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GAP2

Executive summary

The burden of bone fractures on healthcare system

Bone fractures are becoming a growing health and economic worldwide burden. This issue is a result of different factors, especially the rise in life expectancy. For instance, in the United States, annual fractures are expected to rise by 50% from 2005 to 2025. This results in a consequent dramatic increase of the associated costs in the considered time interval, which is expected to reach $25 billion (Burge et al., 2007). The problem has high relevance in European countries as well. Based on data from the Italian Ministry of Health, it is predicted that 40% of the Italian population, i.e. those aged 65 and older, will experience a fracture of the femur, vertebrae, or wrist in the future.

The fragility of the bone, which makes the patient more prone to fractures, is hard to evaluate with existing methods, making the prevention of bone fractures really complicated. While there is a significant research effort on this issue, complete understanding of the phenomena underlying microfractures is still missing, as well as a suitable model for fracture prediction. It is therefore becoming more and more fundamental to find new techniques that allow bone fragility diagnosis and, most importantly, analysis of bone structure and microarchitecture, to provide an estimation of fracture risk.

Meanwhile, on the treatment side, research on bone regeneration is continuously expanding, focusing on synthetic bone substitutes made by artificial scaffolds seeded with cells able to proliferate and reconstruct a true bone tissue. In order to optimize scaffold geometry and ensure a faster recovery, microarchitecture analysis and characterization is again crucial.

Our approach

GAP2 is a research project aimed at enhancing both the prevention and treatment of bone fractures through a synergistic approach.

A State-of-the-art research represented a starting point to gain a general vision on bone fractures, the main diagnostic tools and their limitations, as well as to investigate the existing strategies for bone regeneration and their criticalities. The study focused then on the great potential of Artificial Intelligence (AI), exploring cases of Artificial Neural Network (ANN) applied to medical images in order to facilitate fracture diagnosis, risk prediction, and optimize the design of bone scaffolds. This is a pivotal point to explore in the research, especially due to the intrinsic limitations of the current technology of medical imaging techniques and medical tools, represented by the absence of clinical imaging methods that allow to obtain sufficiently detailed images of the structure of the bone.

This was tackled by the GAP2 project through the exploitation of Synchrotron-imaging techniques of in-vitro bone scaffolds and femoral head samples (from patients undergoing femoral neck osteotomy for primary hip replacement) at the ELETTRA Synchrotron in Trieste, where a technology able to couple μ-CT (X-ray tomography with micrometric resolution), synchrotron X-ray sources (more powerful and detailed than traditional ones), and mechanical testing can be found. A high number of human femoral head samples coming from healthy, covid-affected, or osteoporotic patients were subjected to micro-compression tests under displacement control and simultaneously scanned by the synchrotron monochromatic beam, thus acquiring high-resolution images. Samples of mineralized scaffolds (where bone has started to regenerate) were tested as well. The images were then processed to help optimize the subsequent tridimensional microstructure recognition, including identification of micro-cracks and lacunae, which are features of the bone with a presumed impact on bone toughness.

Despite the high time cost necessary to obtain enough accurate images, these have been the starting point of the practical research to finally be able to investigate whether a correlation between the different scales of bone structures exist, hence enabling physicians to properly identify more frail subjects and enhance prevention.

However, because of the high resolution of this imaging technique and the elevated number of images in the dataset, performing this recognition task following the gold standard (consisting in experts manually labelling each pixel of each image) would be extremely time consuming, if feasible at all. Therefore, algorithms play a crucial role for statistically relevant studies. Nonetheless, the definition of well-performing algorithms can be quite challenging, especially for the recognition of the structure of the scaffold and of the mineralized tissue in the samples, as their identification is dependent on multi-scale and multi-dimensional observations framed in the local context of the surrounding measures. The absolute univocity of this classification is disputable as it can vary not only from person to person but also in between repetitions by the same operator. Luckily, the recent interest and developments in Artificial Intelligence brought in the shed new powerful and readily available tools, such as deep Convolutional Neural Network (CNN). The use of such tools requires carefulness to achieve robustness/universality and avoid biased results, along with the technical skills needed to select and tune the proper neural network architecture, prepare adequately the dataset, and perform the training (fitting) of the model. Still, the balance between the necessary amount of skills, knowledge and time is far more approachable than what is demanded by manual labelling or the development of advanced algorithms. Furthermore, Neural Networks can be very flexible, relatively easy and quick to adapt to new setups, imaging techniques, recognition tasks, or geometries involved. A proof-of-concept is presented in the last phase of our project, which saw the development of two CNNs, one for the detection of the scaffold structure and the other for the recognition of the mineralized tissue. Both models outclass the previously available automated methods, enabling to perform analysis on statistically significant datasets in extremely reduced times and with results comparable to the ones previously achieved by manual segmentation.

Future perspectives

The perspective research for our project can be divided into two main fronts:

- Expanding the available data through new experiments and improving the performance of the Neural Network through a revision of its architecture and training, adapting them to increased availability of computational resources.

- Researching on correlation and further uses of the extracted microstructural features, which will eventually lead to clinically relevant results able to impact and improve lives by the prevention and cure of bone-fragility-related diseases.

Key Words

Bone fractures – Osteoporosis – 3D Bioprinting – Synchrotron Imaging - Convolutional Neural Network

Project description written by the Principal Academic Tutor

The increase in fragility fractures, their dramatic impact on the healthcare system and the financial management raise a big red flag, especially in the mostly affected European countries. With the increase in life expectancy, the prevalence of chronic conditions, such as osteoporosis, is also set to rise, leading to an exponential upsurge in fragility fractures. Nowadays, an additional wake-up call rises from the outbreak of Covid-19 pandemic: a slowdown in bone formation is hypothesized as a direct outcome of the infection, which, together with the increased sedentary lifestyle of the hospitalized patients, lead to a reduction in bone mass and strength. Here, GAP2 comes into play, providing a cutting-edge multidisciplinary approach oriented at designing novel patient-specific solutions to counteract the fracture crisis. GAP2 will bridge the gap between the research and the market level by designing disruptively new technical and biological strategies for the design and development of optimal constructs (i.e. scaffolds) for bone regeneration. This is feasible by adopting advanced additive manufacturing solutions: these constructs will be based, for the first time, on high-resolution synchrotron images of human bones. To tune the impressive number of parameters considered when dealing with implantable devices, an artificial intelligence approach will be implemented. GAP2 aims at designing practical solutions to address the fragility fracture crisis from a multi-scale and patient-specific perspective, exploiting synchrotron power to obtain high-resolution images of human bone micro-architecture, providing bio-inspiration in the development of bone scaffolds. The intrinsic complexity in the scaffold realization phase could be only overcome by means of the high versatility provided by the additive manufacturing technique. Additionally, to shed some light in the puzzling number of parameters to be considered in addressing the fragility fracture issue, neural networks will be an essential aid. This will be evident from two sides: definition of clinical indexes for fracture prevention and realization of physical constructs for bone reconstruction.

Team description by skill

Elena is a MSc Biomedical Engineering student, specialized in the field of Electronic Technologies. Her contributions revolved around the computational aspects and the State-of-the-art analysis.

Giordano is a MSc Space Engineering student, with a background on Industrial Engineering. His contributions revolved around the mechanical aspects concerning scaffold and bone properties, as well as the development of the training dataset for the Neural Network.

Marco is a MSc Material Engineering and Nanotechnology student, specialized in the field of Nanomaterials and Nanotechnology. His contribution revolved around all the aspects related to the development and training of the Neural Network model, as well as the mechanical testing at ELETTRA Synchrotron in Trieste.

Chiara is a MSc Biomedical Engineering student, specialized in Biomechanics and Biomaterials. Her contributions revolved around the biological and mechanical aspects of bone tissue and regenerative treatments, as well as the mechanical testing at ELETTRA Synchrotron in Trieste.

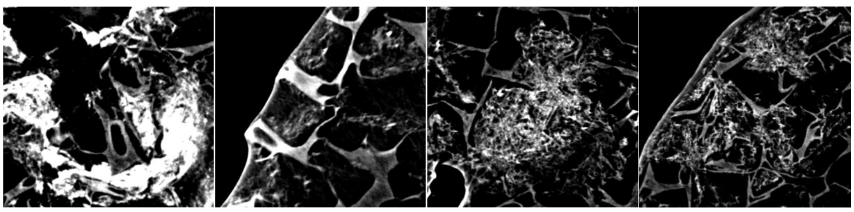
Goal

The aim of the project is to face bone fractures crisis adopting a twofold approach, considering both fracture prevention and treatment.
On the prevention side, the main goal is to work for the implementation of a micro-scale fragility index for fracture prevention to be used in clinical practice to estimate the probability of fractures. To achieve this result, it is necessary to first identify current clinical parameters used to define bone fragility and macro and micro scale effects of bone pathologies on bone architecture. Microarchitecture analysis would be assisted by the development of an Artificial Neural Network (ANN) for the recognition of micro-defects and damages on high-resolution synchrotron images of human bones.
Regarding the treatment side, the objective is to provide strategies to design and develop tailor-made 3D-printed bone substitutes for bone regeneration, to be produced with advanced additive manufacturing techniques, made by a scaffold seeded with mesenchymal stem cells (MSCs), which will focus the healing process where it is most needed, whilst providing adequate mechanical properties similar to the host bone. To ensure a better recovery, scaffold morphology needs to be optimized and customized according to the patient's specific needs. This could be possible after having investigated the influence of scaffold architecture on bone tissue growth thanks to the development of AI tools to be applied to synchrotron bone constructs images. The Artificial Neural Network would focus on the recognition of different regions of the scaffold, based on geometrical and structural parameters favoring bone regeneration and providing mechanical strength.
Our work could provide a methodology for the microscale study for several bone conditions, ranging from osteoporosis to rare bone diseases, with concrete benefits not only for patients but ultimately for the health sector and the entire society.

Understanding the problem

Following the increase of life expectancy and so the rise of chronic diseases, such as osteoporosis, the number of fragility bone fractures is exponentially growing. Osteoporosis affects about 5,000,000 people in Italy, of which 80% are postmenopausal women and mortality from fracture of the femur is 5% in the period immediately following the event and 15-25% after one year. The problem has been further triggered during the last two years by the outbreak of Covid-19 pandemic, as the infection, together with the sedentary life of patients in hospitals, has caused significant loss of bone mass and strength in hospitalized population.

Bone fractures represent a critical condition for humanity from multiple aspects, such as: an increasing impact on women, the psycho-social burden due to the increase in risk with increasing age, and the dramatic impact on the financial management of the Healthcare system due to the necessary hospitalization.
The key aspects on which it is necessary to work to reduce the impact of this issue are prevention and treatment. Fracture prevention, with an efficient fragility diagnosis and estimation of the fracture risk, is currently hard to achieve. This is related to the complexity of bone structure, ranging in scale from centimeters to nanometers, and to the lack of a complete understanding of the relation between bone defects at the micro-scale and the occurrence of fractures. When prevention is not enough, innovative treatments, such as tissue engineering approaches, could foster faster and better recovery of the patients through the implantation of bone scaffolds that promote bone tissue regeneration. Nevertheless, strategies to optimize and customize their morphology are still the object of several studies, and they also require a deeper understanding of the influence of the microstructure. Research on these topics is significantly expanding, thanks to the exploitation of micrometric imaging techniques for the analysis of bone structure and microarchitecture. However, the traditional analysis process of these high-resolution datasets would be completely unfeasible due to the sheer amount of data contained. Therefore, new accurate and fast image processing techniques must be developed.

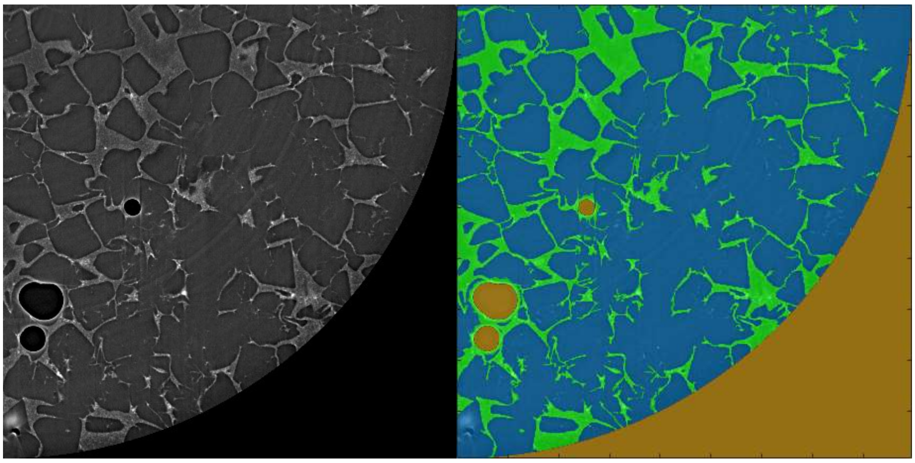


Several examples of microstructural details obtained from Synchrotron X-ray tomography.

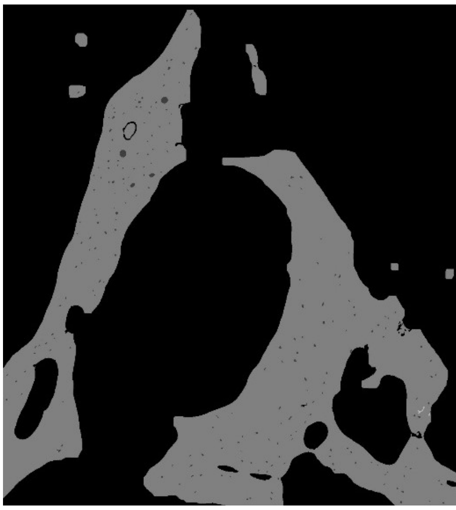
Exploring the opportunities

The main objective of the project could be tackled by the exploitation of a Convolutional Neural Network, a type of Artificial Intelligence model which saw a great rise in popularity and fast developments in recent years.

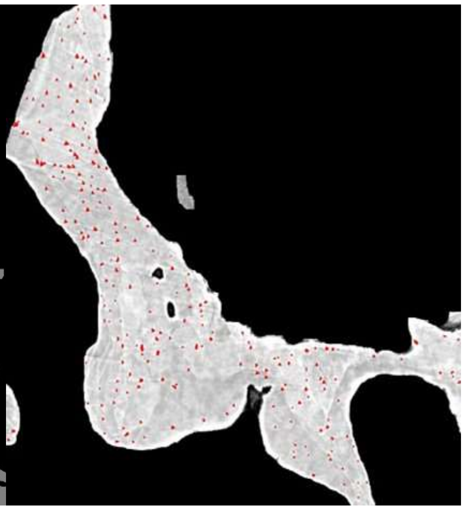
Nowadays, the golden standard is founded on manual segmentation, which is extremely time consuming, necessitates experts and is nevertheless affected by a personal bias, which can lead to different segmentations produced by the same person. A Neural Network algorithm addresses these problems all at once, ameliorating and streamlining the process of microstructural characterization. Nevertheless, it necessitates of a properly selected dataset in order to guarantee an optimal training, and thus efficiency and accuracy of the network itself. The dataset images have been manually segmented exploiting various software alternatives, such as ITK-SNAP and MATLAB Medical Imaging Toolbox.



Manual segmentation: scaffold (green), background (blue), exterior (orange)



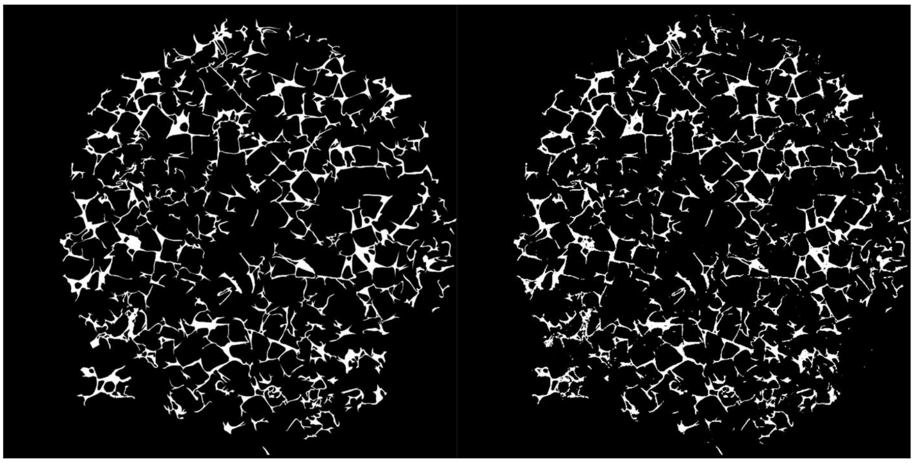
ITK-SNAP, lacunae segmentation



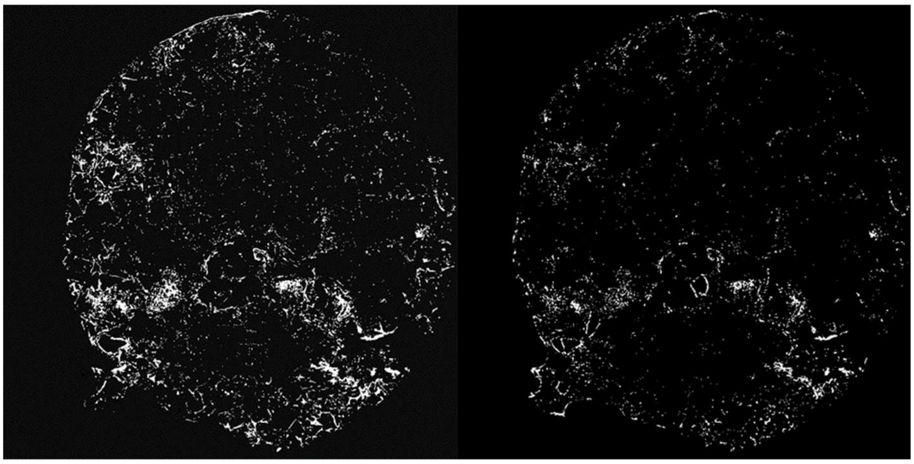
MATLAB, lacunae segmentation

Generating a solution

The solution proposed by the GAP2 project aims at improving and advancing in the field of bone and scaffold microstructural high-resolution imaging, as well as the automatized processing of the obtained images. This includes the development of an Artificial Intelligence algorithm capable of properly identifying and isolating the mineralized bone structure and the scaffold structure. More precisely, a Convolutional Neural Network for image segmentation was trained with a set of manually segmented slices coming high-resolution Synchrotron X-Ray Tomography of silk fibroin scaffold samples, which were left to mineralize after seeding with Mesenchymal Staminal Cells. The network can thus help in identifying which morphological parameters are useful to induce bone mineralization and cell specialization, hence optimizing the regenerative performance of the fabricated scaffolds. By coupling the imaging with integrated mechanical testing, the relation between mechanical properties and the microstructure can also be resolved, which in terms will help both the prevention of bone fractures, by recognizing risk indexes in the bone microstructure of the patients, and the treatment, by the fabrication of sturdier scaffold structures.



Corresponding manually segmented (left) and CNN segmented (right) mask of the scaffold.



Corresponding manually segmented (left) and CNN segmented (right) mask of the mineral bone.

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