Osteosarcoma is a malignant, **primary bone tumor** affecting children and young adolescents. According to the National Cancer Institute, it is estimated that bone and joint cancer represents only 0.2% of new cancer cases in the USA each year. Since they are sporadic, the first symptoms of cancer can be easily ignored or misdiagnosed. Osteosarcoma develops in bone during its remodeling, which is prompt by mechanical stimulation. Cancer cells produce a malignant osteoid, a fibrous cellular substance that gives bones their flexibility in normal (healthy) conditions. However, the malignant tissue created by the osteosarcoma possesses different properties (i.e., stiffness) compared to **healthy bone**. Bone and its unique microenvironment promote cancer cells to utilize adhesion molecules, matrix proteins, and many other factors to survive. This allows their spreading, first, within the surrounding tissues **(invasion)** and then to distant locations through the circulatory system. In the end, the cancer is often at a late stage when diagnosed and has already spread to other organs (i.e., lungs).

Some research works on osteosarcoma have already been undertaken via the implementation of cell cultures and laboratory animal-based models. However, the primary mechanism governing its progression has not been solved yet. In addition, the existing simple three-dimensional tissue models still do not fully exploit the potential of biomaterials, which in turn can be helpful to control cancer cell invasion. At this point, there is an urgent need to develop an in vitro platform to investigate the biomaterial-assisted invasion of osteosarcoma cells. This would help understand the first steps of metastatic spread and the impact of participating healthy bone tissue in cancer progression under mechanical stimulation. An in vitro 3D model of osteosarcoma consisting of healthy and malignant tissue could solve this puzzle. To recapitulate the in vivo environment, the model would need to be subjected to mechanical loading, which would mimic the signal-enhancing bone remodeling.

The overall aim of the project is to develop a novel biomaterial-assisted tissue-engineered in vitro model consisting of healthy and malignant bone tissue to study the invasion of cancer cells. The project will focus on the impact of biomaterial properties and mechanical stimulation on cancer progression. Therefore, it is proposed that a healthy bone model will be fabricated using a three-dimensional printing approach from polyester-based scaffolds seeded with bone- and vascular-forming cells. Simultaneously, osteosarcoma will be inserted into the hydrogel matrix to establish a 3D bone tumor model. This hybrid 3D model, consisting of a bone tumor model inserted into a healthy-mimicking tissue, will be also subjected to cyclic mechanical loading. The invasion of cancer cells will be assessed by fluorescence microscopy. Prior to this, specific tissue-forming cells will be labeled with different fluorescent dyes to allow their recognition during evaluation. Additionally, hydrogel platform and polyester-based scaffolds will undergo multistage characterization during the lifetime of the project, using techniques such as Scanning Electron Microscopy (SEM), Computed Tomography (CT), Dynamic Mechanical Analysis (DMA), fluorescent microscopy, or confocal microscopy.

Ultimately, the implementation of the project will allow expanding the knowledge on biomaterials and the fabrication of healthy and malignant bone tissue models. The developed hybrid model could also be used in the future for new ideas improvement and therapeutic strategies development.