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

Elastomers with tunable degradation as small diameter blood vessel substitutes for peripheral artery disease

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	Nadia Lotti	DICAM

Research project

	Synthetic description
Summary (max 1000 chars)	The research project aims to develop a small diameter (< 6 mm) synthetic vascular graft suited for arterial revascularization in patients with peripheral arterial disease (PAD) and critical limb ischemia (CLI). To allow limb savage and prevent vascular death, open surgery bypass revascularization using autologous vein grafts is the gold standard. When it is not possible, any other approach gives unsatisfactory result. Therefore, developing a tunable synthetic vascular prosthesis made up of new or modified elastomers represents a possible solution. The graft will be composed by a nanofibrous scaffold of elastomers with controlled hydrolysable properties; the scaffold will be biocompatible and will present a fast degrading internal portion and a slow degrading external part for blood contention; a non-thrombogenic surface will be established through plasma ionized gases technology; thanks to its controlled hydrolysable properties a rapid graft integration with human blood and vascular cells will be achieved. The small diameter synthetic vascular grafts should be able to substitute the autologous vein when donor site morbidity and limited autograft availability occur.
Objectives (max 1000 chars + max 5 relevant references)	- Prototyping a small diameter (< 6 mm) synthetic vascular graft suited for arterial revascularization in patients with peripheral arterial disease (PAD) - Synthetic vascular grafts with properties that prevent rupture at implantation, early thrombosis, poor host cell colonization, and late aneurysmal failure - Realization of the synthetic vascular graft which allows to treat patients with PAD whenever donor site morbidity and limited autograft availability occur - Realization of the synthetic vascular graft will allow to reduce the cost and complexity of the *ex vivo* tissue engineering manufacturing process
Rationale and scientific background y (max 2000 chars+ max 5 relevant references)	Peripheral artery disease (PAD) is an occlusive disease of the lower extremities affecting over 200 million people worldwide. In the past decade, its prevalence increased by 28.7 and 13.1%, in low- to mid-income and high-income countries, respectively. Infrapopliteal arterial and below-the-knee (BTK) PAD are the primary causes of critical limb ischemia (CLI). If left untreated, patients with CLI have a poor prognosis; 30% of patients will have major amputation and 25% may have died in one year. Revascularization is therefore the backbone of treatment for CLI.

	However, in these patients the endovascular procedures, i.e., percutaneous balloon angioplasty (PTA), bare metal stenting and drug eluting stents (DES) are burdened by significant failure in short-term follow-up. Accordingly, the inter-society consensus for the management of PAD, does not recommend endovascular treatments when long lesions, extremely calcified arteries, chronic total occlusions, intra-pedal disease, and multi-level stenosis are present. Open surgery bypass remains the best choice for such patients. Autografts is the gold standard for this clinical application, but donor site morbidity and limited autograft availability warrants the search for an effective alternative. Nonresorbable synthetic grafts, e.g., expanded polytetrafluoroethylene (ePTFE), polyethylene terephthalate (Dacron) and polyurethane (PU), and allogeneic fresh or cryopreserved arterial and vein grafts have poor patency at diameters less than 6 mm. Tissue engineered vascular grafts in which auto or allogenic progenitors, or mesenchymal stem cells were grown *ex vivo*, have shown great promise in animal studies and arteriovenous shunt clinical trials. However, high fabrication costs and long production times for patients limit the clinical adoption of these grafts. Decellularized tissue engineered grafts eliminate patient waiting periods and perform well in large animals, but production time and costs remain high. Therefore, in this clinical setting, the use of autologous vein grafts remains unparalleled. As a whole the patency rates for BTK PAD bypasses range from 65% at five years in the case a vein graft substitute to 25% at three years for the synthetic graft. Thus, there is a strong, yet unsatisfied, patient demand for small diameter (< 6 mm) vascular bypass grafts every year.
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 [1], in the surface modification of polymers using plasma technology [2], 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 [3, 4]. Previous experimental and clinical experiences indicate that: - Small diameter grafts (< 6 mm) derived from nonresorbable synthetic materials have poor clinical performance with low patency and high rates of thrombosis in short-term follow-up. They are also highly susceptible to intimal hyperplasia at the sites of anastomosis [3]. - Small diameter grafts from cryopreserved vein or arterial allografts have poor clinical performance due to aneurysmal dilatation in mid-term follow-up [5]. - Decellularized cadaveric matrices have limited clinical potential due to loss of original vessel mechanical properties; it has been also documented a poor host cell infiltration and healing at 6–12 months post-implantation.
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 external well established collaborations. The DICAM group has the task of selecting and synthesizing the elastomers necessary for the realization of the graft prototype. The DEI group has the task of using plasma ionized gases technologies to increase graft biocompatibility, hydrophilicity, surface roughness, and to make non thrombogenic the graft surface as well. The DIMES group has the task to assess the graft biocompatibility, thrombogenicity and integration with human blood and vascular cells. External collaborators will have the task to generate nanofibrous scaffolds from selected synthetized elastomers (ISOF, CNR), to characterize the mechanical properties of the graft prototype (Cesena, Cavalcanti Lab) and to test the scaffold for graft suturability and blood pressure resistance (Vascular Surgery unit at DIMES). **Task 1: Selecting and synthesizing the elastomers.** The synthetic approach will enable, throughout a rational optimization of reaction conditions, the synthesis of new materials with a particular molecular structure. A careful

combination of monomeric units and their disposition along the chain will provide polymers with unique properties, which are often absent in homopolymers. The final properties of the material will be modified by acting on the kind, relative amount, distribution and architecture of the co-monomeric units along the polymer chain. Homopolymers and copolymers employed for the fabrication of the scaffolds will be biocompatible, hydrolysable with tailored degradation kinetics and elasticity to support long-term cyclic strain.
In particular, the reabsorption of polymers will be differentiated so that the part of the polymer turned towards the blood flow will be rapidly degraded compared to the external portion, which will have the task of supporting the blood pressure acting as a leak-proof sheath to prevent bleeding.
Fast degrading is expected to be essential for effective host vascular tissue remodelling. This will greatly improve the graft porosity thus enabling immediate host cell infiltration after implantation.
The new synthesized materials will be employed for the fabrication of nanofibrous scaffolds.

Task 2: Fabrication of the nanofibrous scaffold with the required chemical-physical and mechanical properties.
Electrospinning is the ideal technique for producing nanofibrous scaffolds of high porosity and large surface area, which simulate the morphological properties of the extracellular matrix. Hence electrospinning is an excellent candidate for producing vascular grafts scaffolds. In the present research project, the new synthetic polymers will be electrospun into nanofibrous scaffolds using different solution parameters (e.g. viscosity, surface tension and conductivity) and process parameters (e.g. electric field and flow rate).
The manufacturing conditions will be finely tuned in order to allow the generation of cylindrical tube simulating the vascular wall architecture.

The achievement of the tasks 1 and 2 will be verified through the following methodologies: setting up and optimization of the synthesis procedures; optimization of the electrospinning process; analysis of the effects of the operating conditions; chemical, physical, and morphological characterization of the produced nanofibrous mats. The mechanical properties of the tubular graft will be investigated in a custom-built measurement system that will experimentally determine the strains during the stretching of the tubular graft in a dedicated actuator/sensor bioreactor system. In silico models will be developed for predicting the actual relationship between the pressure imposed to the specimen lumen and its deformation. Scanning electron microscopy will be used to determine the architecture of the graft and its morphological characteristics.

Task 3: Graft surface modification and engraftment with anti-thrombogenic properties
To guarantee the cell free arterial graft would be non-thrombogenic, additional studies will be performed using plasma (ionized gases) technologies. In fact, the major cause of early failure in biomaterial-based engineered vessels is lumen occlusion by formation of thrombus. To this regard, it has long been known that the presence of certain peptide sequences e.g. REDV (Arg-Glu-Asp-Val) promote a non-thrombogenic surface and at the same time favors endothelial cell progenitor engraftment, spreading and proliferation. Also surface coating with heparin coating has been demonstrated useful to prevent graft thrombosis. Plasma ionized gases technologies will include the treatment of the internal surfaces of the nanofibrous grafts in order to increase biocompatibility, hydrophilicity, surface roughness, and entertainment of antithrombotic substances. Atmospheric pressure cold plasmas will be used for this. The experiments will be performed with high voltage, high frequency discharges in DBD (Dielectric Barrier Discharge) plasma actuators and plasma jet actuators.
The achievement of an anti-thrombogenic surface will be assayed through the interaction of blood platelet-rich plasma with the cell free arterial graft within a closed-loop configuration, strain-based pulsatile conditioning actuator/sensor bioreactor system. The sample will be analysed by means of scanning electron

	microscopy (SEM) and the number of platelets adherent to the surface as well as their state of activation will be recorded. **Task 4: Biocompatibility assay and analysis of the graft integration with human blood and vascular cells.** Blood mononuclear cells (BMNCs) will be isolated from human volunteers. Human mesenchymal stem cells (MSCs) will be isolated from cryopreserved femoral arteries to be discharged for having exceeded 5 years preservation. BMNCs and MSCs cultures will be established seeding blood or vascular cells on 1x1 cm^2 pieces of the graft prototype; after 6, 24, 72 hrs cell viability will be assayed using a vital dye; additional samples will be processed for immunofluorescence using FICT labelled monoclonal antibodies (CD44, CD45) to visualize cell penetration in the graft by confocal microscopy; additional samples will be analysed using SEM to determine the cell engraftment in the scaffold, graft porosity and material degradation.
Innovation potential (scientific and/or technological) (max 1000 chars)	The aim of this research project will bring innovation from a clinical, scientific and technological perspective. - Clinically, there is a strong, yet unsatisfied, patient demand for small diameter (< 6 mm) vascular bypass grafts every year and peripheral artery disease is a common disease. Therefore, fabricating a synthetic vascular prosthesis made up of new or modified elastomers represents a clinical innovation. - A further innovation is the generation of synthetic materials whose overall characteristics can be tailored to the mechanical and physiological biomedical requirements with the flexibility offered by the chemical structure design. Accordingly, the reabsorption of polymers will be differentiated, as described in the research project outline, from the luminal side to the external one of the scaffolds; consequently, the polymer portion of the graft effacing the blood flow will degrade rapidly while the outer portion will degrade slowly to prevent blood bleeding. Fast degrading is expected to be essential for effective host remodelling. This will greatly improve the graft porosity thus enabling almost immediate host cell infiltration in an *in vivo* application. - Another innovation would be the use of plasma ionized gases technologies to increase graft biocompatibility, hydrophilicity, surface roughness, and making non thrombogenic the graft surface as well. This approach has not yet been sufficiently explored in the vascular field. - Finally, this approach is expected to reduce the cost and complexity of the tissue engineering manufacturing process. For example, using the plasma technology approach to make the graft surface resistant to platelet adhesion and activation it would make unnecessary to isolate, seed and grow an endothelium. This means that the cost and complexity could be significantly decreased by eliminating the need for a blood vessel biopsy, specific growth media, endothelial specific identity testing. Finally, eliminating the need to keep the tissue *ex vivo* alive allows for extended storage time, simpler shipping conditions, storage at the surgical site, and elimination of a quality control viability test.
Expected results and applications to human pathology and therapy (max 1000 chars)	- Development of modified and/or synthetized elastomers with controlled hydrolysable properties tailored for cardiovascular applications. - Development of a nanofibrous scaffold composed of modified and/or synthetized elastomers with controlled hydrolysable properties. - Generation of a biocompatible nanofibrous scaffold characterized by a fast degrading internal portion and a slow degrading external part. - Generation of a non-thrombogenic surface. - Achievement of a rapid graft integration with human blood and vascular cells. - Development of small diameter (< 6 mm) tubular vascular grafts suited for open surgery revascularization of patients affected by peripheral artery disease with critical limb ischemia.

Available resources for the project

	Synthetic description
Research environment (labs involved, background, and location)	The involved laboratories are: - Clinical Pathology Lab at DIMES (Policlinico S. Orsola Malpighi) 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 "S. Cavalcanti" ICM Lab at DEI, UOS Cesena, equipped with custom built bioreactor suited for vascular graft studies - ISOF CNR equipped with electrospinning processing technique facilities
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) - Actuator/sensor bioreactor systems (ICM Lab) - Instruments for the processing of polymeric materials (Electrospinning facility with humidity and temperature control and coaxial and multiple needles) ISOF CNR
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, France and Geprovas
#2	Research collaboration	E Gostjieva, WG 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

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2. 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, Vol. 121 (2015), pp. 271-279.
3. 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.
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5. Mirelli M, Buzzi M, Pasquinelli G, Tazzari PL, Testi G, Ricchi E, Conte R, Stella A. Fresh and cryopreserved arterial homografts: immunological and clinical results. Transplant Proc. 2005; 37(6):2688-91.