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DELIVERABLE 3.4:
Validated prototype of the OoC

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Type1[1]:	R
Dissemination Level[2]:	PU

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DELIVERABLE D3.4: *Validated prototype of the OoC*

Table of Contents

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1. DOCUMENT HISTORY

Version	Date	Authors/ who took action	Comment	Modifications made by
0.1	30-10-2023	Rosa Monge (BOC), Ignacio Ochoa (UNIZAR)	First draft sent to PIs	Carlos Sánchez Somolinos (CSIC)
1.0	31-10-2023	Carlos Sánchez Somolinos (CSIC)	Submitted to Commission	

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2. LIST OF ABBREVIATIONS

ER: exploitable result

GBM: glioblastoma

LCEs: liquid crystal elastomers

MDR: multidrug resistance

OoC: Organ-on-Chip

TMZ: temozolomide

TME: tumor microenvironment

WP: Work package

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3. DELIVERABLE DESCRIPTION AND SUMMARY

This deliverable outlines the consortium's efforts to validate the effectiveness of valves designed and manufactured in work package 2 (WP2) within a real-use environment focused on the application of Organ-on-Chip (OoC) technology.

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4. INTRODUCTION

4.1 General introduction of OoC in PRIME

Organ-on-Chip (OOC) technology has emerged as a revolutionary breakthrough in the field of biomedical research, promising to transform the way we study human physiology, disease mechanisms, and drug responses. This innovative platform leverages microfabrication techniques to create miniature, three-dimensional cell culture models that replicate the structure and function of specific human organs and tissues. These microfluidic devices enable researchers to closely mimic the complex microenvironment within the human body, opening new avenues for in vitro experimentation that bridge the gap between traditional cell culture systems and in vivo studies.

The development of Organ-on-Chip technology has been driven by a pressing need to improve the efficiency, relevance, and ethical standards of biomedical research. Traditional approaches often rely on animal models that may not fully recapitulate human biology, leading to translational challenges and ethical concerns. Organ-on-Chip models offer a compelling alternative by providing a more physiologically accurate representation of human organs and tissues. These models can simulate intricate biological processes, including fluid flow, cell-cell interactions, and tissue-specific functions, thereby enhancing our ability to study diseases, screen potential drug candidates, and investigate complex physiological phenomena. This kind of technical approach is key to simulate complex environments or diseases like glioblastoma (GBM). With a dismal prognosis and limited therapeutic options, glioblastoma represents a pressing concern in the field of oncology and neuroscience.

Glioblastoma stands as one of the most aggressive and challenging brain tumors known in adults but also in child. This devastating malignancy arises from the astrocytes, glial cells of the central nervous system. GBM has a highly intricate and dynamic tumor microenvironment (TME) that plays a pivotal role in tumor progression, therapeutic resistance, and clinical outcomes. GBM is characterized by its rapid growth, infiltrative nature, and resistance to conventional treatments (Stupp protocol). The Stupp Protocol typically involves a combination of surgery, radiation therapy, and chemotherapy with the goal of extending survival and improving the quality of life for patients with GBM. The initial step in treating GBM is surgical resection, which involves the removal of as much of the tumor as safely possible. However, complete removal is often challenging due to the infiltrative nature of GBM. Following surgery, patients undergo radiation

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therapy. This involves using high-energy X-rays or protons to target and kill any remaining cancer cells in the brain. Simultaneously with radiation therapy, patients receive a chemotherapy drug called temozolomide (TMZ). This is an oral chemotherapy drug that can penetrate the blood-brain barrier and target cancer cells in the brain. After the completion of radiation therapy, patients continue to take temozolomide as adjuvant chemotherapy. Although Stupp Protocol has improved the median overall survival of GBM patients compared to previous treatment strategies but, it's important to note that GBM remains a challenging cancer to treat, and the prognosis is still relatively poor, with almost all patients facing disease recurrence due to multidrug resistance (MDR) mechanisms.

It is postulated that multidrug resistance (MDR) in GBM is a consequence of its intricate microenvironment and cellular interactions. Among the extensively studied phenomena in GBM, ischemia, characterized by a deprivation of oxygen and nutrients, stands out prominently. As GBM tumors exhibit rapid growth, they frequently surpass their blood supply, resulting in regions within the tumour with low oxygen levels and nutrient starvation. The presence of hypoxia and autophagy can activate signalling pathways that facilitate the survival, invasiveness, and resistance to therapeutic interventions of tumour cells. The simultaneous simulation of these pathophysiological events was challenging *in vitro* until the advent of OoC microfluidic devices.

However, even with the utilization of OoC microsystems, various challenges and limitations persist, rendering the simulation of such common pathophysiological events complex. The necessity of employing materials with low gas permeability complicates the fabrication of these devices, rendering them inaccessible to many laboratories. Furthermore, the manipulation of various types of fluids (with or without oxygen and/or nutrients) and the requirement for valves to control fluid flow have limited the widespread adoption of these cultures by researchers. Regarding the issue of valves and flow sensors, the primary limitation encountered is their reusability, driven by their high cost and the significant risk of contamination. This contamination risk includes cross-contamination with remnants of previous samples and microbial contamination by inadequately cleaned and sterilized components.

The proposed solution within the PRIME project involves the development of integrated valves for OoC devices, rendering them single-use and capable of remote actuation via light, thereby enabling their integration into portable devices.

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4.2 Objectives

The overarching objective of this project deliverable is to show the validation efforts performed to demonstrate the capability of the microfluidic platform developed within the project for practical use in simulating diverse in vitro treatment schemes for glioblastoma.

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5. VALIDATION OF THE TECHNOLOGY IN A REAL APPLICATION

5.1 Definition of specifications, design and fabrication of the monolithic chip for OoC

After defining the specifications, different designs and manufacturing methods have been developed to create the devices that have been used throughout the PRIME project. In general, all the devices had the common characteristic and the same basic principle of use, which is that the OoC model presented is based on the generation of gradients inside it. In this way, in general terms, it was necessary to use a device that had a central chamber where a hydrogel with cells embedded in it would be introduced and that was connected to one or two lateral channels through which culture medium could be perfused that would serve to provide to cells with both oxygen, nutrients or drugs.

5.2 Final setup

The integration of the valve element and the OoC is the main objective of the work carried out. The different elements were carried out separately in order to be able to advance in parallel in the different developments. During the last part of the WP3, we have worked in the integration of the separated modules in a fully integrated monolithic operating platform. The final platform is composed of different layers of materials that allow us to define both the fluidic inlets/outlets and the channels and chamber for cell culture, in addition to the integration of the valve developed on this same platform.

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6. RESULTS

6.1) Assessing the impact of TMZ treatment on In Vitro models of Glioblastoma

Throughout the course of this project, we have described a novel in vitro model of glioblastoma developed using spheroids, enabling us to investigate mechanisms of resistance to the most commonly employed chemotherapy treatment, Temozolomide (TMZ). These models have facilitated the identification of a set of genes that are overexpressed in treatment-resistant spheroids when compared to their sensitive counterparts.

As a preliminary step before developing the microfluidic platform coupled with the active valves developed in WP2 to study the response to TMZ using Organ on Chip (OoC) technology, we have created microfluidic chips entirely constructed from thermoplastic materials. This material is highly scalable through mass manufacturing techniques and provide significant adventages. These models have enabled us to describe increased GBM tumor cell death in the presence of oxygen compared to hypoxic conditions following a clinical treatment scheme.

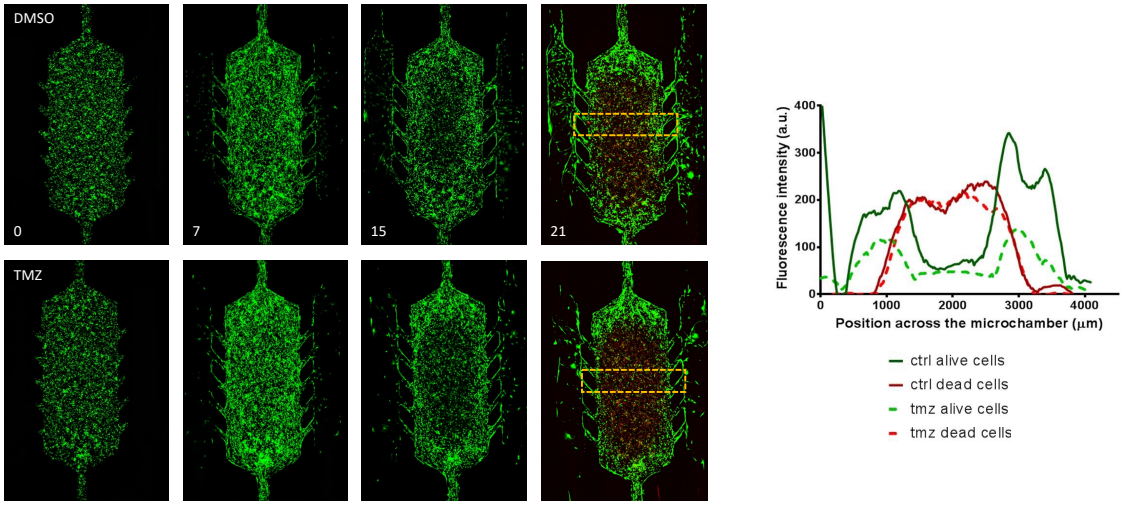

Figure 6.1: Long term assays of TMZ efficacy in GBM organ on chip models using the clinical treatment scheme.
Fluorescence microscopy images of the microfluidic chips after 7, 15, 21 days in culture with TMZ (100 uM) or their controls (DMSO, vehicle used to dissolve the TMZ). Live-dead assay (left). Viable cells are stained in green with calcein, for live cell visualization. Dead cells are stained in red with propidium iodide. Graphic with the fluorescence distribution across the chip in both colors (dead and alive cells) for treated and non-treated chips (right).

Building upon the results obtained in this phase of the project, and leveraging other techniques developed in collaboration between the University of Zaragoza and BEOnChip, we posed the challenge of creating a family of chips capable of eliminating the physical interferences posed by the pillars within microfluidic devices. These pillars were necessary to confine hydrogels within the central chamber of the chips without encroaching upon the lateral channels. This innovative

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development, identified as "Exploitable result 4" (ER4) within the project, has evolved into a new product line for the company BEOnChip.

Continuing with the strategy to develop microfluidic devices compatible with the microfluidic platform that integrates light-actuated control systems (valves) using liquid crystal elastomers (LCEs), we designed a chip in which a central chamber was connected to a lateral channel for the flow of cell culture media and also for treatments, all without the need for rigid pillars. In these models, we also assessed the effectiveness of TMZ treatments and observed results similar to those described in earlier phases of the project.

Despite our initial focus on a modular platform during the second reporting period, in the final phase of the project, we have designed, manufactured, and biologically validated the functionality of monolithic platforms. These platforms integrate light-actuated hybrid valves with cell culture chambers. They have been fabricated and validated for a future use in GBM treatment with TMZ.

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7. CONCLUSIONS

This project has made significant advancements in the development of new designs and models of microfluidic devices for cell culture. It has even led to the creation of a new product line for BEONCHIP. Furthermore, progress has been made in understanding the response of GBM cells to TMZ treatment. New potential applications have been explored, capitalizing on the impermeability of the materials used in chip and platform fabrication.

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