別紙４－１（課程博士（英文））

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Abstract（Doctor）

Title of Thesis	PDMS SlipChip for Evaluation of Effective Drug Concentrations in Cancer Cells

Approx. 800 words

Osteosarcoma (OS) is the most common primary bone cancer, predominantly affecting children and young adults, with treatment efficacy often hampered by chemoresistance linked to the tumor microenvironment (TME) and the overexpression of multidrug resistance (MDR) transporters such as P-glycoprotein (P-gp). Hypoxia, a hallmark of the TME, further intensifies drug resistance, making the determination of minimum effective drug concentration (MEC) essential for advancing personalized cancer therapy. This study presents the development and optimization of a polydimethylsiloxane (PDMS)-based SlipChip microfluidic platform designed for high-throughput evaluation of drug efficacy in osteosarcoma cells. The SlipChip enables rapid and reproducible generation of concentration gradients at nanoliter scales, facilitating real-time assessment of drug responses under tumor microenvironment conditions.

Initially, the biocompatibility of the PDMS material was validated through the culture of SAOS-2 osteosarcoma cells under both normoxic and hypoxic conditions. The porous nature of PDMS facilitated efficient oxygen exchange, enabling accurate simulation of hypoxic TME. Cell viability, morphology, and proliferation were comparable to those observed in conventional tissue culture platforms, with hypoxic conditions in the PDMS chips resulting in reduced proliferation and smaller cell size, consistent with physiological tumor behavior.

Technical advancements in device fabrication included the use of photolithography and soft lithography to produce PDMS SlipChips, with systematic modulation of curing temperatures to optimize surface energy and interlayer adhesion. The study found that lower curing temperatures increased surface energy, thereby enhancing adhesion and sealing, while higher temperatures resulted in stiffer, less adhesive PDMS. Lubrication with silicone oil, particularly 50 cSt at high spin speeds, was optimized to ensure reliable sealing and smooth slipping between layers. A custom 3D-printed stage with magnetic sealing and micro-gauges was developed to guarantee precise alignment, leak-free operation, and consistent microwell overlap. The design also addressed bubble formation by optimizing the width ratio (WR) of microwells to microchannels, with WR=5 achieving the lowest bubble

coverage and most effective gradient formation.

Functional validation of the platform was demonstrated through gradient generation using Rhodamine 6G and green food dye, with fluorescence intensity measurements confirming the accuracy and reproducibility of concentration gradients.

The optimized SlipChip platform exhibited robust sealing, eliminated leakage and bubble-related inconsistencies, and supported healthy 3D cell culture. It enabled the study of ascorbic acid effects on osteosarcoma cell growth, with both SlipChip and traditional culture dishes showing similar cell death responses to the stimulus. These results confirm the platform's suitability for biological assays and its potential for high-throughput drug screening.

In conclusion, the PDMS SlipChip microfluidic platform developed in this research offers a reliable, high-throughput solution for evaluating drug efficacy in osteosarcoma cells. Innovations in fabrication, sealing, lubrication, and alignment have addressed key challenges in microfluidic gradient generation and cell-based assays. The platform successfully supports healthy cell culture and enables precise, reproducible drug screening, paving the way for personalized cancer therapy by facilitating rapid determination of MEC and real-time assessment of chemoresistance mechanisms. Future work will focus on applying the optimized SlipChip system for doxorubicin testing and further refining device performance for broader clinical applications.