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 new in vitro models to evaluate drug absorption and metabolism

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. **Degree in Biomedical Engineering or Chemistry or Material science.**

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

Cultural area	Skills
Medicine, biology, or related disciplines	Pharmacology competences are preferred: knowledge of pharmacokinetics (how drugs are absorbed and metabolised in the body), pathology (the study of disease) and toxicology (how the body is affected by chemicals). Basic knowledge of cell behaviour and disease modelling are preferential.
Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines	Knowledge of material chemistry and biomaterials (polymer chemistry, natural polymers and hydrogels are preferred). Knowledge of mechanical and rheological characterization techniques is preferred. Good analytical skills. Basic knowledge of the mechanism of drug delivery.

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	Claudio Borghi	Department of Medical and Surgical Sciences
	Co-tutor: Giovanna Cenacchi	Department of Biomedical and Neuromotor Sciences
	Co-tutor: Arrigo Francesco Giuseppe Cicero	Department of Medical and Surgical Sciences
Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines	Maria Letizia Focarete	Department of Chemistry "G. Ciamician"
	Co-tutor: Stefania Rapino	Department of Chemistry "G. Ciamician"

Research project

	Synthetic description
Summary (max 1000 chars)	The development of new drugs is characterized by a long and very expensive multistep process with a large failure, potentially exposing patients to possible health risk related to side effects and pharmacological interaction during the registration trials. Traditional in vitro models (monolayer cells) do not strictly represent the complex kinetic of drugs in human tissues. On the other side, animal models are progressively considered unethical and, one more time, not always strictly representative of human physiology and pathophysiology. In this context, **the development of new in vitro models reproducing the histological structure and functional activity** of tissues are strongly needed, both to improve prediction of drug effects in humans, to plan more targeted clinical trials, and to

	improve the selection of more bioavailable and safe drugs and drug-association to be clinical developed. This could be particularly useful **to evaluate the absorption and metabolism of new and old (combined) drugs**, especially in liver, bowel and lung tissues.
Objectives (max 1000 chars + max 5 relevant references)	The main **research objectives** of this PhD project are: • **to realize new in vitro models by means of 3D bioprinting technology as tools in drug discovery and development**. This aim will be achieved through the development of a drug-screening platform by employing commercial bioprinters to simultaneously deliver a hydrogels, biochemical substances and specific cells. • **to evaluate adsorption and metabolism of new but also old drugs** wih the 3D in vitro models As regards **educational activities,** the project aims to provide student with knowledge on: (i) development of a platform of polymeric biomaterials; (ii) use of a bioprinter for 3D organ model fabrication (iii) use of material characterization techniques; (iv) experience in biological laboratory techniques; (v) experience in imaging techniques; (vi) experience in pharmacology and pharmacokinetics. This project covers basic research, technological development and has clinical relevance.
Rationale and scientific background y (max 2000 chars+ max 5 relevant references)	Drug discovery process is one of the most challenging field of research, where accurate *in vitro* human representative models, that provide reliable prediction of drug efficacy and safety *in vivo,* is definitely needed. At present, in fact, most conventional *in vitro* drug discovery experiments are performed in 2D monolayer cell culture systems, which do not mimic *in vivo* conditions for accurate evaluation of cellular responses to drugs. 2D cell culture systems often provide non-predicted or misleading results since drug effects are often altered. Engineered human tissues and organs hold a tremendous potential for predicting the effectiveness of drug responses, reducing, at the same time, cost, time, and failure rates in clinical trials. Body tissues and organs such as hearts, livers, kidneys, etc., are incredibly complex systems, made up of different cell types in a specific microenvironment that provides a kind of 'niche' for the cell. This environment contains key factors for cells to interact, grow and differentiate, and possesses the right mechanical properties. Advances in biomedical technologies and biomaterials, as well as in molecular and cellular biology, biochemical assays and imaging technologies, now enable researchers to engineer 3D human tissues and organs by 'playing' with (i) the combination of multiple types of cells, inserted in (ii) ECM-like hydrogels made of natural and/or synthetic polymers. These are the basic principle of 3D bioprinting, which can be defined as simultaneous deposition of living cells and biomaterials, with a specific layer by layer organization, using a computer-aided process. Additional components that can be deposited together with proteins, peptides, growth factors, biologically active particles, etc. With respect to other 3D *in vitro* systems, bioprinting presents the advantage of precise positioning of cells, controlled density, deposition of co-cultures [1-3]. Bioprinting finds application in several biomedical fields such as tissue engineering and regenerative medicine, transplantation, drug screening, cancer research. In particular, it is presently having a revolutionary impact on medical and pharmaceutical research and application [1-3]. Finally, bioprinting can be integrated with microfluidics to develop devices acting as miniaturized bioreactors for organ-on-a-chip technology.
Preliminary results if existing (max 1000 chars+ max 5 relevant references)	The Polymer Science and Biomaterials Group (Department of Chemistry, UNIBO) has recognized expertise on structure-polymer correlation of natural and synthetic polymeric biomaterials. The group has strong knowledge of material design, material processing through conventional and advanced innovative technologies and nanotechnologies, material characterization and study of

	biodegradability. The group has demonstrated the capability to develop polymeric systems for drug delivery and as tissue models for tissue engineering [4-7]. Moreover, it is a partner in recent national and regional projects in the biomedical field [8]. Regarding the specific topic of this project, the group has already performed preliminary study of the printability of natural polymeric hydrogels (collagen, alginate), to find a correlation between rheological properties and material processability. The cardiometabolic Unit (Dept. of Medical and Surgical Sciences, UNIBO) has a large experience in clinical pharmacology and pivotal international multi-center Phase II and III Clinical Trials on conventional and biological drugs.
Research project including methodology (max 5000 chars)	The research project aims at developing 3D in vitro models to evaluate adsorption and metabolism of new but also old drugs, by employing commercial bioprinters to simultaneously deliver a polymeric matrix typically in the form of hydrogel, precise amounts of given biochemical substances and specific living cells. 3D bioprinting will be operated in sterile conditions and in appropriate environmental conditions (temperature and humidity) to maintain cell vitality. The obtained 3D models will be used to evaluate adsorption and metabolism of the drugs by means of biological tests and integration of the 3D model with a microfluidic circuit. The activities will be based on the collaboration among the following research groups: The Polymer Science and Biomaterials Group, the Functional Imaging and Cellular Chemistry Group, the Cardiometabolic Clinical Trial Unit, and the Subcellular Diagnostics and Pathology Group**.** This PhD candidate will spend 50-60% of his/her time at the Department of Chemistry (prof. Focarete and prof. Rapino) and the 40-50% of his/her time at the medical Department (prof. Borghi, Cicero and Cenacchi). Furthermore, an international secondment of 3-6 months at Regemat (Spain) (a biotech company focused on 3D printing technologies for regenerative therapies and drug discovery and strongly research-oriented) is planned to enhance the knowledge of the candidate on biomaterials processing and use of the 3D bioprinting technology. Preliminarily to the experimental activities the student will need to acquire deep knowledge on the key aspects of *in vivo* environment and of native tissues and organs, together with a fundamental understanding of **clinical pharmacology and therapeutics.** The activities will deal with: **Activity 1:** DEVELOPMENT OF MATERIALS AND TECHNOLOGY Methodology: - Materials investigation: different kind of commercial hydrogels with different crosslinking mechanisms will be studied (alginate, gelatin, collagen, etc.), before being used in 3D bioprinting. Rheological characterization of the material properties in the sol and gel phase will be studied. - The effect of sterilization (mainly autoclave) on material properties will be evaluated - Acquiring knowledge on the design and development of a 3D bioprinting process - Development of different 3D construct architecture and morphology, resembling the key characteristics of native organs without cells to optimize construct pattern. - Chemical, physical, and morphological characterization of the produced plain materials in order to identify the most promising operating

	conditions. - Compression tests for the investigation of the mechanical properties of the produced materials. Stress-strain curves will be recorded to achieve information on Young's modulus, stress at break and strain at break. **Activity 2:** FABRICATION OF 3D CELL-ENCAPSULATED HYDROGEL-BASED TISSUE CONSTRUCTS Methodology: - Cell culture and material preparation - Bioprinting process investigation: the effects of the operating conditions on cell viability will be studied. - 3D Bioprinting construct fabrication **Activity 3:** BIOLOGICAL AND HISTOLOGICAL EVALUATION Methodology: - Culture with and without drug addition - Cell survival, proliferation, morphology - Semiquantitative protein expression by Western Blotting analysis - Histological analysis before and after drug treatment and immunohistochemical analysis with specific antibodies to define the cell phenotype - Ultrastructural study to characterize submicroscopical variation in cell organelles (transmission electron microscopy) and to verify the 3d structure of designed histological tissue (scanning electron mcroscopy) **Activity 4:** DRUG ADSORPTION AND METABOLISM This activity involves the integration of the 3D tissue model onto a microfluidic device for the drug metabolism study and biological characterization of cell-embedded miniaturized organ models. All results will be compared with conventional 2D platforms containing the same cells and drugs in order to validate the accuracy of data obtained by the 3D bioprinting method. The above described activities will be performed by selecting an organ model and a drug model as a proof of concept. **Activity 5:** PLANNING A FIRST-IN-HUMAN TRIAL WITH THE IDENTIFIED AND DEVELOPED COMPOUNDS Methodology - Evaluation of the legal procedures preliminary to the first test in humans - Selection of the drug that can more easily and safely administered to humans - Creation of a pharma-toxicologic dossier - Design of the protocol and of all the related documents to be submitted to the local ethical review board
Innovation potential (scientific and/or technological) (max 1000 chars)	The PhD project has ambitious scientific and technological innovation potentialities: - In the pharmaceutical field, 3D bioprinted organ models may replace conventional 2D cell and animal models enabling a more accurate prediction of drug therapeutic/toxic responses, in addition to a reduction in the cost of drug discovery. - 3D bioprinting allows the development of *in vitro* pathological models, that can

	be fabricated with diseased human cells, and might contribute to study tissue pathology or to test new therapeutics. - 3D bioprinting introduces a disruptive approach: the creation of personalized *in vitro* models, created from patient-derived cells, to realize a reliable patient-specific disease model, towards applications in personalized precision medicine. - Finally, 3D bioprinting has potentiality also in tissue engineering and regenerative medicine. However, bioprinting of complex functional organs for clinically relevant applications is still far from being realized and only single tissues have been successfully bioprinted (hollow vessels, cartilage, etc.)
Expected results and applications to human pathology and therapy (max 1000 chars)	The development of 3D culturing technologies to engineering miniaturized human organ models is expected to overcome some of the key challenges to implement the drug discovery processes. In fact, by recreating the microstructure and the biological functions of target tissues and organs, these systems are expected to complement or even substitute current drug testing platform that include *in vitro* molecular and cell tests, and *in vivo* models. This will allow to predict efficacy and safety of drug candidates earlier in the drug discovery process, thus speeding up the introduction of new drugs at a reduced cost. The present PhD project will be focused on **evaluation of the absorption and metabolism of new and old drugs** in models of liver, bowel and lung tissues.

Available resources for the project

	Synthetic description
Research environment (labs involved, background, and location)	This candidate will start working together with a second year PhD student having a background in Material Chemistry, already involved in 3D bioprinting for drug development (grant from Farmindustria). Therefore the candidate might also have a medical background and in this case, in order to allow the candidate acquiring the technical part of the project, initial effort will be dedicated to improve the candidate understanding and practising about the technology. This project activities are carried out mainly between two groups: - The group at the Chemistry Department, prof. Focarete and prof. Rapino, (via Selmi 2) will provide the training and expertise on polymers, hydrogels, processing technologies, as well as material characterization techniques and drug evaluation. - The group at S. Orsola-Malpighi University Hospital, prof. Borghi, dr. Cicero, prof. Cenacchi, will provide training and supervision on clinical pharmacology and will provide inputs for the design of the 3D model. **Laboratories of the Polymer Science and Biomaterials group at the Chemistry Department "Ciamician", UNIBO:** Laboratory equipped with main facilities for polymeric biomaterial preparation. Expert use of state-of-the-art solid-state characterization techniques of polymeric materials. Rheology for hydrogel properties testing. Mastering the electrospinning processing technique using first class equipment, use of simple polymer processing equipment. **Laboratory of Functional Imaging and Cellular Chemistry Group at the Chemistry Department "Ciamician", UNIBO**. This Laboratory, coordinated by Stefania Rapino (that will be co-tutor of the PhD student), will collaborate in the project with the availability of 3D Bioprinting instrumentation and suitable environment (sterile, controlled environmental conditions) for material fabrication and testing.

	Cardiometabolic Clinical Trial Unit at the Department of Medical and Surgical Sciences, UNIBO The clinical unit coordinated by Prof. Claudio Borghi includes a multidisciplinary team of clinical researchers, including the certified expertise in clinical pharmacology, that support the safety evaluation of first phase clinical trials of the S. Orsola-Malpighi University Hospital. This expertise will be employed to support the candidate during the development of the activities reported at point 5. **Subcellular Diagnostics and Pathology Laboratory at the Department of Biomedical and Neuromotor Sciences** The Lab, reference center in Italy and in Europe, is involved in ultrastructural diagnostics and research in the pathogenetic mechanism in human diseases: neoplastic and non neoplastic diseases of skeletal muscle, myocardium, central nervous system, kidney, focusing on the study of cellular organelles and structures involved in the main cellular function (proliferation, survival, apoptosis, autophagocytosis, pathological storage) in normal and pathological condition both ex vivo and in vitro.
Main equipment (facilities and location)	**Laboratories of the Polymer Science and Biomaterials group** are equipped with: - Instruments for the processing of polymeric materials (**Electrospinning facility** with humidity and temperature control and coaxial and multiple needles, Hot press, Miniature mixing-injection molding machine, Vacuum oven, Spin Coater, Temperature-controlled shaking bath); - **3D printing FDM apparatus (Makerbot), 3D Bioprinting (Regemat)** - Instruments for the thermo-mechanical and rheological characterization of polymeric materials (Differential scanning calorimeter and Modulated-DSC, Thermogravimetric analyzer coupled with Mass spectrometer, Dynamic mechanical thermal analyzer, Dynamometer, Rheometer); - Instruments for the chemical, morphological and structural characterization of polymeric materials (Polarized Optical Microscope with hot stage, Scanning Electron Microscope with EDS, Atomic Force Microscope, Transmission Electron Microscopy, Wide angle X-ray Diffractometer with heating device); Optical tensiometer; UV-Vis, ATR-IR and NMR spectrometers, Gel Permeation chromatography. **Laboratories of Functional Imaging and Cellular Chemistry Group** are equipped with: - **3D Bioprinting (CellLink)** **Cardiometabolic Clinical Trial Unit** is equipped with dedicated rooms, clinical services for put- and inpatients, non-invasive instrumental devices for the study of endothelial function, vascular function and a fully equipped laboratrory for blood chemistry and genetics. **Lab of Subcellular Diagnostics and Pathology** is equipped with devices in order to perform specimen preparation to ultrastructural study both with transmission electron microscope and scanning electron microscope (tissue and cell culture): Cryostat, Ultramicrotome, Western blotting, image analysis for morphometric studies. Cell biology and molecular studies on cell cultures are performed at CRBA lab (Azienda Ospedaliero-Universitaria S.Orsola-Malpighi)
Additional funding (title, amount, start date,	Funding already available at Dept. of Chemistry will cover the cost for laboratory testing (access to characterization instruments, lab consumables,

duration)	sterilization services): • Industrial funding and European project overhead: 100'000 Euro

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

	Project	Location and team
#1	Partner in a H2020 European project proposal preparation. The company is a biotech company focused on regenerative medicine and pioneer in the new and promising area of bioprinting, that uses 3D printing technologies for regenerative therapies and drug discovery.	REGEMAT 3D, Granada, Spain
#2	Partner in a H2020 European project proposal preparation.	Brighton Centre for Regenerative Medicine, University of Brighton, UK, (prof. Santin)
#3	With the team at Maastricht University it is presently active a research collaboration and an exchange of students. This collaboration will contribute to biofabrication process.	MERLN institute for Technology-Inspired Regenerative Medicine at Maastricht University, the Netherlands (prof. Moroni)

References:

[1] J. Park, et al."3D Miniaturizatin of Human Organs for Drug Discovery", *Adv. Healthcare Mater.*, 2018, *7*, 1700551

[2] I.T. Ozbolat et al. "Application areas of 3D bioprinting", *Drug Discov Today*, 2016, *21*, 1257-1271

[3] F. Pati et al. "3D Bioprinting of Tissue/Organ Models", *Angew. Chem. Int. Ed.*, 2016, *55*, 4650 –4665

[4] C. Gualandi, et al. "Easily synthesized novel biodegradable copolyesters with adjustable properties for biomedical applications", *Soft Matter*, 2012, *8*, 5466

[5] Patent: "Substrate of polymeric material and method of carrying out thereof" (WO2011056154) and related product Mycutis (trademark)

[6] M. Alessandri et al. "Influence of biological matrix and artificial electrospun scaffolds on proliferation, differentiation and trophic factor synthesis of rat embryonic stem cells", *Matrix Biology* 33 (2014) 68–76

[7] Chen et al, "Tailoring chemical and physical properties of fibrous scaffolds from block copolyesters containing ether and thio-ether linkages for skeletal differentiation of human mesenchymal stromal cells" *Biomaterials,* 2016, *76*, 261-272

[8] Project coordinated by IRST: "An in vitro and *ex vivo* model of biomimetic regenerative devices to treat bone metastases and soft tissue tumors" (BIOBOS PROJECT 2018-2020); (2) POR-FESR Project (European Funding for the Regional Development): "Step-by-step: integrated approach for the patient with acute neurologic lesions" 2016-2018; (3) POR-FESR Project "TECNO_EN-P: generation of "smart materials" to be applied in biomedical devices for the selective removal of cells and soluble or suspended substances in biological fluids" 2016-2018.