SUBMISSION FORM OF PROPOSALS FOR DOCTORAL RESEARCH PROJECTS

Objective of the Doctoral Programme in Health Sciences and Technologies

The objective of the new interdepartmental Doctoral Programme in Health Sciences and Technologies is to train the next generation of leaders in industrial, clinical, and academic research. Our goal is to develop an organic research programme at the interface between engineering and medicine, which is inspired by the quantitative and integrative approach of physical sciences, and by the latest development in biomedical research, drive the development and clinical translation of disruptive health technologies.
The doctoral training programme will prepare students in conducting research which:
- Extend the comprehension of how human physiology and pathology work in term of physical and chemical mechanisms, and how these mechanisms respond when perturbed by external factors such as therapies, changes in life style, and environmental factors;
- Develop new technologies that by leveraging on this mechanistic understanding pursue a wide spectrum of applications relevant to human health, including prevention, diagnosis, prognosis, treatment, and rehabilitation.

Procedural aspects on the submission of proposals for doctoral research projects

Every year the PhD process will start with the submission of proposals for doctoral research projects. Each proposal must be submitted jointly by two supervisors, one providing the clinical expertise, the other the technological expertise. The Project Selection Committee will select a number of projects that is three times the number of available scholarships and should be distributed in similar proportion between medical-led or technology-led proposals. The resulting list of projects will be included in the call for student applications that the Executive Committee will compile soon after. Each student, depending on their degree, will be able to apply only for a sub-set of projects; among them each student will be allowed to select three projects, and name them in order of preference; however, in some cases it might not be possible to satisfy all requests, and some students might be offered a research project different from those they selected.

Doctoral training program

In order to be admitted to the selection, a student needs a five-year higher education degree, which includes at least one module for each of the following disciplines: mathematics, physics, computer science, biology, physiology, and anatomy.

Max number of proposals for each member of the Academic Board: 3 (three)
Max number of selected projects for each member of the Academic Board: 2 (two)
Max number of selected projects for 2019: 12 (twelve)

Title of the project

Development of a hydrogel construct engrafted with thrombogenic agents to prevent endoleaks' occurrence following Endovascular Abdominal Aortic Aneurysm Repair

Student's degree (you can choose more than one, if needed)

Yes/Not	Cultural area
Yes	Medicine, biology, or related disciplines
Yes	Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines

Student's skills (you can fill more than one field, if needed)

Cultural area	Skills
Medicine, biology, or related disciplines	Basic knowledge of normal vascular biology.

Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines	Knowledge of material chemistry (polymer chemistry preferred) and biomaterials. Knowledge of material characterization techniques. Basic knowledge of low temperature plasma engineering.

Tutors (2, from different cultural areas and with at least 1 from the Academic Board)

Cultural area	Name & Surname	Department
Medicine, biology, or related disciplines	Gianandrea Pasquinelli	DIMES
Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines	Carlo Angelo Borghi	DEI

Research project

	Synthetic description
Summary (max 1000 chars)	Endovascular aortic aneurysm repair (EVAR) is currently the most common method for the elective treatment of abdominal aortic aneurysm (AAA), a common disease of elderly. Although the technical success rate for abdominal aortic endografting is high, reinterventions due to endoleak (EL), a persistent flow of blood into the aneurysm sac after device placement, are frequent. Since persistent type II EL (ELII) is associated to increased probability of AAA rupture and vascular death, AAA sac embolization with metallic coils is undertaken to reduce its development. This treatment exposes patients to unwanted metallic load and interference, as metal spirals are radiopaque, in diagnostic imaging procedures that are used in post-procedural follow-up. In the present research project, we propose to develop an innovative injectable, biocompatible hydrogel in which thrombogenic agents are engrafted to augment hemostasis locally. This construct may allow a selective intraprocedural sac embolization to reduce post procedural pELII and possible AAA rupture.
Objectives (max 1000 chars + max 5 relevant references)	- Synthesis of a biocompatible hydrogel acting as a three-dimensional platform in which biologically active agents can be engrafted to augment hemostasis locally. - Identification of a biological agent or a combination of several agents that can trigger thrombosis locally. - Hydrogel engraftment with thrombogenic substances. - Realization of an injectable compound that can be placed through a catheter guide in the aneurysmal sac, in order to fill the residual free lumen of the sac, not occupied by the endograft, through polymer expansion, thereby preventing the onset and the persistence of type II endoleak in the follow up of an abdominal aortic aneurysm EVAR treatment.
Rationale and scientific background y (max 2000 chars+ max 5 relevant references)	The abdominal aortic aneurysm (AAA), a common disease of elderly, is the pathological, segmental enlargement of the abdominal aorta to greater than 1.5 times its normal size. In most adults, an aortic diameter >3.0 cm is generally considered aneurysmal. For people aged between 65 and 80 years the incidence of AAA is 7.6% in men and lower (4.2%) for women. According with the American and European guideline the indication for the AAA treatment is given when the AAA reach the diameter of 5.5 and 5.2 in men and women

	respectively. If left untreated, it has been estimated that a third of these aneurysms would eventually rupture with overall mortality rates of up to 90%. First undertaken in 1987, endovascular aortic aneurysm repair (EVAR) is currently the most common method for the elective treatment of AAA. EVAR consists in the internal coverage of the aorta using a metallic stent-graft covered with an impermeable (polytetrafluoroethylene or polyester) fabric. Sealing of the device against the aortic wall proximally and distally the aneurysm excludes the aneurysm sac from the systemic circulation thus preventing subsequent rupture. Sealing is achieved by the radial force and by the active fixation though hooks and barbs of the stent-graft on the aortic wall. Although the technical success rate for abdominal aortic endografting is high (99%), endograft-related reinterventions are common, with an incidence that ranges from 11 to 30%. Among the causes of reinterventions, endoleak, a persistent flow of blood into the aneurysm sac after device placement with consequent failure to completely exclude the aneurysm, is the most frequent. Five types of endoleaks exist. Type II endoleaks (ELII) are the most common (estimated incidence of 8.6 to 23%) and consist in retrograde flow to the aneurysm sac from branches such as the lumbar and inferior mesenteric arteries. The clinical impact of ELII has not been completely clarified; however persistent ELII (pELII) that is present ≥6 months since intervention, was found to predict the EVAR-related complications; such leaks are also associated with increased probability of AAA rupture during follow up after EVAR. Some pre-treatment morphological factors, such as multiple patent aortic branch efferent vessels (≥6) and AAA sac thrombus volume (<40%), increases the risk of pELII after EVAR [1]. A few preliminary clinical studies have provided evidence that AAA sac embolization, using either metallic coils alone or 5 mL of fibrin associated with at least one coil, may reduce the incidence of ELII, thus lowering the post procedural risk of AAA rupture [1]. Further it has become evident that the volume of the implanted coils is critical to treat pELII effectively and a concentration of >0.17coil/cc was considered the best treatment to prevent post-procedural pELII. Major limitation of this approach is the presence of artifacts caused by the presence of coils, that are radiopaque, at imaging follow-up. In fact, the presence of coils can hamper pELII when duplex ultrasound (DUS) and computed tomography angiography (CTA) are performed. There is therefore the necessity to develop a material that is injectable but easily controllable to avoid distal embolization, expandable thus filling the aneurysmal sac, thrombogenic to increase AAA sac thrombus load > 40%, radiolucent so as not to interfere with imaging performed in the post procedural follow-up. In the present research project, we propose to develop a biocompatible hydrogel three-dimensional platform in which biologically active agents can be engrafted to augment hemostasis locally. This construct, once injected in the aneurysm sac, may allow a selective intraprocedural sac embolization to reduce post procedural pELII and possible AAA rupture.
Preliminary results if existing (max 1000 chars+ max 5 relevant references)	The members of the research group have consolidated experience in the synthesis of polymers [2], in the surface modification of polymers using plasma technology [3], in the study of the interaction between cells and tissues with micro or nanostructured biomaterials aimed at application in the cardiovascular field in an in vitro and in vivo setting [4]. Previous clinical experiences indicate that: - AAA sac embolization, using metallic coils, has been performed in patients considered to be at high risk of developing type II endoleak. The risk characteristics, defined on the basis of a retrospective study, are the presence of 6 or more efferent vessels from lumbar and inferior mesenteric arteries and / or a volume of thrombus in the sac less than 40% of the total volume of the sac [5]. - A further study explored the effectiveness of sac embolization by comparing patients at risk for pELII in which sac embolization was performed vs untreated patients; at 1 year a lower incidence of pELII was found in patients undergoing embolization [1]. - Since the end of 2016 our vascular surgery unit has begun to embolize patients at risk with a dose of metallic coils proportional to the volume of

	the aneurysmal sac; preliminary results show a further reduction of pELII at 6 months, thus suggesting that the number of coils is critical for preventing pELII and AAA rupture.
Research project including methodology (max 5000 chars)	The research activities will be based on the collaboration and contribution of the participating research groups with the contribution of well established external collaborations. The DICAM group has the task of synthesizing the hydrogels necessary for the realization of the three-dimensional platform. The DEI group has the task of using plasma ionized gases technologies to increase hydrogel biocompatibility, adherence, and engraftment of the thrombogenic biological agents. The DIMES group has the task to identify biologically active agents can be engrafted to augment haemostasis locally and to assess the construct biocompatibility and thrombogenicity. External collaborators will have the task to test the usability of the injectable construct; preliminary tests will be performed in a simulator currently used to train vascular surgeons at EVAR repair (vascular surgery unit at DIMES). **Task 1: Synthesis of the hydrogel.** Hydrogel with tailored structure will be designed, according to two different synthetic approaches, with two characteristics in common: i) step-growth polymerization will be employed, in order to avoid the presence in the final material of non-reacted residual monomer, which may be toxic; ii) melt polymerization will be preferred to reduce both the environmental impact and the toxicity of the solvent. The first approach aims to obtain linear multiblock biodegradable non-toxic hydrogel copolymers, starting from a hydrophilic soft block, such as polyethyleneoxide with suitable molecular weight, and a biodegradable hydrophobic segment, properly designed to get the desired mechanical response. Through the second synthetic approach, we propose to obtain, by melt one-pot synthesis, hyperbranched polymers starting from multifunctional monomers, the gel point being controlled by varying polymerization conditions and monomers ratio. **Task 2: Identification of a biological agent or a combination of several agents that can trigger thrombosis locally.** Hemostasis is a dynamic process occurring in distinct integrated phases, including the formation of the platelet plug and the propagation of the clotting through the coagulation cascade; in this step there is a sequential activation of fibrin lattices that reinforce the platelet plug. Distinct approaches will be explored to promote local hemostasis in clinical practice. Thrombin is a naturally occurring enzyme that converts fibrinogen into fibrin, an integral step in clot formation. It is reconstituted from a lyophilized powder and can also be used in conjunction with a gelatin matrix agent that provides the thrombin with an immediate scaffold for clot formation. As an example, thrombin granules are useful for promoting hemostasis at vascular graft suturing sites. Human thrombin and a recombinant thrombin are available for clinical use. Fibrin sealants are typically a two-component system that includes a solution of concentrated fibrinogen and factor XII, and a solution of thrombin and calcium. When mixed together just prior to use, a fibrin clot forms. Fibrin sealant can be used to control bleeding at vascular anastomotic sites. They are also used to control bleeding from cut surfaces. **Task 3: Hydrogel engraftment with thrombogenic agents.** Plasma processes will be used to allow the adhesion between the selected thrombogenic agents and the hydrogel copolymers through the introduction of specific functional groups on the hydrogel surface. Chemical processes will be used to link thrombogenic proteins to hyperbranched polymers. The achievement of the tasks 1, 2 and 3 will be verified through the following methodologies: setting up and optimization of the synthesis procedures; analysis

	of the effects of the operating conditions; chemical and physical characterization of the produced hydrogel. Confocal immunofluorescence microscopy will be used to visualize the distribution and composition of biological thrombogenic agents in the hydrogel three-dimensional platform. **Task 4: Biocompatibility and haemostatic assays.** To assess the biocompatibility, human umbilical vein endothelial cells (HUVECs) will be carefully seeded onto unmodified hydrogel copolymers or hyperbranched polymers, and hydrogels with engrafted thrombogenic agents. On days 1, 3, and 7, HUVEC seeded on top of hydrogel constructs will be visualized using a LIVE/DEAD® fluorescent probe membrane integrity assay (viable and dead cells are labelled green and red, respectively). Thrombogenicity assay will be performed in a chamber loop system in accordance with ISO 10993-4. Whole blood will be isolated from human volunteers and used within 1 hr from harvesting. **Task 5: Evaluation of the clinical usability.** The hydrogel construct will be assayed for its ease in handling and delivering in a realistic simulated environment similar to that experienced during endovascular procedures. Customized silicone vessels duplicating the anatomy and pathology of a AAA will be manufactured to make more realistic the simulation analysis. Vascular surgeons will be able to determine the practical usability of the hydrogel construct.
Innovation potential (scientific and/or technological) (max 1000 chars)	The rationale of the research project is to develop a unique product, which is not yet present in the biomedical market and is innovative enough to overcome the limits of the current systems used to embolize the aneurysmal sac. At the present, prevention of persistent ELII is achieved through AAA sac embolization, using either metallic coils alone or 5 mL of fibrin associated with at least one coil. This procedure exposes the patient to a consistent metal load whose health effects are difficult to predict over time. The compound that will be developed will avoid the patient the unwanted metal load. In addition, it will be biocompatible, injectable, capable of expanding at the site of the inoculum and capable of efficiently triggering the embolization of the aneurysmal sac. All these properties are not found in the current devices used to prevent ELII. Finally, the material will be radiolucent and therefore will not interfere, as metal spirals do now, in diagnostic imaging procedures that are used in post-procedural follow-up.
Expected results and applications to human pathology and therapy (max 1000 chars)	- Synthesis of a hydrogel with tailored structure suited for embolization in cardiovascular applications. - Development of an injectable, biocompatible three-dimensional platform engrafted with one or several biological agents able to trigger thrombosis locally. - When delivered in a specific setting the synthetic and biological construct components will work synergistically to promote clot formation at the aneurysm sac. - The hydrogel expansion will provide a matrix on which a blood clot can form. - The biological agent will activate platelet and pertinent coagulation factors promoting the conversion of fibrinogen in fibrin. - Polymerized fibrin will enhance stability of the expanded construct. - The construct will be injectable and biocompatible thus allowing its clinical application for the embolization of the aneurysmal sac during endovascular abdominal aortic aneurysm repair.

Available resources for the project

	Synthetic description

Research environment (labs involved, background, and location)	The involved laboratories are: - Clinical Pathology Lab at DIMES (S. Orsola-Malpighi University Hospital) equipped with cell culture, histopathological and ultrastructural facilities. - LAMAC at DICAM equipped with techniques for synthesis of polymeric materials and material characterization - LIMP at DEI where the design, development and testing of the plasma sources and the materials' treatment with the plasma and characterization will be performed
Main equipment (facilities and location)	- Light microscopes, fluorescent microscope (clinical pathology facility, DIMES) - Confocal fluorescent microscope, transmission and scanning electron microscope (BIGEA, outsourcing facility with DIMES specific contract) - Vacuum chamber, Dielectric Barrier Discharge plasma actuator, Schlieren system (LIMP) - Glass polymerization reactor, FT-IR spectrofotometer fitted with ATR, GPC, capillary and plate to plate rheometers, calorimeters (LAMAC) - Vascular simulator and 3-D print faculties (Simulation Lab, Industrial Bioengineering, DIMES)
Additional funding (title, amount, start date, duration)	The research group has availability of commercial funding (without that will be dedicated to this project).

International collaborations for the project (also in view of the Student's secondment)

	Project	**Location and team**
#1	Research collaboration and exchange of students	Nabil Chakfè. CHRU de Strasbourg, Geprovas, France and Geprovas
#2	Research collaboration	Elena Gostjieva, William G Thilly. Dept of Biological Engineering, MIT, Boston, USA
#3	Research collaboration and exchange of students	Frédéric Heim, Université de Haute Alsace, Mulhouse, France and Geprovas

1. Mascoli C, Freyrie A, Gargiulo M, Gallitto E, Pini R, Faggioli G, Serra C, De Molo C, Stella A. Selective Intra-procedural AAA sac Embolization During EVAR Reduces the Rate of Type II Endoleak. Eur J Vasc Endovasc Surg. 2016; 51:632-639.
2. Fabbri M, García-Fernández L, Vázquez-Lasa B, Soccio M, Lotti N, Gamberini R, Rimini B, Munari A, San Román J. Micro-structured 3D-electrospun scaffolds of biodegradable block copolymers for soft tissue regeneration. European Polymer Journal. 2017; 94:33-42.
3. Fabbri M, Gigli M, Costa M, Govoni M, Seri P, Lotti N, Giordano E, Munari A, Gamberini R, Rimini B, Neretti G, Cristofolini A, Borghi CA. The effect of plasma surface modification on the biodegradation rate and biocompatibility of a poly-butylene succinate-based copolymer, Polymer Degradation and Stability. 2015; 121:271-279.
4. Pasquinelli G, Freyrie A, Preda P, Curti T, D'Addato M, Laschi R. Healing of prosthetic arterial grafts. Scanning Microsc. 1990; 4(2):351-62.
5. Gallitto E, Gargiulo M, Mascoli C, Freyrie A, De Matteis M, Serra C, et al. Persistent type II endoleak after EVAR: the predictive value of the AAA thrombus volume. J Cardiovasc Surg 2018;59(1):79-86.