Microalgae are a large and diverse group (hundreds of thousands of species exist) of unicellular eukaryotic microorganisms of different shapes and between 2–50 μm in diameter. Those microorganisms are abundant in nature and occur both in freshwater and seawater all over the world. Most species contain chlorophyll and use sunlight as an energy source to convert carbon dioxide into biomass and oxygen in the process of photosynthesis. This remarkable ability of microalgae to continuously produce oxygen and valuable bioproducts can be used in various fields of biomedicine and bioengineering. Many bioapplications use microbe entrapment (immobilization) in hydrogels (water-containing polymeric networks) as a method for the simplified separation of cultured cells and their products from the growth medium. Immobilization in hydrogels protects the cells from shear forces and provides the microorganisms with special tolerance for environmental stresses, hence supporting their growth. Among the immobilization methods, 3D bioprinting has been obtaining the highest popularity in recent years. One of the newest additive manufacturing techniques is coaxial direct ink printing, which simultaneously extrudes two or more inks or other solutions arranged concentrically in a single fiber.

Immobilization may serve as an excellent tool to create engineered living materials (ELMs). ELMs are designed by entrapping biological cells within non-living matrices. ELMs have been gaining the attention of scientists around the world who recognize that often the most robust and functional materials are those that mimic nature. However, unerring biomimicry requires a deep understanding of natural mechanisms and interactions between living and non-living matters at the microscale scale. Also, the appropriate geometry and internal structure are necessary to optimize the functionality of ELMs. By combining the functions of living cells and the structure of nonliving matrices, ELMs can detect changes in the environment and adjust their functions accordingly. The unique properties of microalgae to change behavior and photosynthesize in response to light may prove suitable for creating a living material that produces oxygen in a controllable manner.

This project (Fig. 1) will use 3D bioprinting and microalgae-containing materials to develop living 3D materials capable of sustainable oxygen production. For the first time, we will introduce branched hydrogel lattices laden with microalgae as an ideal platform for the design of a new class of oxygen-generating ELMs. The alginate and gelatin methacrylate-based hydrogels will serve as a scaffold and support for microalgal (*Chlamydomonas reinhardtii*) cells, together forming a bioink. Microalgal hydrogels will be designed to have a network of patterned hollow channels. Coaxial direct ink printing will be used to print two materials simultaneously in a core/shell manner using a coaxial nozzle. The inner nozzle of the coaxial nozzle will be used to extrude sacrificial ink or crosslinking solution, while the outer nozzle will be used to print a sheath material - bioink. Living and nonliving components together will form a material that has the photosynthetic ability of microalgae and the robustness of the hydrogels. The microalgae-containing core-shell constructs will provide an advanced platform to investigate 3D static (batch) and dynamic (perfused) human umbilical vein endothelial cell (HUVEC) culture under controlled oxygen microenvironments. We will study oxygen production by microalgae and its transport to a flowing cell medium. We anticipate that dynamic oxygenation will be a better oxygen supply for mammalian cells than the traditional static process and will help them grow and maintain activity. Next, we will use mathematical modeling of diffusion, shear stress within the nozzle, and perfusion (CFD) to support experimental results. Finally, the mechanism governing oxygen release by microalgae immobilized within channeled scaffolds will be proposed.

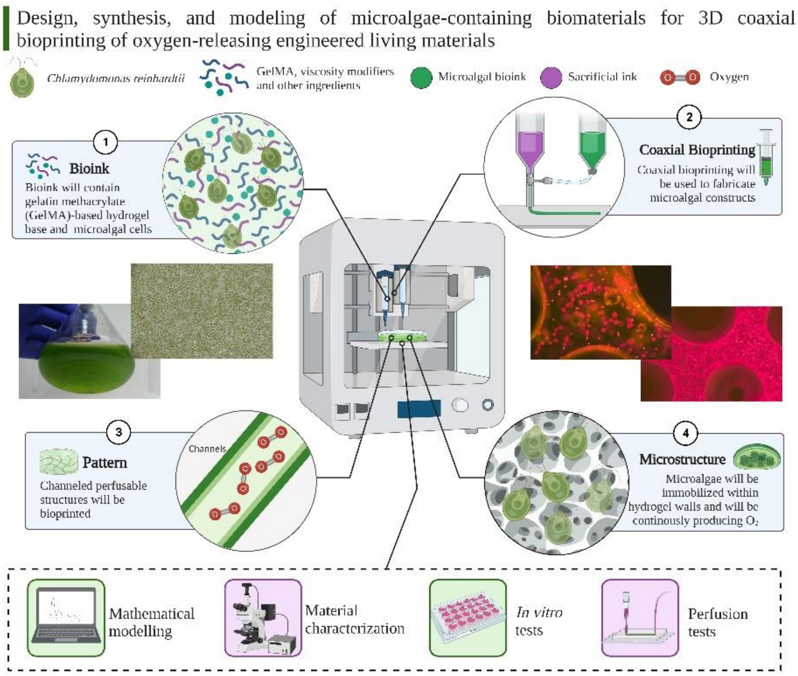

Figure 1. Schematic summary of the project tasks. Created using Biorender.com

The bioprinted living materials offer diverse possibilities for novel product applications including biomimetic structures (e.g. artificial leaves), tissue models, and wound-healing dressings. Also, the combination of oxygen-generating microalgae ELM hydrogels with 3D coaxial printing has the potential to create an innovative strategy for hypoxia treatment.