BIOENGINEERING AND MEDICAL-SURGICAL SCIENCES

UNITO - 3D models of healthy and bone metastatic niche

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Context of the research activity	Bone is a fundamental organ for its mechanical and metabolic functions. Bone tissue engineering is a key field in regenerative medicine requiring for both physiological and pathological conditions accurate 3D modeling. Several animal models, mainly murine, have been developed to simulate the various pathological conditions of bone and study potential therapies. The use of animal models has always been the subject of ethical, economic and environmental debates; therefore, research is trying to find alternative models that save animals. To this end, this project proposes to develop an in vitro 3D system that mimics the bone microenvironment and in particular the hematopoietic stem cell niche. Under physiological conditions these niches guarantee the correct reservoir of blood cells and are made up of endosteal, immune and endothelial cells. In case of osteotropic tumors, cancer stem cells are attracted to these niches and can completely subvert them, thus altering the functionality of the bone tissue. At the level of pre-metastatic niches, cancer stem cells can evade the control of cells of the immune system, such as Natural Killer (NK) cells, which represent an initial defense barrier against cancer stem cells. Therefore, the study of the interaction among cancer stem cells, immune cells and bone niche represents a potential source of identification of target molecules capable of blocking metastasis. Biocompatible materials such as polycaprolactone (PCL), ß - tricalcium-phosphate (ß-TCP) and bioglasses will be mixed at different concentrations, to identify a mixture capable of stimulating osteoblasts to create the endosteal niche, which must be vascularized by the endothelial cells, responsible for the perivascular niche. B-TCP is known for its osteoinductive properties, while copper-rich bioglasses have pro-angiogenic action. Using 3D printing, the mixtures will be printed to build a 3D scaffold with a trabecular, bone-like structure. Subsequently, these scaffolds will be populated with different cell types to study the complex interactions between bone cells, endothelial cells, tumor stem cells and NK cells. Tumor stem cells will be obtained from different NSCLC cell lines and we will study their survival and localization capabilities in the scaffolds, previously populated by endosteal and endothelial cells. Finally, NK cells will be added to evaluate their effect on tumor stem cells and the possible formation of "metastatic lesions". In parallel, the same type of experiments will be carried out without

	tumor cells as a normal control of the 3D structure. A bioreactor, which allows the flow of nutrients to be controlled and maintained constant within structures, will be used to maintain the crops for 30 days. The various factors released by the cultured cells will be assayed by ELISA. Some scaffolds will be fixed with new generation fixatives with low environmental impact and analyzed under the electron microscope and by immunohistochemistry to visualize the presence of bone niches, tumor stem cells and NK cells. NK cells will be recovered from the scaffolds to study their phenotype, and understand the state of activation or inhibition they undergo after interaction with tumor stem cells.

Objectives	The main aim of the research is the regeneration of pre-metastatic niche in a perfused 3D printed scaffold - Design of the 3D printed bone-mimicking scaffold. PCL polymeric matrix filled with a specific concentration of ß-TCP and with or without the presence of a bioactive glass, containing pro-angiogenic elements. The geometry and orientation of the filaments will be optimized to produce structures with standard and controlled porosity, similar to the bone one. - Assessment of optimal strategy to repopulate the 3D scaffold. Cultures of osteoblasts and endothelial cells (H-MEC) will be optimized on the 3D scaffold, maintained in a bioreactor, which allows, in combination with customized holder, direct perfusion of the samples. This type of culture has a duplex advantage because it allows a continuous re-circulation of medium and nutrients inside the structures and also gives hydrodynamic stimulation to co-cultures. All these features will be tuned to better reproduce the physiological environment of the bone niches. To understand whether the presence of bioglasses is effective in promoting vascularization, VEGF will be added or not in the co-culture with osteoblasts. After 30 days of culture, scaffold will be fixed with PAF and processed for IHC and SEM analyses. At 14- and 30-days of culture, supernatants will be collected to dose angiogenetic factors through ELISA. - Assessment of the ability of immune cells, such as activated NK cells to interact with components of the bone niche and cancer stem cells. After repopulation of the niche re-created in the scaffold, cancer stem cells derived from non-small cell lung cancer and immune cells, such as activated NK cells will be added on the scaffold to study their localization in the niche, and their interaction with osteoblasts and endothelial cells. Even in this case IHC and SEM will be performed. Inhibitor targeting molecules expressed by cancer stem cells, will be tested to verify their effect of CSC colonization of bone niches.

Skills and competencies for the development of the activity	The candidate is expected to be graduated in Biology with competence in functional genomics. The ideal candidate should have previous experience in cell biology, with at least one year of experience in: 1) in vitro cultures of bone, endothelial, cancer and immune cells 2) using flow cytometer to analyze cell immunophenotype 3) materials available to mimic the bone tissue and knowledge of methods to test their biocompatibility. 4) gene expression analysis Conference/workshop participation related the topic of this fellowship represent a plus.