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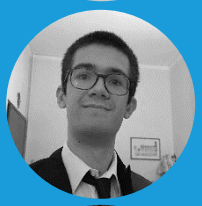

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BASTA

Executive summary

Osteoporosis and fragility fractures

Osteoporosis is a systemic disorder which reduces bone strength, increasing its susceptibility to fragility fractures. It is a global health issue, affecting over 200 million people, and a socio-economic burden, as it requires invasive and expensive treatments. Traditional medications are insufficient for effective healing of large-sized fractures, making the development of new solutions crucial. One of the most promising approaches is bone tissue engineering, with the design of bone scaffolds, which constitute a temporary support for bone healing, helping to regenerate a functional tissue.

The BASTA project

The BASTA (Bio-inspired and Adaptive Strategies to TAckle fragility fractures) project aims to design an advanced bone scaffold, more efficient than existing commercial products. A systematic review of commercially available bone scaffolds was crucial to identify the main limitations that prevent a widespread adoption in clinics. They are poorly efficient, as they need to be used in combination with other medical devices, and they are predominantly made of ceramic materials, which are suboptimal for load-bearing applications due to their brittle nature.

The requirements for the new system were drawn from an analysis of the needs of relevant stakeholders. The main requirements are fast bone healing, patient customization, and compatibility with advanced validation techniques like synchrotron imaging for quality control and cellular response analysis.

Drivers of innovation

Innovation in the BASTA project is driven by the need to address key limitations of current bone scaffolds:

- **Bio-inspired design**: inspired by the hierarchical structure of real bone, the scaffold incorporates both macro-scale porosity (*trabeculae*) and micro-scale network of pores and channels (*lacunae* and *canaliculi*).
- **Advanced fabrication:** two-photon-polymerization, a cutting-edge 3D printing technology, was used to replicate structures with a sub-micrometric resolution, necessary to include the network of lacunae and canaliculi. The micro-scale network was designed following two alternative patterns, a simple cubic regular lattice and a randomly generated network.
- **Validation via high-precision imaging**: synchrotron imaging and deep-learning techniques were employed to assess the printing accuracy and the positive outcomes of cellularizing the constructs.

Future perspectives

The future of this innovation includes enlarging scaffold dimensions for clinical use, characterizing the mechanical properties of the material used, and continuing to employ cutting-edge technology like synchrotron imaging and AI-based evaluation. This combination could lead to more effective treatments for osteoporotic fractures, reducing the socio-economic burden and improving patient outcomes.

Key Words

Bio-inspired bone scaffolds, 3D printing, Two-photon polymerization, Synchrotron imaging, AI-based image segmentation

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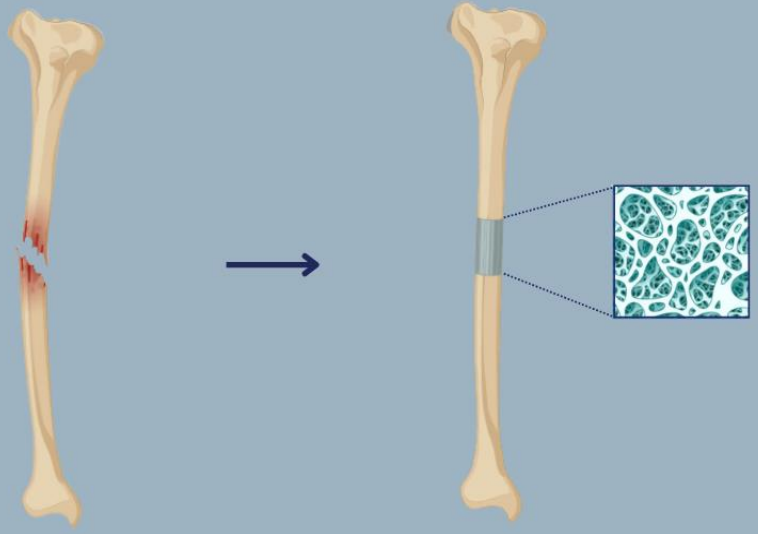
BASTA

Bio-inspired and **A**daptive **S**trategies
to **TA**ckle fragility fractures

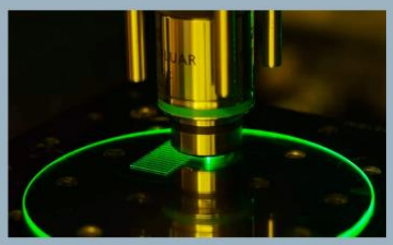


Osteoporosis
Total of 200 million patients worldwide
It often leads to fragility fractures

Bone scaffolds
They promote bone regeneration

DESIGN

2 bio-inspired architectures
recall the hierarchical structure of natural bone



MANUFACTURING

Two-photon polymerization 3D printing
with sub-micron resolution

ANALYSIS

Synchrotron imaging and AI-based segmentation
for quality control

Project description written by the Principal Academic Tutor

The development of new materials with enhanced performance has always presented a challenge for scientists and engineers. A promising approach is to study natural materials that possess unique properties and attempt to replicate their characteristics using new artificial solutions. This approach, known as bio-inspired design, has been utilized to develop materials that mimic the adaptable internal structure of bone tissue, which can specifically adjust to environmental demands. While recent efforts have been made to apply bio-inspired design in the healthcare system, there is still a long way to go to overcome the burden of fragility fractures.

Here, BASTA comes into play, providing a cutting-edge multidisciplinary approach oriented at translating bio-inspired strategies to the design of optimal constructs for bone repair. BASTA will adopt patient-specific adaptive solutions, by merging inter-disciplinary competences in biomechanical engineering, high-resolution synchrotron imaging and artificial intelligence. Indeed, the generation, for the first time, of a novel AI-based bone phantom will be the key for improving the accuracy of medical diagnoses, as well as the design of scaffolds for bone repair. Additionally, the combination of advanced 3D bio-printing techniques and the use of AI algorithms can enable researchers to simulate bone remodeling in pathological patients.

Team description by skill

Giulia is a MSc Biomedical Engineering student, specialized in the field of biomechanics and biomaterials. Her contributions include the printing process of the bone scaffold and the post-processing of synchrotron images.

Silvia is a MSc Biomedical Engineering student, specialized in the field of biomechanics and biomaterials. Her contributions include the printing process of the bone scaffold and the post-processing of synchrotron images.

Marco is a MSc Biomedical Engineering student who followed the curriculum in bionanotechnologies. During the project he carried on experiments at the synchrotron.

Andrea is a MSc Physics Engineering student, specialized in photonics. His contributions revolved around the synchrotron campaign.

Mauro is a MSc Physics Engineering student, who followed the curriculum of nanophysics. His contributions to the project focused on the synchrotron campaign.

Linda is a MSc Biomedical Engineering student, specialized in biomechanics and biomaterials. Her effort in the project revolved around the printing of the bone scaffold and the experimental campaign.

Stefano is a MSc Aeronatical Engineering student. He gave contributions to the design of the scaffold and the post-processing of the data acquired at the synchrotron.

Goal

The aim of the BASTA project is to develop a bone scaffold, a biological construct for bone repair interventions, to treat bone fractures with a patient-specific approach.

Bone scaffolds are an alternative solution to standard grafts based on human or animal bone tissue. These techniques suffer from drawbacks like propension to trigger immune rejection and limited availability of bone tissue. Bone scaffolds, instead, are not yet commercially widespread due to technological acerbity. The goal of the project is to design a bone scaffold that can become popular in clinical practice by fulfilling the biological requirements of such interventions: the scaffold must both maintain physical integrity of the transplant site and promote bone ingrowth to heal the bone defect.

The main goal of realizing such a scaffold was then divided into sub-objectives, the first of which was to design the construct. The approach followed for this purpose was bio-inspired: starting from the study of natural materials with unique properties, the attempt is to replicate these characteristics in innovative, artificial ways. In the specific case of the BASTA project, the bio-inspired approach was oriented at developing an architecture that mimics the hierarchical internal structure of bone tissue.

The second objective was to physically realize the designed scaffold, which was printed by a high-resolution 3D printer, based on the process of two-photon polymerization. Subsequently, the scaffold was cellularized, so that the cells necessary for the new bone formation can grow and differentiate.

The third step was to study the quality of the printed architecture of the scaffold and if the proliferation of the cells proceeded as expected. For this purpose, high-resolution images were taken during two experimental campaigns at the Synchrotron of Trieste. The printing-quality assessment, consisting of reconstructing the 3D geometry from synchrotron slices, was fuelled by U-net based deep learning model. A qualitative analysis of the mineralization, i.e. the cell-guided growth of the mineral components in the bone scaffold, was performed through optical microscopy.

Understanding the problem

As the average age of the population is increasing, and so the rise of age-related disorders, such as osteoporosis, bone fractures are exponentially growing. Osteoporosis affects about 200 million people globally. Limiting to the Italian situation, there are 5 million osteoporotic people, of which 80% are postmenopausal women. The recovery process from a fracture is often slow, difficult and may lead to a disability or even to the death of the patient. Up to 25% of patients who suffer a femur fracture will die within a year. Of those who survive, approximately half are totally or partially dependent. Chronic pain, functional limitation, social dependence, psychological disorders, reduced mental health and social isolation combine to cause a serious deterioration in the quality of life.

Thus, it is evident that further work is needed in finding a solution for fracture treatment, stimulating bone regeneration at the fracture site. Autologous bone graft is still considered the gold standard for treating bone fractures but variable clinical outcomes, morbidity at the donor site and surgical costs must be taken into consideration. Bone tissue engineering (BTE) aims to provide viable alternatives to solve these issues. BTE strategies, called bone scaffolds, have gained wide attentions in the research field, and the some of them have already achieved the clinical application. However, commercial scaffolds need to be used in combination with other medical devices to stimulate an acceptable outcome. Having to use two different devices may both be more expensive and render the surgery more complicated. More and more researchers claim that this shortcoming is attributable to the fact that bone scaffolds do not comprise a pore network which resemble the architecture of native bone. Furthermore, commercially available bone scaffolds are sold in default dimensions which do not match the bone defect, making the surgery longer. Lastly, the great majority of bone scaffolds are made in ceramic materials, which are not suitable for load-bearing applications.

Therefore, new micro-featured, customized and load-bearing bone scaffolds must be developed.

Exploring the opportunities

The main objective of the project could be tackled by the exploitation of two-photon polymerization technology, a type of 3D-printing approach which saw a great rise in popularity and fast developments in recent years.

3D printing strategies have been leveraged in the production of bone scaffolds as they permit to develop constructs in a customized way. However, their main limitation lies in the resolution. Indeed extrusion-based approaches, the most employed, can provide features only at the millimeter scale. Two-photon polymerization is a particular 3D-printing approach which overcome this limitation, reaching a submicrometric resolution. Different and complex construct can be printed, but a human bone-inspired geometry seems the most reasonable solution. The usage of a biocompatible and stiff material is essential to provide a suitable environment for the cellular colonization of the structure. Then, IP-Visio, a specific polymeric resin, was selected as it can guarantee the required specifications.

Generating a solution

The solution proposed by the BASTA project originated from observing the structure of natural bone, which exhibits a hierarchical structure with porosity at different levels. Thanks to high-resolution bone scans obtained via synchrotron imaging, it was possible to extract quantitative information about the geometry of natural bone down to the micro-scale level. The team adopted a bio-inspired approach at two levels to design the solution. At the first level, a structure incorporating the macro-scale porosity of human bone was derived directly from the scan of a bone specimen, originally gathered from a femoral head. At the second level, the natural complex micro-network of small pores and interconnecting channels, called *lacunae* and *canaliculi*, was integrated into the structure.
This second level of design followed two alternative patterns. The first is a simplification of nature, consisting of a simple cubic lattice of lacunae. The second aims to replicate the natural complexity more closely, using a randomly generated network of lacunae.

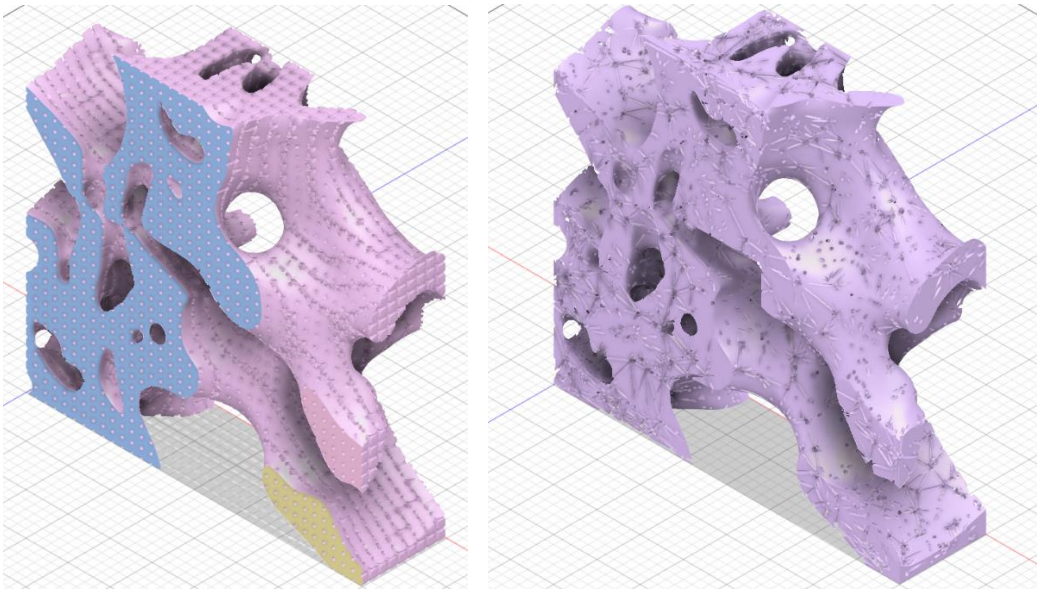


Figure 1: Regular (on the left) and random (on the right) bone scaffold design

The fabrication of this geometry was made possible using a cutting-edge 3D printing technology known as two-photon polymerization. This technique is one of the few that allows replication of structures with sub-micrometric resolution.

The design and realization of a bone scaffold with two-level porosity are innovative, as previous solutions could only mimic the meso-scale due to a lack of detailed information about the micro-scale and the limitations of lower-resolution manufacturing methods.

The printed constructs underwent quality control at the Elettra Synchrotron in Trieste during an initial imaging campaign to verify their printing fidelity. This process involved deep-learning-based slice segmentation and 3D reconstruction. Following satisfactory results, human mesenchymal stem cells were seeded onto the scaffolds and cultured in vitro to encourage differentiation into bone cells, which are responsible for the deposition of hydroxyapatite (the main mineral component of bones).

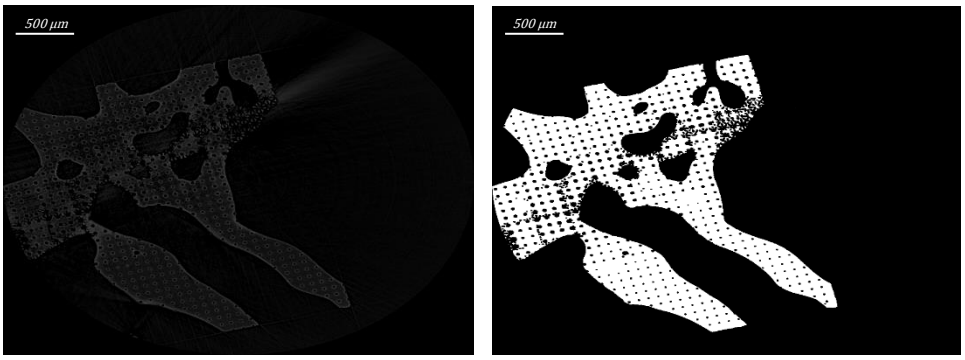


Figure 2: Raw data from the synchrotron (on the left) and corresponding ground-truth binary mask (on the right)

A second imaging campaign at Elettra Synchrotron aimed to study the cellular response, but the hydroxyapatite crystals were not clearly visible. As a result, an analysis with optical microscopy was performed to further evaluate mineralization, providing additional qualitative insights into scaffold performance.

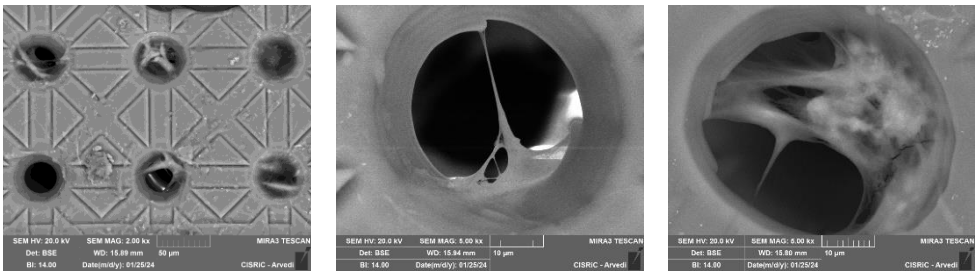


Figure 3: Microscope images of the cellularized scaffolds

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