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Laser diode-based ultrafast crosslinking of cell-encapsulated gelatin methacrylate hydrogels

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Introduction

The photo-crosslinking hydrogels are widely used in tissue engineering. The shorter crosslinking time results in the higher cell viability and functionality and the more accurate structure for desired scaffolds. However, the photo-crosslinking time of hydrogels is normally tens of seconds under the high-intensity light source and a few minutes with a low-intensity. We present an ultrafast photo-crosslinking method for gelatin methacrylate (GelMA) hydrogel using an inexpensive laser diode. The crosslinking process has been completed within ten seconds and the cell viabilities at day 0 and day 4 were around 90%.

Materials and Methods

10% w/v GelMA [1] and 1% w/v VA-086 photoinitiator (Wako Chem. Ltd., Osaka, Japan) were dissolved in phosphate-buffered saline (PBS). NIH 3T3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% penicillin streptomycin (All from Life Technologies, Carlsbad, CA, USA) at 37ºC and 5% CO_2. A laser diode with 405 nm wavelength and 300 mW power was purchased from an online sotre. The laser diode was placed 10 cm away from a well plate and supplied by a DC power source with 2 V and 1 A as shown in Figure 1. Before crosslinking, 8 x 10^6 cells/mL cells were mixed with the GelMA prepolymer solutions. 1 mL of GelMA prepolymer solution with cells were added to each well and illuminated by the laser for 10 seconds. The crosslinked samples were washed three times with PBS and fresh DMEM media was added for culturing. To measure cell viability at day 0 and day 4, crosslinked samples were treated with Live/Dead cell viability assay (Biotium, Hayward, CA, USA) for 30 minutes. Cell viability was accessed by the fluorescent confocal microscope (FV1000, Olympus, Tokyo, Japan).

Results and Discussion

Figure 2A and 2B shows the fluorescent images of cells in the crosslinked GelMA hydrogels at day 0 and day 4. Cells were successfully encapsulated in the GelMA within 10 seconds. Figure 2C shows the result of cell viability analysis. The cell viability is very high till day 4, and most of the cells were attached, demonstrating that the developed technique is capable of crosslinkng hydrogel faster than any other conventional techniques and is biocompatible for a variety of tissue engineering applicatios.

Conclusion

In this abstract, we present a rapid photo-crosslinking technique, which can crosslink cell-laden hydrogel in ten seconds. The fluorescent images of a cell viability assay prove the around 90% of viability 4 days after the laser-based crosslinking of GelMA. This inexpensive crosslinking method has great potential in the rapid fabrication of micro and nano structures of hydrogels for bioprinting and tissue engineering.

References

[1] Nichol JW, Koshy ST, Bae H, Hwang CM, Yamanlar S, Khademhosseini A. Cell-laden microengineered gelatin methacrylate hydrogels. Biomaterials 2010;31:5536–44. doi:10.1016/j.biomaterials.2010.03.064.

Figures

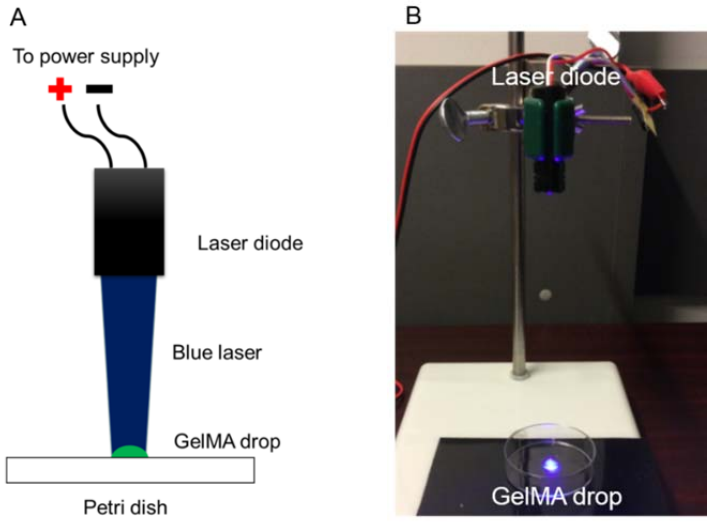

Figure 1. System setup (A) Schematic and (B) Photo of the simple laser crosslinking system

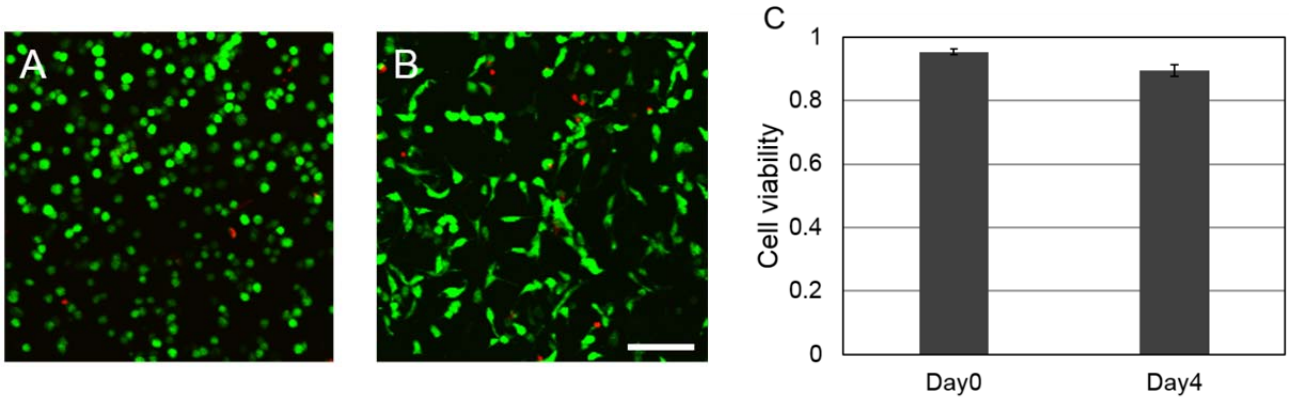

Figure 2. Cell viability analysis. (A) and (B): Fluorescent images of the laser crosslinked cell laden GelMA at (A) day 0 and (B) day 4. (Scale bar: 100 µm) (C) Cell viability at day 0 and day 4.