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

Electrospun scaffolds for the regeneration of tendons and ligaments

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

Yes/Not	Cultural area
NO	Medicine, biology, or related disciplines
YES	Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines. **Degree in Mechanical Engineering or 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	Desirable (but not mandatory) experience: • in the area of cell testing and biocompatibility • in the field of orthopaedics
Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines	Mandatory: some background in polymer chemistry + biomaterials + biomechanics Desirable: • material testing • electrospinning

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	Prof Francesco Traina	Dept. of Orthopaedic-Traumatology and Prosthetic surgery, Rizzoli Orthopaedic Institute
Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines	Main supervisor: Prof. Maria Letizia Focarete Co-supervisor: Prof. Luca Cristofolini	Dept. of Chemistry "Giacomo Ciamician", UniBo Dept. Industrial Engineering (DIN), UniBo

Research project

	Synthetic description
Summary (max 1000 chars)	Degenerative or traumatic lesions of tendons and ligaments are difficult to repair. Post-operative failures affect between 15% and 40% of cases (depending on initial indications). We developed a prototype of an electrospun scaffold replicating the hierarchical morphology and the mechanical properties of tendons and ligaments. This PhD project will further develop the prototype by increasing the bioactivity and enhance the integration of the constituent material with the surrounding tissues, and will bring this technical solution towards clinical application. The following aspects will be investigated: optimization of the polymeric biomaterial and its functionalization to improve cell adhesion, recruiting and differentiation and to prevent inflammatory response, optimal technique for effective sterilization; means of surgical attachment to the host tissue. The collaboration between the technical area (engineering and chemistry) with the clinical counterpart (orthopaedic surgery) will be a key point of this project.

Objectives (max 1000 chars + max 5 relevant references)	The overall objective of this PhD project to bring a promising electrospun scaffold for the repair of tendons and ligaments from the current state of technical development (between TRL3 and 4) towards clinical application. The following specific objectives will be tackled: • Optimization of the sterilization technique to ensure effective sterilization of the nanofibrous structure and preservation of the desired mechanical properties and biocompatibility properties • Optimization of the surgical technique to attach the scaffold to the host bone, and/or the residual tendon/ligament, in collaboration with the orthopaedic surgeons to ensure adequate mechanical strength and lack of stress concentrations and surgical practicability • Increasing scaffold bioactivity and integration in the surrounding tissues to prevent adverse response of host tissue and avoid inflammatory reactions. This project covers some basic science (interaction between nanofibrous scaffold and host tissue), it focuses on technological development (implementing and testing different solutions on the scaffold) and has clinical relevance (develop the best solution for implantation).
Rationale and scientific background (max 2000 chars+ max 5 relevant references)	The fact that different orthopaedic surgeons chose different strategies for the repair of damaged tendons and ligaments is an indicator that there is no consolidated and satisfactory technique. In fact, the post-operative outcome is far from satisfactory. Surgical treatments fail in 15% and 40% of cases (depending on the initial indications). Artificial implants fail mostly because of biomechanical mismatch (inadequate stiffness, limited strength etc). Xenografts often do not get properly integrated or even create rejection. Allografts offer better similarity, but are limited due to cost and availability. Autografts solve some of the problems above, but are associated with morbidity of the donor site and are limited in stock. Bioresorbable scaffolds are a very promising option, as they are not limited in availability [1]. We recently developed a technique to manufacture electrospun scaffolds that replicate the morphology and the mechanical properties of the human natural tendons and ligaments [2,3]. To bring this project towards an animal trial and a future clinical application, there are some clear points that need to be fine-tuned. Specifically, as the key to success of such devices is integration with the host tissues, this project aims to understand how to prevent such common clinical complications, and to develop the extremities and the interfaces so as to grant success in case of implantation. ***References*** [1] Sensini, A., and Cristofolini, L., 2018, "Biofabrication of electrospun scaffolds for the regeneration of tendons and ligaments," MDPI Materials, 11(10-1963), pp. 1-43. [2] Sensini, A., Gualandi, C., Cristofolini, L., Tozzi, G., Dicarlo, M., Teti, G., Mattioli-Belmonte, M., and Focarete, M. L., 2017, "Biofabrication of bundles of poly(lactic acid)-collagen blends mimicking the fascicles of the human Achille tendon," Biofabrication, 9(1), p. 015025. [3] Sensini, A., Gualandi, C., Zucchelli, A., Boyle, L. A., Kao, A. P., Reilly, G. C., Tozzi, G., Cristofolini, L., and Focarete, M. L., 2018, "Tendon Fascicle-Inspired Nanofibrous Scaffold of Polylactic acid/Collagen with Enhanced 3D-Structure and Biomechanical Properties," Scientific Reports, 8(1(17167)), pp. 1-15.
Preliminary results if existing (max 1000 chars+ max 5 relevant references)	This project spins from the exciting results we obtained recently on this concept: • We identified the most promising blends of collagen PLLA to produce bundles mimicking the collagen fibrils in tendons. The morphology (dimensions and spatial arrangements of the nanofibers) and mechanical properties (strength, stiffness, toughness) were assessed. Viability and morphology of cultured tenocytes was confirmed [1]. • Crosslinking was fine-tuned to provide adequate control over collagen stability. Cell viability was tested with human fibroblasts up to 21 days. The Resazurin assay confirmed an excellent cellular metabolism; fluorescent microscopy indicated cell elongation along the scaffolds [2]. • High-resolution XCT scanning, confirmed that the morphology of the

	scaffolds, and the directionality of the nanofibers closely mimics that of collagen fascicles in human tendon [3]. • We developed a method to assemble multiple bundles in a hierarchical structure that replicates the multiscale morphology of tendons. High-resolution XCT scanning confirmed we obtained the desired morphology, inter-fiber spacing, and alignment of the nanofibers. The scaffold had comparable stiffness to the natural tendon, and excellent cell viability and infiltration of human fibroblasts [4]. ***References*** [1] Sensini, A., Gualandi, C., Cristofolini, L., Tozzi, G., Dicarlo, M., Teti, G., Mattioli-Belmonte, M., and Focarete, M. L., 2017, "Biofabrication of bundles of poly(lactic acid)-collagen blends mimicking the fascicles of the human Achille tendon," Biofabrication, 9(1), p. 015025. [2] Sensini, A., Gualandi, C., Zucchelli, A., Boyle, L. A., Kao, A. P., Reilly, G. C., Tozzi, G., Cristofolini, L., and Focarete, M. L., 2018, "Tendon Fascicle-Inspired Nanofibrous Scaffold of Polylactic acid/Collagen with Enhanced 3D-Structure and Biomechanical Properties," Scientific Reports, 8(1(17167)), pp. 1-15. [3] Sensini, A., Cristofolini, L., Focarete, M. L., Belcari, J., Zucchelli, A., Kao, A. P., and Tozzi, G., 2018, "High-resolution x-ray tomographic morphological characterization of electrospun nanofibrous bundles for tendon and ligament regeneration and replacement," Journal of Microscopy, 272(3), pp. 196-206. [4] Sensini, A., Gualandi, C., Focarete, M. L., Belcari, J., Zucchelli, A., Boyle, L., Reilly, G., Kao, A. P., Tozzi, G., and Cristofolini, L., IN PRESS, "Multiscale hierarchical bioresorbable scaffolds for the regeneration of tendons and ligaments," Biofabrication.
Research project including methodology (max 5000 chars)	The focus of the activities will be on optimizing and testing electrospun hierarchical scaffolds made of blends of natural (collagen) and synthetic (PLLA) polymers so as to ensure that they become suitable for implantation. This PhD candidate will spend 30-40% of his/her time in the biomechanical laboratory of prof. Cristofolini, 30-40% of the time in the Chemistry lab of prof. Focarete and the remaining 20-40% of in the clinical settlement, in collaboration with Rizzoli Orthopaedic Institute. Furthermore, an international secondment of 2+3 months at the University of Portsmouth is planned to complement the preparation of this candidate providing high-resolution imaging and cell culture, whereas an international secondment of 2+3 months at Erlangen University is planned to complement the preparation of this candidate on scaffold functionalization and biomineralization. **Activity 1** – CLINICAL TRAINING. Building the understanding of lesions of the tendons and ligament, about the current surgical techniques for reconstruction, and about the post-op failure mechanisms. This activity will be particularly intense during the 1st year, to acquire new clinical understanding. However, during the entire duration of development and validation activities will be closely connected to the clinical environment. . Task 1.1: Basic knowledge: In this phase the candidate will receive materials and specific training in order to comprehensively understand the indications for tendon and ligament reconstruction, their clinical relevance, epidemiology, treatment options, radiological assessment, and methods of outcome evaluations. . Task 1.2: Specific training: in this phase the candidate will become familiar with the different failure scenarios of tendons and ligament reconstructions. Furthermore, he/she will participate on the outpatient, hospital department and first aid activities so as to get involved and aware about the real clinical problems. Through this activity, he/she will develop a first concept of what is needed to improve the implantable devices and the surgical technique.

Activity 2 – STERILIZATION TECHNIQUE. Within this activity the candidate will first get familiar with the sterilization techniques that can be applied to this family of resorbable materials. Tests will be carried out to identify a technique and the parameters that grant adequate reduction of the bioburden, without compromising the mechanical properties and the biocompatibility of the scaffolds

. Task 2.1: Preparation of prototype scaffolds for sterilization. Here simplified scaffolds will be prepared that provide the same morphological complexity of the intended final device, but with a lower production cost and labour.

. Task 2.2: Sterilization. The scaffolds will be subjected to different sterilization techniques. A first candidate is gamma radiation. Different doses will be tested. If gamma radiation provides unsuitable, other techniques (e.g. ETO) will be assessed.

. Task 2.3: Evaluation. The sterilized scaffolds will be extensively characterized to assess that their performance is not compromised by sterilization:

- Morphological (SEM) will be carried out to assess that the stylization does not compromise the fibrous structure and the porosity of the scaffold.
- Therma properties (DSC) will be used to ensure that the material stability and properties are not modified by the sterilization.
- Mechanical properties: tensile tests will be used to measure the stiffness, strength, toughness before and after sterilization. Protocols already available at DIN will be adapted for this purpose.
- Cell viability: to confirm that the sterilization does not compromise the material, cell culture with human fibroblasts will be assessed.

Tasks 2.2 and 2.3 will iterate until the optimal solution is defined.

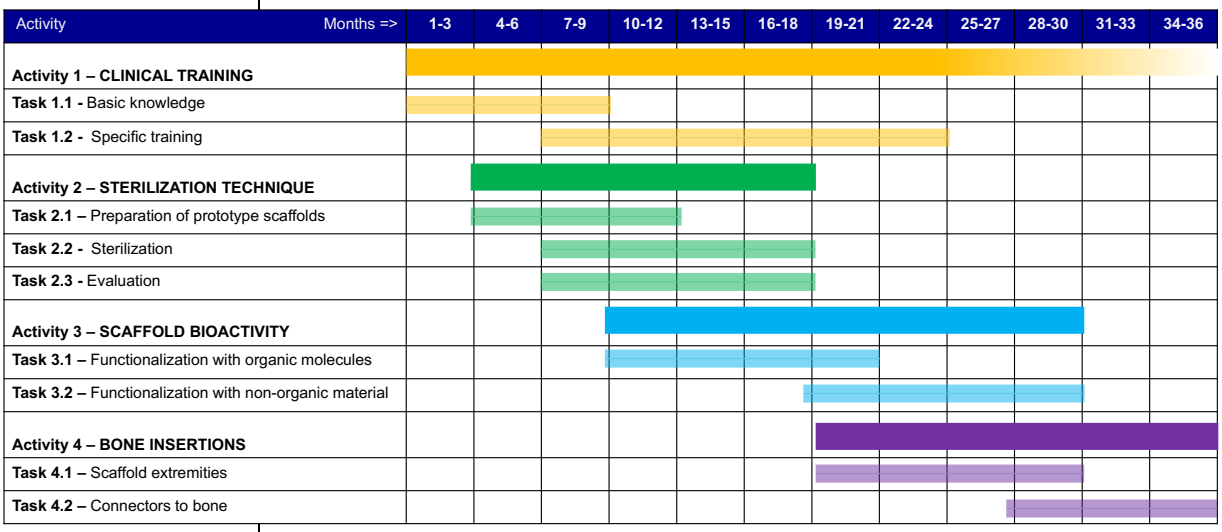
Activity 3 –SCAFFOLD BIOACTIVITY AND INTEGRATION WITH HOST TISSUE. This activity will be carried out in parallel with the previous ones, with the aim to optimize the constituent material and increase the bioactivity of the scaffold and its integration with the surrounding tissues. Within this activity the candidate will get familiar with scaffold functionalization techniques.

Task 3.1: Functionalization with organic molecules, belonging to the family of beta-lactams, that have been recently used in combination with electrospun fibers of PLLA, by the group at the Chemistry Dept. to strongly promote cell adhesion mediated by integrins. The most active compounds will be selected and used as candidates for the loading on the electrospun scaffold.

Task 3.2: Functionalization with non-organic material. Activities within this task are correlated to the previous activity (Bone insertion). The scaffold extremities will be functionalized with osteoconductive Bioglasses (BG) or hydroxyapatite (HA) to improve bioactivity and increase the precipitation of bone-like apatite layer upon immersion in SBF. This activity is planned in collaboration with Erlangen University.

Activities 2 and 3 overlap and partially iterate.

Activity 4 – BONE INSERTIONS. The activities within this activity are fundamental to adapt the scaffolds and define the surgical technique that will provide adequate insertion in the host bone. This is currently one of the main surgical challenges. The input of the clinical supervisor is extremely important in this phase.

	. Task 4.1: Scaffold extremities. To encourage tissue integration at the enthesis, the polymeric scaffold (both the plain scaffolds and the functionalized scaffold described below in Activity 4) will be enriched with **osteoconductive inorganic materials**, following a protocol already tested at the Dept. of Chemistry. In brief, the capability of the scaffolds to promote uniform deposition of nanocrystalline apatite will be tested through immersion of the scaffold in simulated body fluid (SBF), a solution with an ionic composition similar to that of blood plasma, or in a slightly supersaturated calcium phosphate solution (CaP) buffered with Hepes. Scaffold mineralization will be assessed by means of morphological analysis (SEM equipped with energy dispersed X-ray spectrometer (EDS)., TEM), and structural analysis (X-ray diffraction). . Task 4.2: Connectors to bone. Different devices for connecting the scaffold to the host bone will be explored, including resorbable screws, 3D printed fixtures, and other osteoconductive solutions. Mechanical tests will be performed first on simplified constructs, then on bone-scaffold assemblies (using bone tissue from farm animal). The focus will be on the connection strength, interface micromotions and stress concentrations. Protocols available at DIN will be adapted for this purpose, including use of Digital Image Correlation (DIC). **Gantt chart** 
Innovation potential (scientific and/or technological) (max 1000 chars)	This proposal mainly aims at technological innovation: this PhD project will provide significant advancement in the development and validation of nanofibrous scaffolds. In particular, currently no hierarchical scaffold is available for the regeneration of tendons and ligaments. This research will deliver unpreceded solution with highly biomimetic scaffolds. The main points of innovation will be: • Advancing the development of electrospun hierarchical bioresorbable scaffold • Developing and validating technological and surgical solutions for the attachment of such scaffolds to the host tissues • Developing and testing innovative solutions to prevent inflammatory response and tissue adhesion Furthermore, it is foreseen that this project will deliver scientific innovation providing new insights in the way host tissues (tendon, ligament, bone and enthesis) react to nanofibrous scaffolds
Expected results and applications to human pathology and therapy (max 1000 chars)	This project is meant to develop a better solution for the repair and regeneration of lesions of tendon and ligaments, so as to overcome the critical limitations of the current commercial devices and surgical techniques. This will allow, in a perspective, to deliver better treatments both to young patients (typically affected by traumatic lesions) and elderly ones (presenting degenerative lesions). While in the duration of this PhD project it is not realistic to start a clinical trial on humans, it will definitely open the way to a dedicated animal trial.

Available resources for the project

	Synthetic description
Research environment (labs involved, background, and location)	This candidate will have an engineering background. While this will facilitate him/her in grasping the technical part of the project, some time and effort must be dedicated at the beginning to improve his/her understanding of the clinical problem. This project is rooted between three groups: - The group of **Prof. Focarete** (Chemistry Department) will provide the training and expertise on electrospinning, polymers, and treatment and modification of polymers. - The group of **Prof. Cristofolini** (Department of Industrial Engineering) will provide "training through research" in the area of biomechanics and material characterization. - The group of **Dr Traina** will provide training and supervision on the surgical procedures for tendon and ligament repair, on complications, and will supervise the design of the implantation technique. Prof Focarete and Prof Cristofolini have been collaborating in the recent years for the development of the first prototype of this implantable scaffold. The success of collaboration is documented by a number of joint publications. Also: Prof. Traina and prof. Cristofolini have been intensively collaborating for years on research projects at the intersection between orthopaedic clinical application and biomechanics research. A strong integration of the two research groups has been achieved by involving the clinical staff in lab activity, and the lab staff in clinical research. This PhD candidate will enjoy this extremely stimulating interdisciplinary environment, and will share his/her research time between clinics (in tight collaboration with Rizzoli Orthopaedic Institute) and biomechanics lab. **The Polymer Science and Biomaterials group at the Chemistry Department "Ciamician", 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. In collaboration with the Biomechanics lab of prof. Cristofolini the group has acquired knowledge to develop scaffolds with optimized biomechanical properties by playing on the choice of the more appropriate material and on the selection of the best morphological properties of the scaffolds. The **Department of Industrial Engineering** includes a large Biomechanics lab that is extremely active in the field of orthopaedic biomechanics. The focus of the biomechanics group directed by prof. Cristofolini within DIN is on the multi-scale biomechanical characterization of skeletal structures and orthopaedic devices, and on the integration of *in vitro* tests and numerical modeling. Their group, in collaboration with the Electrospinning group, recently developed and characterized innovative regenerative scaffolds. Furthermore, this group is acknowledged internationally for the applications of DIC to biomechanics. The **Dept. of Orthopaedic-Traumatology and Prosthetic surgery** and revisions of hip and knee implants of the Rizzoli Orthopaedic Institute is nationally recognized for the treatment of severe orthopaedic conditions including joints tendon and ligament reconstructions (mainly in the lower limb). Its activity is mainly focused on surgical treatment of complex cases, analysis and data collection of multiple type of joint replacement surgery through different surgical approach and procedures. Comparison between different procedures and cases are routinely performed in order to continuously improve the patient's provision of care.

Main equipment (facilities and location)	**Laboratories of the Polymer Science and Biomaterials group** (via Selmi 2, Bologna) 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. The **Labs of the Department of Industrial Engineering** (Via Terracini 24-28, Bologna) are equipped with the testing facilities required for this project, including: - An industrial electrospinning machine and a proprietary one that can be adapted for specific purposes. - Five universal testing machines - Under construction: a proprietary multiaxial simulator for biomechanical testing - State-of-the-art digital image correlation (DIC) system. - Equipment and procedures for safe storage, preparation, testing and disposal of biological tissue specimens (both human and animal)
Additional funding (title, amount, start date, duration)	No significant costs are expected on behalf of Rizzoli Institute, most of the research and training costs will be covered within the Department of Chemistry and the Department of Industrial Engineering. Funding already available at Dept. of Chemistry will cover the cost for laboratory testing (access to characterization instruments, lab consumables, sterilization services): • Industrial funding and European project overhead: 100'000 Euro • Additional funding will be sought with an orthopaedic manufacturer that has approached us for the exploitation of this idea. Funding already available at DIN will cover the cost for laboratory testing (synthetic and biological specimens, access to testing machines, lab consumables, dedicated testing fixtures): • Industrial funding on related activities (static and dynamic testing of orthopaedic implantable components): 130'000 Euro • PON 2017 "Bone++" on innovative orthopaedic devices (2019-2021): 320'000 Euro • UniBo "Proof of Concept" on a related patent: under evaluation: 40'000

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

	Project	Location and team
#1	MicroCT and XCT imaging of scaffolds (2 months)	University of Portsmouth (UK), Zeiss Global Centre, dr G Tozzi
#2	Cell culture and simulation of enthesis (3 months, interlaced with the 2 above)	University of Portsmouth (UK), Dept. of Bioengineering, dr M. Roldo and prof. Gordon Blunn
#3	With the team at Erlangen it is presently active a research collaboration and an exchange of students. This collaboration will contribute to scaffold biomineralization to encourage tissue integration at the enthesis.	Institute for Biomaterials at the Department of Materials Science and Engineering, University of Erlangen-Nuremberg (prof. Boccaccini)