

# BUTTERFLIES Project

Bio-Polymers & Additive Manufacturing

# Newsletter 3

## Developing two photon printing for chitosan organoid scaffolds

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# Developing two photon printing for chitosan organoid scaffolds

Vital3D Technologies and its BUTTERFLIES partners are developing a laser printing process for biodegradable chitosan scaffolds intended to support organoid research. The team has configured and delivered a research printing platform, examined the architecture of reference scaffolds and completed initial exposure tests on a chitosan formulation. These activities establish the experimental basis for the next phase of material and process development.

Organoids are three-dimensional cell models used to study aspects of tissue behaviour. The scaffolds that support them need carefully designed internal spaces, allowing cells to organise and nutrients to reach the growing tissue. BUTTERFLIES is exploring how two-photon polymerisation, or 2PP, can produce these complex porous structures in chitosan-based materials. In this process, focused ultrashort laser pulses solidify selected regions within a light-sensitive material, building a structure according to a digital design.

The project builds on Vital3D's FemtoBrush technology, which changes the shape of the laser focus to adjust the volume processed during printing. This provides a route towards increasing throughput while retaining fine features where the design requires them. Within BUTTERFLIES, the focus is on adapting this capability to the behaviour of new chitosan formulations and the geometries needed for organoid scaffolds.

The Vital Light 3D research system was configured for the project and demonstrated at Vital3D's laboratory in Vilnius, where MTC personnel also received training. The printer was delivered to MTC in September 2026. On-site commissioning and a printing demonstration remain subject to completion of MTC's site-specific acceptance procedures for laser research equipment. Printing work can meanwhile continue at Vital3D.

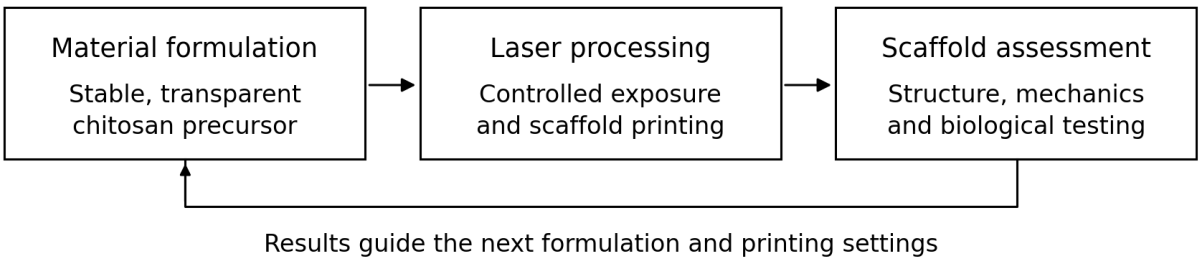


Figure 1. Material development workflow. The scheme includes completed, ongoing and planned activities; it does not represent a validated chitosan printing process.

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To establish a reference for subsequent chitosan experiments, porous scaffold geometries were first printed in a commercial photoresin. MTC examined the samples using scanning electron microscopy and X-ray computed tomography. These complementary methods reveal both the surface features and the internal pore network, including whether pores remain open throughout the scaffold height. The screening showed that laser settings influence pore connectivity and structural consistency. Selected settings will now undergo repeatability checks and further validation. These results concern the reference material; its suitability for chitosan still needs to be established.

The first chitosan formulation supplied by EMPA was successfully prepared and exposed in the printing setup. Camera observations showed lines forming during laser exposure, indicating a local material response consistent with photopolymerisation. However, the mixture also solidified spontaneously and lost transparency, preventing the recovery of complete three-dimensional printed structures. Follow-up tests linked this instability to the presence of the photoinitiator, the additive that helps initiate the light-driven reaction. The findings were shared with Empa to guide formulation improvement.

This feedback is an essential part of the development process. A usable formulation must respond to the focused laser while remaining stable and transparent during preparation and printing. The next material batches will therefore be assessed for stability before progressing through line-writing and multilayer printing trials. The team has identified a working target of at least 24 hours of stability after mixing.

Over the coming months, the partners plan to complete on-site commissioning, test revised chitosan formulations and establish suitable processing conditions. Subsequent work will examine the mechanical properties, chemistry and biological compatibility of printed samples, alongside the fabrication of more complex scaffolds and microchannels. Later in WP4, Vital3D will develop an inflow printing approach that continuously supplies material during scaffold formation, together with a new scanning system. The aim is to increase scaffold production speed while preserving geometry and material performance. These capabilities remain development objectives to be demonstrated in the project.

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