Biomedical Technologies and Innovation Doctoral Programme (BIOTIN)

Title of the PhD Project	**3D printed boron doped bioactive glasses for soft tissue engineering applications and their zebrafish response**
Acronym	**SOFT-BOR**
Research Fields of the Project	Biomaterials, materials science, artificial intelligence, computer engineering, biomedicine
Keywords	3d printing, bioactive glasses, soft tissue regeneration, machine learning, in vivo studies
Host Institution, Department and Campus Location	Boğaziçi University, Institute of Biomedical Engineering, Kandilli Campus, Çengelköy, İstanbul
PhD Awarding Institution and Graduate Programme	Boğaziçi University, Institute of Biomedical Engineering, PhD in Biomedical Engineering
Name and Affiliation of Main Supervisor	Assoc. Prof. Duygu Ege (BOUN)
Name and Affiliation of Co-supervisor	Asst. Prof. Deniz T. Yücesoy (*IZTECH)* Prof. Güneş Özhan (*IBG)*
Research Environment and Infrastructure	In our Institute of Biomedical Engineering, we have required facilities for production and in depth- characterization of the bioactive glasses and 3D printed scaffolds. There is a 3D extrusion based bioprinter (Axolotl Biosystems) in our laboratory. A mechanical testing device (Lloyd Instrument, LF Plus) is present which is capable of measuring compression and tension of soft biomaterials. At Boğaziçi University, there is a rheological testing machine (Anton Paar), Fourier Infra-red Spectroscopy (FTIR) (Perkin Elmer), X-Ray Diffraction Spectroscopy (XRD) (Rikagu), X-ray Photoelectron Spectroscopy (XPS) (Thermoscientific), Scanning Electron Microscopy (SEM) (Phillips-FEI XL-30), dynamic light scattering (DLS), and contact angle measurement device. There is a cell culture laboratory present in our laboratory. We are capable of measuring cell viability and analysing cell morphology and differentiation via staining. We have a cell culture incubator, laminar flow cabinet, flourescence microscope (Zeiss) and centrifuge in an isolated room with a Hepa filter to carry out the cell culture studies in a sterile environment. Izmir Institute of Technology has the required know-how for the computational and statistical analysis aspect of the study. This part of the study will be purely computer based with use of commercially available design of experiments (DOE) tools and open 3D printing databases (such as https://cect.umd.edu/3d-printing-database). İzmir Biomedicine and Genome Center has the required facilities for in vivo studies with

	zebra fish. In the zebrafish facility at the Vivarium center, there is anesthetic apparatus, class2A Biosafety Cabin, Operation equipment, procedure room, stereo microscope, surgery set and technical support. Additionally, for studying gene expressions, there is a real-time PCR detection system is present in the center. *http://web.boun.edu.tr/duygu.ege/*
Scientific Context of the Project	Bioactive glasses are conventionally used for hard tissue applications. Recent research showed promising application of bioactive glasses also for soft tissue engineering applications. Borate glasses could heal wounds untreatable with conventional methods. In addition to this, they are also promising for other applications such as muscle and nerve tissue regeneration. In this study, first boron doped bioactive glasses will be prepared by sol-gel technique. Then, prior to 3D printing, a design of experiments analysis will be conducted utilizing custom codes and available softwares, e.g., Design-Expert®software (DX7), to determine the printing parameters. With these results, the polymeric phase of the 3D printing model will be determined by the student. Then, rheological studies will be carried out to determine accuracy of the determined variables from the DOE analysis. The scaffolds will be 3D printed. Then, they will be characterized by techniques including XRD, SEM, mechanical tests, contact angle measurements, DLS, FTIR, and cell culture studies. Finally, in vivo studies will be carried out with zebra fish to study effect of the produced scaffold for the healing of a damaged tissue. Histochemical studies will be conducted to study the effect of scaffolds on wound healing. The zebrafish has a pronounced ability to regenerate its tissues and organs. To unravel the potential contribution of boron doped bioactive glasses in tissue regeneration *in vivo*, we will exploit stab wound injury of the adult zebrafish brain, which is a well-established approach to investigate vertebrate brain neurogenesis and regeneration. Following stab injury, fluorescently labeled boron doped bioactive glasses will be delivered into the brain using cerebroventricular microinjection (CVMI). Afterwards, the tissue will be analyzed with respect to expression of glial and neuronal markers that are indicative of reactive regeneration. Here, immunoflurescent staining of tissue sections and/or quantitative real-time PCR on RNA isolated from the brain tissue will be performed.
Brief Workplan	Month 1-8: Production of glasses Month 8-16: DOE studies, Rheological studies Month 16-22: 3D printing at BOUN, 3D printing and characterization at FAU Uni, Germany Month 22-32: Characterization, Cell culture studies Month 32-40: In vivo zebra fish model study Month 40-48: In vivo zebra fish model study

Biomedical Technologies and Innovation Doctoral Programme (BIOTIN)

Innovative Aspects of the Project	This study is very novel as application of boron-doped bioactive glasses for wound healing is a recent growing and very promising field. Boron doped bioactive glasses are capable of healing wounds of diabetes patients. Therefore, this showed great promise of use of boron doped bioactive glasses also for soft tissue engineering applications. Another important aspect is use of statistical tools for optimization of 3D printing parameters. This is a very key aspect of the study as this would reduce work labour (studying many parameters for optimization) and also reduce cost of 3D printing. Another important aspect is application of zebra fish models for in vivo studies as there is very limited amount of in vivo study for evaluation of boron doped bioactive glass-based 3D printing scaffolds for soft tissue engineering applications.
Training Opportunities of the Project	The PhD student will be trained to produce bioactive glasses via sol-gel technique. This is a key technique to produce nano-sized particles. The student will be trained to characterize these particles with DLS and SEM. Then, student will learn machine learning techniques to pre-determine 3d printing parameters. After this, the student will learn to carry out rheological studies and to characterize these results. This is a very important technique for fabricating 3d printed scaffolds and injectable hydrogels. The student will then be trained to 3D print scaffolds and analyze biocompatibility via cell culture techniques. Finally, student will gain experience with in vivo studies with a zebra fish model.
Interdisciplinary Aspects	This study is very interdisciplinary as it combines fields of biomaterials, materials science, chemistry, data science, biomedicine. In the first part of the study, boron doped bioactive glasses will be developed by sol-gel processing. This stage of the study is the chemistry-based part of the study. This will follow with determination of printing parameters for the boron doped bioactive glass/polymeric scaffold with DOE.. This will follow with rheological study, 3D printing and characterization which is chemistry and materials science-based part of the study. The final stage is biomedicine aspect of the study where in vivo studies will be carried out with the zebra fish model.
Intersectoral Mobility **☒ Short Visit** **☐ Secondment**	*Host: AMGEN Türkiye* *Context of Mobility: Management for the Pharmaceutical Industry*
Intersectoral Mobility **☒ Short Visit** **☐ Secondment**	*Host: Istanbul Health Industry Cluster (ISEK)* *Context of Mobility: Entrepreneurship Training, Thematic Pre-incubation Program*
International Academic Secondment	*Host Supervisor: Prof. Aldo Boccaccini* *Host Institution:* University of Erlangen-Nuremberg, Erlangen, Germany *Host Department: Institute of Biomaterials*

	Duration: 6 months *Estimated Time of Mobility: 2nd year of the PhD*
Main Supervisor:	
Brief CV	**Assoc. Prof. Duygu Ege** **E-mail:** duygu.ege@boun.edu.tr **ACADEMIC DEGREES** Ph.D. Medical Material — University of Cambridge, UK — 2013 M.Eng. Materials Science and Engineering — Imperial College, UK — 2009 B.Sc. Materials Science and Engineering — Imperial College, UK — 2008 Google Scholar: https://scholar.google.com/citations?user=rrnTjdIAAAAJ&hl https://orcid.org/0000-0002-9922-6995
Co-supervisors:	
Brief CV	**Prof. Güneş Özhan** **E-mail:** gunes.ozhan@ibg.edu.tr **ACADEMIC DEGREES** Ph.D. Developmental Biology — Eberhard Kals Universitaet Tübingen, Germany — 2009 M.Sc. Molecular Biology — Georg-August-Universitaet Göttingen, Germany — 2005 B.Sc. Molecular Biology & Genetics — Middle East Technical University, Turkey — 2003 Google Scholar: https://scholar.google.com/citations?user=N8a9_1oAAAAJ&hl https://orcid.org/0000-0002-4806-5917
Brief CV	**Asst. Prof. Deniz Yücesoy** **E-mail:** denizyucesoy@iyte.edu.tr **ACADEMIC DEGREES** Ph.D. Materials Science and Engineering — University of Washington, US — 2018 M.Sc. Materials Science and Engineering — University of Washington, US — 2014 B.Sc. Molecular Biology & Genetics — İzmir Institute of Technology — 2009 ResearchGate: https://www.researchgate.net/profile/Deniz-Yucesoy https://orcid.org/0000-0002-9590-3178