CRESCENDO

Project 12

Name of the PhD course	**Engineering of Products and Industrial Processes**
Name of the PhD coordinator	Prof. Andrea D'Anna
Name/Title of the PhD project	***Design and construction of micro-static morphogenerators for bioprinting of spatially-controlled bacterial population***
Department of reference	Department of Chemical, Materials and Production Engineering (DICMaPI) of the University of Naples Federico II (http://www.dicmapi.unina.it/)
Working conditions, research team, infrastructures, equipment	The Department gathers the expertise of the schools of Chemical Engineering, Materials Engineering and Production Engineering, which have been very active at the University of Naples Federico II since the 60s of the last century. The Department organizes educational and training activities involving approximately 1500 students (undergraduates and postgraduates), more than 140 PhD students, 79 Professors (including Assistant, Associate, Full and Emeritus Professors). Regarding the research projects, there are 26 ongoing projects (with 2ERC, 8 H2020, 6 PRIN) for a total funding 7.7M€. The Department is part of a vast network of national and international collaborations of high scientific profiles and offers its professional skills in support of many national and international companies. The Department has a long history of collaboration and partnership with industry.
Scientific context	plays a fundamental role in influencing the metabolic fluxes on the biosphere. Microbiome activity is essential for the life on our planet and the well-being of organisms, and research is attracting interest and investment from the industry worldwide. Collaborative behaviors are typical of cell activities. *Spatial distribution of bacterial strains (micro-biogeography) in a shared volumetric space, and their morphological structure affect their societal behavior*. The literature reports on several microbiological techniques focused on the culture of single bacterial strains or perfectly-mixed bacterial communities Micro-spatially controlled architectures at high resolution are challenging. The current trend of product engineering is moving towards the design and processing of innovative materials with multifaceted properties tailored for specific applications such as organic- inorganic hybrid materials and multicomponent catalysts Powerful computational tools and advanced experimental techniques offer the possibility to design and fabricate new classes of materials properly structured to show complex properties Along this way, it is even possible to generate "metamaterials", i.e., materials that can modify some of their properties in response of an external stimulus. Examples are origami-inspired materials made through cutting, folding, and buckling techniques that undergo a wide range of shape transformations with variable mechanical properties [5]. Products with multifunctional abilities can be fabricated by combining different simple materials with either top-down or bottom-up approaches. In the first case, subtractive or additive manufacturing techniques (3D printing) are employed. Several limiting factors, however, exist including physical constraints, speed, parallelization, material selection Bottom-up approaches consist in self-assembling simple building blocks so leading to a complex final structure. This technique is widely adopted at small length scales where self-assembly of nano-/micro-sized particles is induced through hydrodynamic interactions, van der Walls forces, etc. but its application on larger length scales can be difficult. Products with spatial-dependent properties made of a single, properly "structured" material are of great technological interest. A proper design of the structured material requires a fine control of its morphology *A general, easy-to-use, flexible technique able to fabricate multi-structured materials with a desired and well-controlled morphology is missing in the state of art*. Such an approach could allow the fabrication of highly structured products. Just as an example, one might fabricate microchannels with complex shape difficult or impossible to build through conventional techniques [9], or products with hierarchical structures with anisotropic mechanical properties
Project Research plan	We plan fabricate a microfluidic device, the morphogenerator, capable of producing fine-scale structures by properly mastering the so-called chaotic advection like in a Kenics static mixer. The *goal is to achieve intercalated layers containing different bacterial strains*. The morphogenerator will be used to analyze the importance of spatial distributions of bacteria and their capabilities to process different foods. This PhD aims at developing a general technique, inspired to the concept of static mixers, to build multi-structured products with complex morphologies. While aim of the static mixer is to promote intimate mixing, here its characteristic fluid-mechanics will be exploited to generate complex morphologies. Indeed, the vision is to "engineer" the elements housed in a channel where two or more input fluids are made to flow to generate a structured material with a specific target morphology to be retained at the solid state. Then, the **first objective** of PhD is to design, develop, and test versatile microdevices, from now on referred as "morphogenerator", to produce a structured material with a desired complex morphology at the microscale. Input liquid streams may consist of different materials with different electrical, thermal, acoustic, rheological properties, but they can also consist of the same material differently loaded with solid particles, fibers, biological matter or blowing agents. Consequently, the final material will have different properties "compartmentalized" according to a morphology engineered for a specific application. The simplicity and versatility of the processing approach will open up new opportunities in a countless number of fields, from biology to medicine, from agriculture to food and packaging. The research activity will be carried out by combining advanced microfabrication techniques, experiments, and numerical simulations, aimed at clarifying the effect of the morphogenerator geometry on the product final morphology. Given the great number of degree of freedoms in the selection of the static mixer geometries, the **second objective** of the PhD will be the development of a "*reverse engineering*" tool able to design the morphogenerator elements capable of inducing the desired final morphology to the product. To accomplish this, machine learning techniques will be adopted, properly trained by sufficiently large databases built from simulations. The tool will be employed to develop innovative products targeting two specific applications: i) the fabrication of microchannels

	with complex shape that are difficult or impossible to build through conventional techniques and ii) the production of hierarchical structures with anisotropic mechanical properties. To accomplish the aims of the project, numerical simulations, 3D printing microfabrication, experimental analysis, and machine learning techniques will be employed. Specifically, the backbone of the project relies on three interconnected units: i) modeling and simulation, ii) microfabrication, iii) experimental testing. Modelling and numerical simulation unit is expected to predict the flow behavior in the morphogenerator, and the resulting morphology of the multi-structured material. The results will be transferred to the microfabrication unit to design the microfluidic device. The produced device will be used to perform experiments with flow conditions suggested by the simulation unit. Upon validation of the model, an intensive simulation campaign will be carried out by varying the static mixer geometries in order to produce a sufficiently large dataset of attainable final morphologies to be fed to machine learning techniques.
Research and Training Innovative aspects	The morphogenerator is a novel device which might achieve submicron resolution. To our knowledge no application on food digestibility is published so far. Real tissues are composed of multiple micrometer-thickness layers of distinct cell types. Although appealing and enabling, the cost-effective fabrication of multi-material, and perhaps multi-cell type, lamellar microarchitectures has proven to be challenging, especially when adjacent thin, perhaps single cell layers, of multiple cell types are desired. Multi-material and multi-layered architectures achieve functionality and/or performance that are not achievable with monolithic materials. Moreover, the functionality and performance of multilayered composites is frequently determined by the proximity, indeed the density, of the constituent layers. Multilayered materials with a high amount of internal surface area can yield higher capacitances in supercapacitors, elevated mechanical strength and fatigue resistance, better sensing capabilities, or improved energy-harvesting potential. A multi-lamellar architecture that features highly accurate control of surface geometry and surface area is also desirable in applications related to the controlled release of pharmaceuticals. Multilayered structures are particularly relevant in nature and in biological applications. Indeed, one of the most pressing challenges in biofabrication is the development of strategies for the facile and high-throughput creation of multilayered and multimaterial tissue-like constructs. In this PhD we aim to develop a *disruptive technology for the continuous creation of fine and complex structures at the micrometer and submicrometer levels* within polymer fibers filling the current gap in literature.
Inter-Multidisciplinary aspects	The project involves competences in engineering (microfluidics, 3Dprinting, Industry4.0), Optical measurements, Food, Microbiology. Indeed, to accomplish the aims of the project, numerical simulations, 3D printing microfabrication, experimental analysis, and machine learning techniques will be employed. Specifically, the backbone of the project relies on three interconnected units: i) modeling and simulation, ii) microfabrication, iii) experimental testing. The general aim of the numerical simulation unit is to predict the flow behavior in the morphogenerator and the resulting morphology of the multi-structured material. The results will be transferred to the microfabrication unit to design the microfluidic device. The produced device will be used to perform experiments with flow conditions suggested by the simulation unit. Once the experimental and numerical results agree for a wide number of morphogenerator designs, an intensive simulation campaign will be carried out to produce a sufficiently large dataset for machine learning techniques.
Secondment opportunities	The PhD will have at least two secondment opportunities that may last from 3 to 6 months depending on the project needs and PhD wishes. In particular, the PhD is expected to collaborate with: - **Dr. Francesco Del Giudice** who is a Senior Lecturer and a **Chartered Chemical Engineer at the Department of Chemical Engineering, Swansea University (**United Kingdom). - **Mr. Mischa Stevens Head of R&D Process Development & Pilot Plant at The Kraft Heinz Company** (The Netherland), https://www.kraftheinzcompany.com/.
Main Supervisor: Prof Pier Luca Maffettone (https://www.docenti.unina.it/pierluca.maffettone)	
Brief CV	Full Professor of Chemical Engineering -Supervising experience certified by more than 20 PhD, more than 10 PostDoc and more than 80 Master Students.
Publications	Prof Maffettone has 202 publication, h-index 40, i10-index 114 (source Google Scholar). Here we list the 5 most relevant: -P. Memmolo, Z. Wang, V. Bianco, D. Pirone, M. M. Villone, P. L. Maffettone and P. Ferraro, Dehydration of plant cells shoves nuclei rotation allowing for 3D phase contrast tomography, Light: Science & Applications, 10, 187 (2021) - D. Tammaro, V. C. Suja, A. Kannan, L. D. Gala, E. Di Maio, G. G. Fuller and **P. L. Maffettone**, Flowering in bursting bubbles with viscoelastic interfaces, Proceedings of the National Academy of Sciences, 118, e2105058118 (2021) -M. M. Villone, P. Memmolo, F. Merola, M. Mugnano, L. Miccio, **P. L. Maffettone** and P. Ferraro, Full Angle Tomographic Phase Microscopy of Flowing Quasi- Spherical Cells, Lab Chip, 18, 126-131 (2018) -G. D'Avino, F. Greco and **P. L. Maffettone,** Particle migration due to viscoelasticity of the suspending liquid, and its relevance in microfluidic devices, Annu. Rev. Fluid Mech., 49, 341-360 (2017) -G. D'Avino, G. Romeo, M.M. Villone, F. Greco, P.A. Netti and **P. L. Maffettone**, Single line particle focusing induced by viscoelasticity of the suspending liquid: theory, experiments and simulations to design a micropipe flow-focuser, Lab Chip, 12, 1638–1645 (2012)
Projects participation	The total number of funded projects participation with microbiome research, followed by a list of maximum 3 EU funded projects (past and/or current) in the microbiome field: 1. YIELD-stress fluids beyond Bingham – closing the GAP in modelling real-world yield-stress materials 2. MORphological biomarkers For Early diagnosis in Oncology 3. An inter-disciplinary high-throughput approach to olefin block copolymers (OBC) 4. Micro-mechanical and robotic tools for the diagnosis and therapy of prostate cancer and 5. Virtual Materials Marketplace