers and cytokines secreted by healthy donors dendritic cells (DCs) following genetically engineered yeasts, we assume these immune cells polarize in a type 1 response. To measure a specific HIV Gag response, we matured DCs derived from an HIV+ patient with transformed yeasts and incubated them with autologous T cells from the same patient. Interestingly, only DCs, which have been in contact with Gag-expressing probiotic *S. cerevisiae* strains, were able to efficiently perform HIV Gag antigen presentation to T cells, as observed by clonal expansion of the former when later incubated with a Gag peptide pool. Our preliminary results show that genetically engineered probiotic strains of *S. cerevisiae* Sb and Sc47 are promising vaccination strategies against HIV. In collaboration with the Graduate School of Public Health (GSPH), University of Pittsburgh, we are characterizing their immunogenicity *in vivo* and *in vitro*. Overall, this work has already resulted in 1 patent submission, 4 scientific publications (2 research articles, 1 review article and 1 genome public submission) and 10 scientific presentations in international scientific meetings and invited talks (4 oral and 6 posters).

Meetings and Publications

Meetings

Poster presentations

Engineering of vaccines against infectious diseases and efficacy evaluation in a humanized mouse platform. Viana IFT, Dhalia R, Palma ML, Douradinha B, Nascimento E, Garcia-Bates T, Duangkhae P, Mailliard R, Rinaldo C, Marques ETA and Bility MT. Infectious Diseases and Microbiology department retreat, University of Pittsburgh, Pittsburgh PA, USA, September 8-9th 2016

Oral presentations

Development of a mucosal vaccine against HIV based on genetically-engineered

Saccharomyces cerevisiae probiotic strains, in American Society of Tropical Medicine and Hygiene (ASTMH) 65th annual meeting, Atlanta GA, USA, November 13-17th 2016

Vaccine Discovery in Sicily: targeting our own problems to help others, in Ri.MED Annual Symposium, Palermo, Italy, October 17-18th 2016

Patents

Probiotic yeast as vaccination vector, Douradinha B., Italian Patent Office, filled 17/11/2016.

Publications *(accepted)*

Lipid droplet levels vary heterogeneously in response to simulated gastrointestinal stresses in different probiotic *Saccharomyces cerevisiae* strains. Zamith-Miranda D, Palma ML, Matos GS, Schiebel JG, Maya-Monteiro CM, Aronovich M, Bozza PT, Bozza FA, Nimrichter L, Montero-Lomeli M, Marques ET Jr, Martins FS and Douradinha B. J Funct Foods. 2016 Mar: 21. (IF 3,97)

Publications *(in progress)*

Non-Immune Cells Differentially Regulate the MHC Class II Expression by Modulating the Proteasomal Degradation of Class II Transactivator. Palma ML, Duangkhae P, Douradinha B, Dhalia R, Rigato PO, Nascimento EJM, Oshiro TM, Mailiard RB, Duarte AJS, Marques ETA Jr. Gene Therapy. Re-submission (IF 3,24)

In vivo and *in vitro* anti-malarial effect and toxicological evaluation of the chloroquine analogue PQUI08001/06. Reis PA, Pais KC, Pereira MF, Douradinha B, Costa NF, Kaiser CR, Bozza PT, Areas ALL, Zalis MG, Ferreira ML, de Souza MVN, Frutuosa VS, Castro-Faria-Neto HC. To be submitted to ChemMedChem (IF 2,98)

Goals for 2017

To set the Fondazione Ri.MED Vaccine Discovery laboratory. The laboratory will be set initially at the Ri.MED/ISMETT joint research lab space in the ISMETT infrastructures. Initially, we will be focused in developing a vaccine against the multidrug resistant bacteria Klebsiella pneumoniae that is currently a major clinical concern in hospital recovering patients and immunocompromised individuals. We are currently establishing a collaboration with GSK Vaccines (Siena, Italy) to identify, produce and test novel antigens against K. pneumoniae. This synergetic collaboration will be strengthened by further collaborations with multidrug resistant bacteria reference laboratories and hospitals (e.g., Policlinico of Palermo), which will provide sera samples of patients who suffered a K. pneumoniae infection, essential for immunogenicity and functional studies.

In parallel, we will continue the HIV vaccine project based on genetically engineered *S. cerevisiae* strains in a regimen of collaboration with the researchers at GSPH (Dr. Mariana L. Palma, Prof. Robbie Mailliard and Prof. Moses T. Bility). They will continue to study the *in vitro* interaction with of the genetically engineered yeasts with

human dendritic cells, either from healthy or HIV+ individuals, to measure the level and type of immune response induced by the transformed strains. Also, they will study the cellular, humoral and mucosal immunization potentials of Gag expressing yeasts in an animal model of humanized mice, where both immunogenicity and challenge studies can be performed.
Moreover, in 2017, we plan to apply to USA Department of Defense (DoD) with vaccine projects against HIV and K. pneumoniae.

Bioengineering a kidney in an ectopic site

Project Leader

Maria Giovanna Francipane, PhD

Brief description

Worldwide, increasing numbers of patients are developing end-stage renal disease, and at present, the only treatment options are dialysis or kidney transplantation. Dialysis is associated with increased morbidity and mortality, poor life quality and high economic costs. Transplantation is by far the better option, but there are insufficient numbers of donor kidneys available. Thus, novel strategies to generate transplantable tissue are a healthcare priority.
The kidney is one of the most complex organ in the human body, consisting of more than 26 different cell types. Over the last few years, our understanding of mouse kidney development and breakthroughs in pluripotent stem cell biology have guided *in vitro* protocols for the formation of human kidney organoids containing at least 8 distinct renal cell types. Whilst impressive, an unbiased comparison of their expression profile with that of human fetal tissue suggests kidney organoids show histological features congruent with first trimester human kidney. Many pressing questions need to be answered regarding these induced pluripotent cells (iPSC)-derived kidney organoids; for example, how stable is the differentiation state *in vivo*? The potential for the formation of teratomas or other neoplasms is a major safety roadblock to clinical application of iPSC-based therapies. Unfortunately, the lack of a suitable transplantation site for long-term *in vivo* studies remains a major issue.
Paradoxically, orthotopic engraftment of kidney tissue in the adult does not provide an environment conducive to vascularization and functional differentiation, with the generation of perfused renal tissues capable of producing urine remaining a major challenge. Among potential endogenous "bioreactors," the lymph node (LN) stands out, exhibiting permissive properties for maturation of several tissues including kidney rudiments. Thus, we are proposing the LN as an *in vivo* system to evaluate the efficiency and safety of iPSC differentiation protocols, and ultimately identify the optimal cell source(s) for successful human kidney tissue engineering.
For this project, we established collaboration with:

- Dr. Carlton Bates, a clinical Pediatric Nephrologist, scientist, and an expert in kidney development, with a particular focus on nephron and ureteric progenitors;
- Dr. Sunder Sims-Lucas, a junior faculty member in the Pediatric Nephrology Division specialized in kidney development (particularly the stromal compartment) and vasculogenesis;
- Dr. Leif Oxburgh, a research scientist expert in kidney development, who has developed innovative techniques to isolate and differentiate purified progenitor cells and iPSCs toward a renal fate;
- Drs. Thomas Kleyman and Lisa Satlin, clinician-scientists/nephrologists with over 30 years of experience in epithelial cell biology and renal physiology;
- Dr. Catherine J. Baty, a junior faculty specialized in lymphatic endothelial cell biology;
- Dr. Jacqueline Ho, a clinical Pediatric Nephrologist, scientist, and an expert in kidney development.

The study is also supervised by Dr. Eric Lagasse, a stem cell biologist and bioengineer, with over 20 years-experience in tissue regeneration including pioneering the use of the LN as a bioreactor for organogenesis.

Impact

Overall, this project will have a wide-ranging impact for tissue engineering approaches for the rebuilding of functional tissues *in vivo* including - but not limited to – the kidney.

Results achieved in 2016

Upon transplantation in syngeneic conditions inside the LN, we previously showed (2014) mouse metanephroi undergo successful organogenesis, developing into nephrons with glomerular and tubular functions[5,6]. During the years 2015-2016, we found that:

1. The mouse LN also supports engraftment of human fetal kidney;
2. The engrafted human kidney is progressively vascularized by the host;
3. Staining for different kidney markers and partial three-dimensional (3D) reconstructions show the grafts have a normal architecture;
4. Injection of 10,000 kDa MW Texas Red Dextran into the recipient mice suggests ectopic nephrons are capable of glomerular filtration into the proximal tubule.

As the use of embryonic tissue/cells may not be a viable source for kidney reconstruction due to the scarcity of this resource, we also investigated the possibility of implanting kidney organoids generated from mouse nephron progenitors and later, human induced pluripotent stem cells (iPSCs). iPSCs were differentiated using the Bonventre protocol7. Several mice were transplanted each with one organoid. The injected organoids engrafted and become vascularized as early as one week after injection. A few WT-1 (podocyte marker) positive structures (potential primitive glomeruli) and several LTL positive tubules could be observed. While LTL was present prior to injection, there were no vessels or WT-1 expression *in vitro*. However, the WT-1 positive structures were not able to further maturate into functional glomeruli *in vivo*. Moreover, overtime, we observed presumptive cartilage inside the LN. Overall, this data indicates that optimal conditions for the stable long-term *in vivo* propagation of human nephron progenitors have yet to be achieved.

Meetings and Publications

Publications

- M. G. Francipane, and E. Lagasse. Toward organs on demand: breakthroughs and challenges in models of organogenesis. Current Pathobiology Reports. July 2016.
- M. G. Francipane, and E. Lagasse. Towards organs on demand: a new platform for successful kidney organogenesis. Atlas of Science. April 2016.

Posters

- M. G. Francipane, S. Sims-Lucas, and E. Lagasse. Regenerating a kidney in a lymph node. McGowan Institute for Regenerative Medicine – 15th Annual Scientific Retreat. Farmington, PA, USA. March 6-8, 2016.

Seminars

- M. G. Francipane, and E. Lagasse. Toward Engineering a Novel Transplantation Site for De Novo Kidney Regeneration. Bridgeside Research Forum, Pittsburgh, PA, USA. February 19, 2016.

Awards

- Article "Pluripotent stem cells to rebuild a kidney: the lymph node as a possible developmental niche" featured on the cover of Cell Transplantation Journal (July 2016 issue).

Conferences attended

- American Society of Nephrology Kidney Week Chicago, IL- 2016. Nov 15-20.

New memberships

- American Society of Nephrology.

New peer-review activities

Joined the Editorial Board of the following journals:

- Austin Journal of Pathology & Laboratory Medicine
- SL Clinical Medicine: Research

Grants submitted

- NIH Grant R01- 1R01DK113261-01. Role CoI.
- Stem Cell Translational Medicine's Young Investigator Award. Role PI.
- DOD Funding Opportunity Number: W81XWH-16-PRMRP-IIRA. Role: CoI
- McCune Foundation. Pediatric Device Seed Funding RFP. Role: CoI FUNDED
- High Impact, Interdisciplinary Science in NIDDK Research Areas (RC2). Funding Opportunity Number: PARP-16-126 (Salary coverage but not Investigator, because ineligible for the funding opportunity). UNDER REVISION

Goals for 2017

This project will focus on two aspects:

1) Using the LN as a technology platform to assess efficacy of newly emerging cell sources in human kidney tissue engineering. To date, we have assessed the *in vivo* outcome of human kidney organoids reproduced following the Bonventre method7. The kidney organoids have proved incapable of reaching full maturity when transplanted into the LN system. Rather, undifferentiated structures including presumptive cartilage

have often dominated the graft. While new organoids will be generated following other published protocols, we anticipate a similar *in vivo* outcome. Indeed, despite significant progress, human iPSC-derived kidney organoids obtained *in vitro* exhibit a high percentage of non-differentiating cells. Moreover, stability of cells which have already differentiated toward a renal lineage *in vitro* is unclear. A possibility exists that defined chemical cues are needed to guide and/or maintain renal cell progenitor maturation *in vivo*. The manipulation of pathways known to direct renal progenitors toward a particular fate with the use of small molecules is the only way to answer these questions. This would require transplantation of multiple groups of mice and the currently available funds are insufficient to pursue this goal. For this reason, I have applied to the CMRF grant (see below). Pending available fundings, we will add the following specific factors to APEL media8, in which the organoids are suspended, prior injection:

To expand the progenitor pool:

- Inhibitors of Smad2/3 phosphorylation, PD169316 and SB203580 as well as their inactive analogue, SB202474. We will use 10-μM dose of each inhibitor9 (Calbiochem).
- Inhibitor of Smad1/5/8 phosphorylation Dorsomorphin (DM). We will use 1-μM dose of DM10 (Sigma-Aldrich).

To drive NPC differentiation:

- CHIR99201. We will use 3μM CHIR99201 (Stemgent).

To differentiate NPCs into specific nephron segments:

- Notch Ligand, Dll1, to drive cells toward proximal fates. We will use 3μg/ml of human soluble Dll1 (PrepoTech).
- Notch Inhibitor, DAPT, to drive cells toward distal fates. We will use 1μM DAPT (Sigma).

We target having 10 mice per drug, 5 to be sacrificed at 3 weeks, and 5 to be sacrificed at 6 weeks post transplantation. Harvesting at different time-points is important to measure the stability of the cellular phenotype induced. Proper differentiation of injected nephron progenitors into major segments of nephrons will be assessed by immunohistochemistry staining for specific markers. Tubular function will be assessed by *in vivo* measurements of concentration of typical urine metabolites such as creatinine in the cyst fluid, representing ultrafiltrate that has been modified by tubular transport. To investigate whether the cyst fluid originates from the host circulatory system, we will inject fluorescence-conjugated low-molecular-weight dextran in host mice, and evaluate dextran accumulation in the cyst 2 hours later.

We anticipate that co-injection of small molecules will result in the expansion of the overall graft size and/or more efficient differentiation (including reducing the number of iPSCs that remain undifferentiated). If we do not see the desired effects, we would reconsider re-injecting the engrafted LNs with the factors on a daily basis and/or alter factor concentrations. We could also use other factors described in the literature. Incomplete maturation of functional glomerular or tubular structures could suggest that the iPSC-derived organoids may not be fully capable of forming stable renal structures (based on the known robust organizing properties of the LN for developing

kidney and other tissues). This would direct the scientific community to refine new approaches to direct iPSCs toward a renal fate.

2) Advancing our knowledge about the LN microenvironment. Understanding LN remodeling and adaptation upon tissue transplantation could prove valuable in future endeavors to create a "niche" for human kidney cells. In this context, we have evidence that fibroblastic reticular cells (FRCs, a subpopulation of the LN non-hematopoietic stromal cell compartment dynamically controlling LN expansion and contraction) drive vascularization of the ectopic tissue, an important prerequisite for its survival, maturation, and function. Further analysis will elucidate the specific molecular mechanisms triggered by FRCs.

Grants submitted during 2017

UPMC Competitive Medical Research Fund (CMRF) award. Assessing how co-injection of defined small molecules ameliorates expansion and differentiation of iPSC-derived renal lineages *in vivo*. Role: PI. UNDER REVISION.

Grants in preparation

NIH Grant R01. Role to be defined depending on permanent residency application timeline

Exosome for the diagnosis and monitoring of islet transplant rejection and as therapeutic tools in Type 1 Diabetes Mellitus

Project Leader **Marta Garcia-Contreras, PhD**

Brief description

Type 1 Diabetes Mellitus (T1D), the most severe form of diabetes mellitus, is a disorder triggered by environmental factors that results in the autoimmune attack against the insulin-producing ß cells localized in the pancreatic islets of Langerhans. As a consequence, there is a decrease in insulin synthesis that leads to hyperglycemic episodes in T1D subjects. Circulating autoantibodies against beta cell autoantigens are currently the only biomarkers clinically available. However these autoantibodies don't necessarily correlate with the loss of beta cells and are correlated with a relatively late stage of the autoimmune process and therefore are not suitable for disease intervention.

Transplantation of human islets to cure T1D is the only cell source currently used for cell-based therapies. Islet transplantation in recipients with T1D improves blood glucose control, reduces or eliminates the need for exogenous insulin injections to control hyperglycemia, prevents severe hypoglycemic episodes and maintains near normal HbA1c levels. However, the efficacy of transplantation has

been limited due to loss of islet viability and function during pre-transplantation or to islet rejection, or loss of graft function post-transplantation. Enhancement of islet viability prior to transplantation and improvement in long term function may improve clinical outcome.

To both ends, there is a need for non-invasive biomarkers that will allow to early diagnose T1D and to detect beta cell loss, whether this is caused by rejection, autoimmunity or other factors. Markers of beta cell loss may precede appreciable changes in insulin secretion, and thus may allow early intervention and prolong islet graft survival. Likewise, biomarkers of rejection, autoimmunity or other processes that can damage islet cell grafts could serve the same purpose.

Exosomes (EXOs) are small (approximately 30–200 nm in diameter) lipid vesicles derived from multivesicular bodies that are released by virtually all cell types. EXOs have emerged as important mediators in cell communication, transferring proteins, lipids and RNA species (miRNA, mRNA, tRNAs, etc.) between cells. Moreover EXOs have been shown to be enriched in a subset of RNA species found in the parental cell. EXOs are present in different body fluids including serum, urine, cerebral spinal fluid, and saliva and bronchiolar lavage fluid. The RNA content of EXO isolated from different sources can reflect biological events and disease processes. In particular, plasma and serum exosome RNA profiling have been shown to have a potential in the diagnosis and therapy of different diseases. Thus, plasma derived exosomes, enriched in specific RNAs, could provide a disease-specific diagnostic signature allowing for prediction and monitoring of T1D and beta cell loss. Furthermore, EXOs from different sources (ex. Mesenchymal stem cells) have been shown therapeutical effects on autoimmune diseases that could be used as a cell-free therapy for the optimization of pre-transplant culture of human islets, potentially to prolong human pancreatic islets functionality and clinical outcomes.

Impact

This project aims:

1) To identify biomarkers of early diabetes, or graft failure in islet transplantation that may allow early intervention. To use this information to identify the underlying causes of loss of insulin-producing cells.

2) To development cell-free based therapies to improve islet function and tolerance induction.

Results achieved in 2016

We demonstrate for the first time that the miRNA signature found in exosomes isolated from the plasma of T1DM subjects could serve as a potential diagnostic biomarker. In the comprehensive microarray analysis, seven biomarker candidate miRNAs were identified and further analyzed with qRT-PCR. We also found that

exosomes released by MIN6 Insulin-producing beta-cells and human islets under stress conditions hypoxia (3% O2), and inflammatory conditions (cytokine cocktail of IL-1β, 50 U/mL; IFN-γ, 1,000 U/mL; and TNF-α, 1,000 U/mL) express a distinct miRNA signature. A subset of 2/4 and 14/20 miRNAs were differentially expressed in MIN6-derived exosomes and human-islet derived exosomes, respectively, under inflammatory and hypoxic conditions. Furthermore, islets pre-incubated with T1D plasma-derived exosomes for 48h stimulated with 11 mM glucose showed a selective decrease on the second phase of the response. The second phase is important for insulin release kinetics. The results may suggest an impairment in insulin release due to the exposure of T1D plasma-derived exosomes which may be involved in the development/or in the pathology of T1D.
In the therapeutic setting we showed that MSC induces the expression of anti-inflammatory and immunomodulatory molecules when co-cultured with islets *in vitro*. And MSC-dependent or endothelial cell-dependent improvement of human islets function is partially mediated by secreted exosomes. Furthermore, MSC and derived exosomes exhibit a contrasting effect on the proportion of CD4+/CD25+ T cells. We have been able to load MSC-derived exosomes with immunomodulatory drugs.

Patents

UMIP87 "Co-transplantation of encapsulated cell types within scaffolds for cellular therapies for combined intra and peri capsule biologic effect"

CDA Thermo Fisher, and we are preparing an Invention disclosure for submission, to develop a kit for novel exosome content isolation.

Grants

NIH-NIDDK-HIRN-CHIB 1UC4DK104208. Engineering a Human Physio mimetic Islet Microsystem.

Diabetes Research Institute Foundation funding

Others

Junior Membership of the International Society of Extracellular Vesicles

Meetings and Publications

Meetings

- L.B. Boccuzzi, M.G.C. Garcia-Contreras, C.R. Ricordi. Effect of inflammation on miRNA expression in pancreatic beta cells and their exosomes. Miami Winter Symposium 2017 Diabetes. 22—25 january, 2017 | Hyatt Regency Miami, USA.
- P. Buchwald, M. Garcia-Contreras, A. Tamayo-Garcia, C.L. Stabler, C. Ricordi. Effects of Inflammation, Hypoxia, and High Glucose on Isolated Pancreatic Islets and Islet Function: A Metabolomics Study. Miami Winter Symposium 2017 Diabetes. 22—25, 2017 | Hyatt Regency Miami, USA.

- M. Garcia-Contreras, S.H. Shah, R.B. Goldberg, A. Mendez, C. Ricordi. Exosome associated microRNA profiling in Type 1 Diabetes. Miami Winter Symposium 2017 Diabetes. 22—25, 2017 | Hyatt Regency Miami, USA.
- L.B. Boccuzzi, M.G.C. Garcia-Contreras, C.R. Ricordi. Effect of Inflammation on Mirna Expression in Pancreatic Beta Cells and Their Exosomes. ABRCMS. November 9 – 12, 2016. Tampa Convention Center in Tampa, FL
- M.Garcia-Contreras, S.H. Shah, R.B. Goldberg, A. Mendez, C. Ricordi Exosome associated microRNA profiling in Type 1 Diabetes – NIH –Human Islets Research Network – Annual Meeting 24-27 may 2016.
- Andras, IE, Leda, A, Garcia Contreras, M, Bertrand, L , Skowronska, M, Toborek, M. Extracellular vesicles of the blood-brain barrier: role in the HIV-1-induced amyloid beta pathology. The Society on NeuroImmune Pharmacology 22nd Scientific Conference, April 6-9, 2016.

Journal articles

- Andras IE, Leda A, Contreras MG, Bertrand L, Park M, Skowronska M, Toborek M. Extracellular vesicles of the blood-brain barrier: Role in the HIV-1 associated amyloid beta pathology. Mol Cell Neurosci. 2016 Dec 29;79:12-22. doi: 10.1016/j.mcn.2016.12.006.
- Baidal DA, Ricordi C, Garcia-Contreras M, Sonnino A, Fabbri A. Combination high-dose omega-3 fatty acids and high-dose cholecalciferol in new onset type 1 diabetes: a potential role in preservation of beta-cell mass. Eur Rev Med Pharmacol Sci. 2016 Jul;20(15):3313-8.
- Gomez-Meade C. A., Lopez-Mitnik G., Messiah S. E., Garcia-Contreras M., Sanchez J. Vitamin D status in children and adolescents with type 2 diabetes in a sun-rich environment. CellR4 dec 2016; 4 (6): e2214
- Vitamin D status in children and adolescents with type 1 diabetes in a sun-rich environment. Gomez-Meade C. A., Lopez-Mitnik G. V., Messiah S. E., Garcia-Contreras M., Sanchez J. CellR4 2016; 4 (5): e2140

Goals for 2017

Scientific discoveries/ Innovation

- Define RNAseq profiles and levels in serum-derived exosomes from serum samples of islet transplant recipients, both in the immediate post-transplant (acute) and later follow-up (chronic).
- To correlate RNA candidates with clinical history and laboratory measures of graft function, rejection and islet autoimmunity.
- Improve drug-delivery to Insulin-producing beta-cells by drug-loaded exosomes
- Co-transplant islet and Mesenchymal Stem Cell-derived exosomes to determine whether this affords protection from immune-mediated destruction in Islet transplantation.

Meetings

- Rapamycin-loaded Exosomes: A strategy to enhance drug-delivery to Insulin-producing beta-cells. Miles Brooke, Marta Garcia-Contreras, Camillo Ricordi. ISEV 2017. Toronto, Canada.
- Exosomes secreted by Insulin-secreting cells and human islets under stress conditions reveal an altered microRNA profile: Implications for Monitoring Islet transplantation. Marta Garcia-Contreras Alejandro Tamayo, Miles Brooke, Carlo Bosi, Luciarita Boccuzzi, Peter Buchwald1, Paul D Robbins, Camillo Ricordi. ISEV 2017. Toronto, Canada.

Manuscripts under review or accepted for publication in 2017

- A Metabolomics Study of the Effects of Inflammation, Hypoxia, and High Glucose on Isolated Human Pancreatic Islets. Garcia-Contreras, Marta; Tamayo-Garcia, Alejandro; Pappan, Kirk; Michelotti, Gregory; Stabler, Cherie; Ricordi, Camillo; Buchwald, Peter. Manuscript ID: pr-2017-00160c. Journal of proteome Research.
- Plasma-derived exosome characterization reveals a distinct microRNA signature in long duration Type 1 diabetes. Marta Garcia-Contreras, Sanket H Shah, Paul D. Robbins, Ronald B. Goldberg, Armando J. Mendez and Camillo Ricordi. Nature Scientific Reports.
- Human Endothelial-derived exosomes for enhancement of *in vitro* human islet function. Marta Garcia-Contreras, Paul D. Robbins, Armando J. Mendez and Camillo Ricordi. Nature Scientific Reports.
- Purification of Wnt/TCF/LEF/beta-catenin activity from bone marrow-derived mesenchymal stem cell exosomes transporting Wnt3a. McBride, Jeffrey; Rogriguez-Menocal, Luis; Candanedo, Ambar; Garcia-Contreras, Marta; Badiavas, Evangelos Van. Nature Scientific Reports.
- Review: Exosomes as biomarkers and theraputic tools for Type 1 Diabetes Mellitus. Marta Garcia-Contreras, Paul D. Robbins, and Camillo Ricordi. Journal of endocrinology.

Expandable organoid lines: new strategies for the development of autologous cell therapies and for *in vitro* disease modeling

Project Leader

Antonio Lo Nigro, PhD

Brief description

This project aims to elucidate the potential of organoids by developing autologous cellular therapies for pediatric liver diseases and by establishing *in vitro* systems to model drug induced liver injury, hepatitis viral infections, liver inherited diseases and liver cancer.

Impact

The further development of this technology in the context of liver pathologies may provide:

1) an advanced therapeutic medicinal product (ATMP), by coupling stem cell and gene therapy as a possible alternative to liver transplantation in the context of pediatric genetic diseases.
   A number of inherited diseases, involving metabolic and genetic defects, lead to early liver cirrhosis. These defects are due to mutated enzymes/transport proteins and result in an altered liver homeostasis and in a heterogeneous class of diseases with different incidences, penetrance, age of onset and clinical outcomes. As for liver diseases not depending on inherited mutations, in many cases liver transplantation is the only possibility for long-term survival. The generation of an organoid biobank from these genetic diseases will help the discovery and preclinical validation of novel drugs in a patient/disease specific way. Moreover, recent "genome editing" technologies allow to specifically correct the mutations, responsible of the specific disease. In this way, we could combine gene therapy with stem cells, opening a new scenario for autologous therapies in genetically inherited liver disease, after the correction of the disease-leading mutations.
2) an *in vitro* platform for modeling drug induced liver injury (DILI).
   DILI leads to the withdrawal of many drugs from the market. It accounts for up to 30 percent of cases of acute hepatitis and is the most common cause of acute liver failure in the United States. Inter-individual differences in hepatic metabolism depends on genetic polymorphisms of genes, belonging to the family of cytochrome P450. For this reason, the possibility to perform predictive *in vitro* test is of a primary importance for pharmaceutical company.
3) an *in vitro* platform for modeling viral hepatitis.
   Chronic liver diseases caused by hepatitis viruses affect more than 500 million people worldwide and often result in death due to cirrhosis or hepatocellular carcinoma. Among the viruses that cause hepatitis there are the hepatitis A-G virus. Recently, the development of multiple direct-acting antivirals (DAAs) led to an effective treatment for HCV infection, now eradicated in most cases within

weeks. However, not all patients are eligible for DAAs treatment and other viral hepatitis, as hepatitis E (HEV) and Hepatitis G virus (HGV) still require effective treatments/vaccinations and/or new immune-therapeutic approaches to eradicate the infections, as hepatitis B virus (HBV).

4) a "living biobank" for modeling liver cancer.
Liver cancer is the third leading cause of cancer-dependent death in the world. Isolation of liver organoids from the above tumors and the comparison to their "healthy" equivalent may provide an *in vitro* model for understanding liver cancer biology and its progression. Their unlimited cellular expansion permits a deeper investigation of the mutations acquired over metastatic progression, may facilitate the development of new diagnostic tools and may provide a source for high throughput screening, for the discovery and preclinical validation of novel cancer drugs in a patient specific manner.

Results achieved in 2016

Several organoid lines were derived from liver biopsies, obtained from patients undergoing liver transplantation. These lines showed a limitless expansion potential, at least for one year, and strongly resemble the ductal epithelia rather than liver parenchyma (i.e. hepatocytes), if cultured in expansion medium. Upon differentiation, organoid-derived hepatocytes acquire expression of phase I detoxification enzymes and secretes albumin and bile acids.
Preliminary analysis showed the expression of several entry receptors for HCV and HBV in liver organoids and that oHep, upon inoculation with HCV JFH1 replicons, supports the replication of this virus, as demonstrated by detection of viral core in their medium and HCV RNA in the supernatants and in the cellular pellet.

Meetings and Publications

Meeting:
Poster presentation: Expandable liver organoids, an *in vitro* model of hepatocyte infections by viral agents. EMBL Symposium on Organoids: Modeling organ development and disease in 3D culture EMBL Heidelberg, Germany 12-15 October 2016.

Publications
PDGFRα + Cells in Embryonic Stem Cell Cultures Represent the *In Vitro* Equivalent of the Preimplantation Primitive Endoderm Precursors. Lo Nigro A*, de Jaime-Soguero A, Khoueiry R, Cho DS, Ferlazzo GM, Perini I, Abon Escalona V,Lopez Aranguren X, Chuva de Sousa Lopes SM, Koh KP, Conaldi PG, Hu WS, Zwijsen A Lluis F, Verfaillie CM. Stem Cell Reports, 2017, IF: 7,023 *Corresponding author
PIWI-interacting RNA (piRNA) signatures in human cardiac progenitor cells. Vella S, Gallo A, Lo Nigro A, Galvagno D., Raffa GM, Pilato M, Conaldi PG. International Journal of Biochemistry and Cell Biology, 2016; IF: 3.905

Goals for 2017

1. To get IRRB approval
2. To perform remaining experiments for writing a patent application "Bi-potential

liver precursors, user-friendly culture method for obtaining them and their applications"
3. To perform remaining experiments for writing the paper "Expandable liver organoids, an *in vitro* model of HCV-mediated infections"
4. To establish living biobanks for liver cancer and liver paediatric diseases
5. To get hands-on experience with CRISPR/CAS9 (genome editing technology)
6. To find necessary collaborations for implementing the project

Mesenchymal and epithelial cells isolation from human placenta

Project Leader	**Mariangela Pampalone, PhD**
Brief description	We are working to optimize the procedure of mesenchymal and epithelial cells isolation from human amniotic membrane with phenotypic characterization of markers and preliminary evaluation of their immunomodulatory capacity.
Impact	Preliminary results show that the cells inhibit the peripheral blood mononuclear cells (PBMC) proliferation, stimulated by CD3 and CD28, by direct contact and by their conditioned medium action.
Results achieved in 2016	We optimized cell extraction protocol in order to improve the yield, the purity and the vitality of mesenchymal and epithelial cells. The cells in culture were monitored by evaluating the morphology and their ability to grow *in vitro* for the possible use at different passages in culture. The cells were analyzed to FACS analysis from the first to the fourth step in culture to monitor the expression of the characteristic markers commonly expressed by mesenchymal cells (CD90 - CD13) and epithelial (CD166 - CD324). During the first extractions, the cells had a percentage of blood component unsuitable to a possible *in vivo* infusion. We showed that mesenchymal and epithelial cells express a higher percentage of its characteristic markers after two passages in culture in concomitantly to a lower expression of characteristic markers of the blood. The protocol of extraction was optimized in order to obtain preparations having <5% of CD14, CD66 HLADR and CD3 after extraction procedure, in order to use the cells for possible *in vivo* infusions without *in vitro* manipulation. We have prepared different batches not manipulated *in vitro* suitable for *in vivo* infusion of mesenchymal and epithelial cells in cirrhotic rats. Moreover, we performed preliminary experiments *in vitro* to assess the immunomodulatory ability of mesenchymal cells in co-culture with PBMC. The PBMC were extracted from human whole blood and, in order to assess their proliferation,

were pre-labeled with succinimide ester of carboxyfluorescein (CFSE). We simultaneously compared the immunomodulatory capacity of the conditioned media to several days with and without exosomes and the immunomodulatory capacity of different concentrations of exosomes, isolated by ultracentrifugation, products from the cells after 48 hours of starvation

Meetings and Publications

Meetings

- 7th FIRST Milan May 13th, 2016.
- SillaJen PHOCUS (HEP024) European Investigators' Meeting, Rome 19
- 21 October 2016.

Goals for 2017

To improve the immunomodulation test on mixed lymphocyte reaction (MLR) evaluating the immunomodulatory capacity of MSCs and AECs (or their derivates) against NK cells, Cytotoxic Lympocytes, dendritic cells and testing the secretion of pro-inflammatory and anti-inflammatory cytochines including TGFβ, IL10, TNF-α and INF-γ.

To test angiogenetic, anti-fibrotic effects of MSCs and AECs *in vitro* and after to use this capacity to improve pancreatic islets transplantation.

To test fibrin as 3D scaffold in order to evaluate his ability to maintain islet cell architecture, beta cell insulin secretion and islet angiogenesis in co-infusion with MSCs or AECs. Bio-engineered scaffolds can potentially provide an alternative extra-hepatic transplantation site for islets by improving nutrient diffusion and blood supply to the scaffold.