3D CELL CULTURE USING MONODISPERSE PEPTIDE HYDROGEL BEADS
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ABSTRACT
We describe a three-dimensional (3D) cell culture system using monodisperse peptide hydrogel beads. We utilized a self-assembling peptide hydrogel to provide cells in vivo-like microenvironment. We succeeded in encapsulating endothelial cells within the hydrogels by using a 3D microfluidic axisymmetric flow-focusing device (AFFD), and showed the encapsulated endothelial cells were viable and were able to migrate within the gels.

KEYWORDS: Cell encapsulation, Monodisperse, Hydrogel, Tissue engineering

INTRODUCTION
Numerous challenges remain to successfully fabricate functional tissue-engineered organs. There is an increasing demand for in vitro models that capture more of the relevant in vivo complexity than traditional 2D cultures, thus novel technologies such as 3D cell culture systems that can provide spatial control of cell position and the cellular microenvironment [1-2]. In vivo, cells are typically surrounded or embedded by 3D extracellular matrices (ECM) and maintain their native phenotype. Therefore, cell culture scaffolds should have properties that mimic the role of the ECM by allowing cells to migrate, grow and differentiate.

Here we utilize synthetic self-assembling peptide nanofibers as a cell encapsulation material due to their high potential for use in tissue engineering applications. Microencapsulation of cells in monodisperse ECM-like hydrogel beads prepared by the AFFD allows us to create easily manipulable tissues that can be useful for building tissues on the microscale, as well as for chemical/drug assays on a chip.

EXPERIMENTAL
We generated cell encapsulated peptide hydrogel beads using the AFFD [3]; the inner fluid consists of an acidic peptide fiber solution (PuraMatrixTM: RADA 16) with suspended bovine carotid endothelial cells, while the outer fluid consists of mineral oil, lecithin and dispersed cell culture medium powder. We infused two solutions into separate inlets to produce the microgel beads encapsulated with cells (Figure 1). The AFFD makes water in oil (w/o) emulsions, and the salt in the oil phase gradually diffuses into the water phase, resulting in the complete gelation of the peptide fibers.

We resuspended the generated cell-containing gel beads in cell culture medium containing 10% fetal bovine serum and culture them at 37°C in a humidified atmosphere with 5% CO₂. We then observed the cell behaviour and viability in the
microgels. Encapsulated cells were fixed and fluorescently labeled with Hoechst33342 and Alexa488-conjugated phalloidin to observe their morphology.

RESULTS AND DISCUSSION

We successfully generated cell encapsulated peptide gel beads at a diameter of approximately 100 μm by AFFD (Figure 2). We were able to produce monodisperse microbeads with a coefficient of variation less than about 5% (Figure 3). We also visualized cell viability using the Live/Dead Assay® and compared cells cultured in bulk gels with microgel beads. (Figure 4). More than 80% of encapsulated cells in microgel beads are alive, which was same as the cells encapsulated in bulk gels, indicating our encapsulation method by our AFFD achieves the encapsulation of cells under mild conditions without reduce the cell viability. Figure 5 shows time-lapse images of cells encapsulated in peptide gels. We observed that encapsulated endothelial cells were able to migrate in 3D peptide gels.

Figure 1. Schematic illustration of the production of monodisperse microgel beads by AFFD device. Peptide fiber solution containing cells (mixture A) are focused at a narrow orifice. Mineral oil containing dispersed powder of cell culture medium (mixture B) infused outer orifice breaks up mixture A into gel beads.

Figure 2. Phase contrast microscopies of micro-encapsulated endothelial cells in peptide gels. Scale bars: 50 μm.

Figure 3. Diameter distribution of microbeads at different flow rate ratio. (0.25w/v% of peptide fiber solution.). C.V. is defined as the ratio between the standard deviation of the diameter and the mean diameter.
CONCLUSIONS

This paper represents the first effort to produce a 3D microscale cell culture system using monodisperse peptide hydrogels. This technique holds promise for further study of fundamental cell-cell communication and angiogenesis mechanisms in reconstructed 3D environments as well as for fabrication of complicated, vascularized and functional tissue grafts that are required in many tissue engineering applications. Moreover, the 3D cell cultured monodisperse beads proposed here can be a useful tool for on-chip chemicals/drugs screening assays.

REFERENCES

