別紙４－１（課程博士（英文））

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Abstract（Doctor）

Title of Thesis	Live Single-Cell Screening Based on Cell Encapsulation and Irradiation of Patterned Light

Approx. 800 words

Single-cell screening has emerged as a powerful tool in life science, offering an effective method for detecting, isolating, and manipulating individual cells based on their specific characteristics. Traditional approaches involving manual identification of single cells from images were labor-intensive and time-consuming. Image-based cell sorting (IBCS) addresses these limitations by employing image processing tools for efficient identification and processing of single cells from large populations. This thesis presents two novel light irradiation approaches for single-cell screening.

Chapter 2 details the development of an automated photopolymerization system for encapsulating suspended single cells in photo-cross-linkable hydrogel. The system utilizes an image processing algorithm to identify individual cells from cell groups in captured images. A Digital Micromirror Device (DMD) transfers generated polymerization patterns, allowing targeted irradiation of suspended single cells in gelatin methacryloyl (GelMA) for encapsulation. Three data transfer methods were developed and evaluated based on their encapsulation rates and processing times.

Chapter 3 explores the potential of GelMA for single-cell screening in comparison to polyethylene glycol diacrylate (PEGDA). HeLa cells were encapsulated using a common polymerized pattern at 1000-2000 mJ/cm². The study revealed that 5% GelMA facilitated superior cell collection within two days due to enhanced profile retention. GelMA also demonstrated greater

biocompatibility with HeLa cells for long-term observation of proliferation and biodegradation. Cell displacement of 16 μm was observed over two days. Additionally, two trypsin-based targeted cell recovery methods were developed. These findings establish GelMA as a promising bioink for single-cell screening, offering advantages over PEGDA in cell encapsulation and targeted recovery.

Chapter 4 introduces a liquid crystal display (LCD) screen-based photopolymerization system for live single-cell encapsulation in hydrogel. This approach addresses the limitations of the DMD-based method, which required complex optical design and skilled operation. The LCD-based system employs 3D CAD software to create photopolymerization patterns, which are then processed using CHITUBOX software to set exposure conditions. The final pattern is uploaded to an LCD printer, allowing for cell encapsulation in GelMA hydrogel using LED light irradiation. This chapter analyzes the formation of photopolymerized patterns and demonstrates the successful collection of both wanted and unwanted cells.

In summary, this thesis presents two approaches—DMD-based and LCD-based—for encapsulating live single cells in hydrogel using patterned light irradiation. Both methods successfully encapsulate cells in polymerized GelMA hydrogel and enable the recovery of both wanted and unwanted cells. The study of encapsulated cell behavior, conducted primarily using the DMD-based approach, provides valuable insights into the potential of these techniques for advanced single-cell screening applications.