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Application of gel suspension printing system in 3D bio-printing

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ABSTRACT

Clinical applications of soft tissue using 3D bio-printing have been limited due to the difficulty of forming bio-ink materials. In this study, we propose a simple and feasible gel suspension printing system with high printing accuracy using a temperature-sensitive material, Pluronic F-127. The detailed printing parameters of the printing system were confirmed. A heart organ model was successfully fabricated using this printing system, and in vitro cell experiments have demonstrated its good biocompatibility. The results showed that the printing system has the practical ability to prepare soft tissues for application.

1. Introduction

Recently, tissue and organ scaffolds containing cells, biomaterials, and bioactive signals have been fabricated using 3D bio-printing technology, which has been widely used in regenerative medicine [1]. However, due to the limitations of 3D printing fabrication technology, it is difficult to achieve the preparation of soft tissues (such as blood vessels, nerves, heart, etc.) using 3D bio-printing technology [2,3]. Due to the difficulty of stacking and solidifying the bio-ink material for soft tissues, and because of the intricate structure of tissues and organs, soft tissues cannot be created without support.

To solve this problem, researchers have developed a gel suspension printing system. The viscous gel medium was used as a support for printing bio-ink. For example, Lee et al. has developed a FRESH printing system, which mainly uses a LifeSuppor medium (including gel microparticle support and fluid phase crosslinking) as the gel medium [4]. It can chemically crosslink with the gel material in the bioink to ensure that the printed scaffold was not deformed, and successfully printed the heart valve tissue and heart organ. However, the formulation of the gel medium is complex and initially only be initially realized in the laboratory. In addition, Samuel et al. used agarose as the gel medium to print soft-solid materials, but the printing accuracy was limited and the production process was complicated [5]. Therefore, a better study direction to support the development of 3D bio-printing of soft tissues is how to develop a gel suspension technology that can not only ensure the printing accuracy, but also be easily processed.

As a hydrogel material with excellent biocompatibility, Pluronic F-127 is widely used as a 3D bio-printing ink for printing tissue engineering scaffolds printing with cells [6]. In addition, it is also commonly used as a sacrificial material as a porogen for tissue engineering scaffolds due to its thermosensitive properties of high-temperature solidification and low-temperature dissolution [7]. Therefore, in this study, Pluronic F-127 is intended to be used as the gel medium, and its gel suspension ability is adjusted by changing the temperature, so as to establish a gel suspension printing system. The printability of the gel suspension printing system and the preparation ability of soft tissue scaffolds were evaluated and investigated.

2. Experimental

2.1. Preparation of gel suspension printing system

Pluronic F-127 polymer was purchased from Sigma-Aldrich to prepare the gel suspension printing system. Then, the polymer of F-127 was dissolved in deionized water at 4 °C for 8 h, and the F-127 solution was prepared with different concentrations (w/v, 10%, 15%, 20%, 25%, and 30%) was prepared. The viscosities of F-127 solutions with different concentrations at different temperatures (0–40 °C) were measured with a viscometer (Shanghai Fangrui Instrument Co., China) under 10 rpm/min to confirm the flowability.

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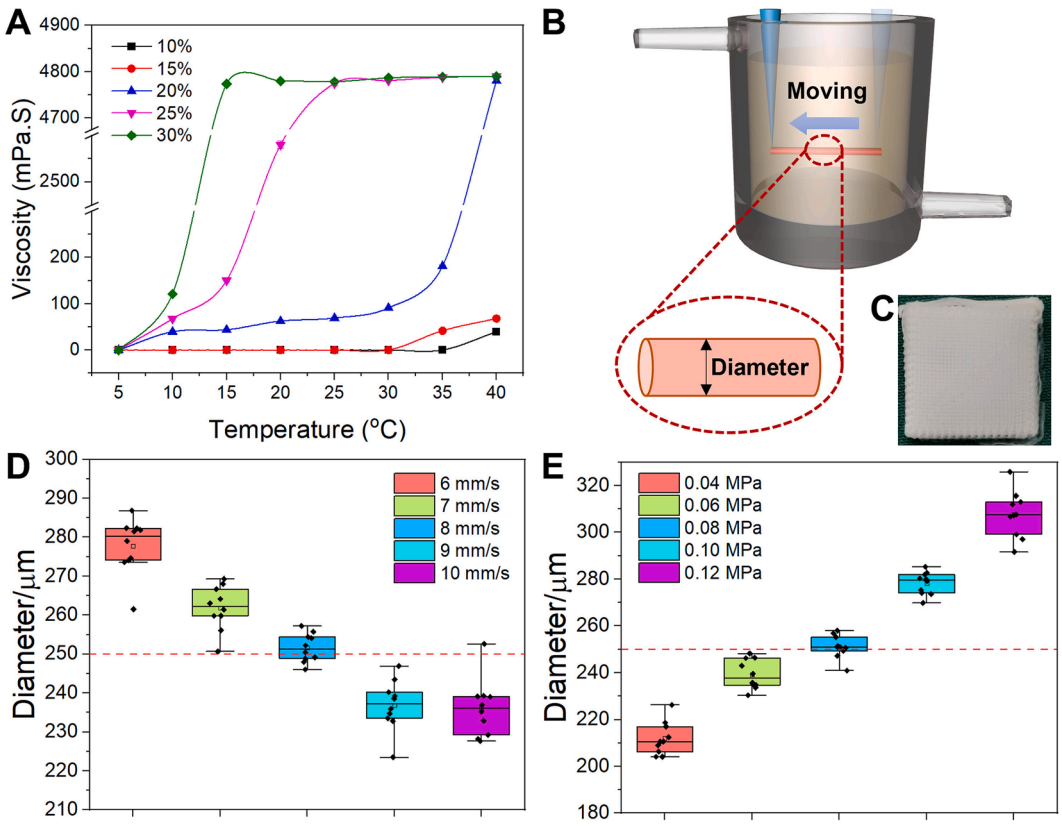

Fig. 1. (A) Viscosity of F-127 solution at different temperature and concentration. (B) 3D printing of bio ink in gel suspension printing system. (C) 3D printing scaffold at 8 mm/s speed and 0.08 MPa air pressure, the scaffold size is 20 mm*20 mm*2 mm. (D) The effect of printing speed on printing accuracy at a printing pressure of 0.8 MPa, (E) The effect of printing pressure on printing accuracy at a printing speed of 8 mm/s. (The print model diameter is set to 250 μm, the closer the actual print diameter is to 250 μm, the higher the printing accuracy).

2.2. Printing performance of the gel suspension printing system

The poly(ethylene glycol) diacrylate (PEGDA, Shanghai Guangyin Biotechnology Co., China) and polyethylene oxide (PEO, Shanghai Aladdin Biochemical Technology Co., China) were mixed to prepare the 3D bio-printing ink, the concentration is 5% (w/v) and 4% (w/v), respectively. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAB, 0.05%, w/v) was added to the ink as photoinitiator.

The temperature of the gel suspension printing system was controlled by the circulating water of the jacket layer of a jacketed beaker. Before 3D printing, the prepared F-127 solution was added to the inner layer of a jacketed beaker and kept at 5 °C to maintain its fluidity, so as to facilitate the pouring operation and eliminate bubbles. Then, the temperature was set to 20 °C to investigate the supportability of the gel suspension system. The ink and F-127 solution were sterilized through a 0.22 μm filter at 5 °C.

The print nozzle is a 24G metal needle with a length of 50 mm, the printing temperature is 20 °C and the printing depth from the top of the gel medium is 60 mm. The printability and printing accuracy of the gel suspension printing system were investigated by controlling the printing speed and air pressure. The printing speed was set to 6, 7, 8, 9, 10 mm/s, and the printing air pressure was set to 0.04, 0.06, 0.08, 0.10, 0.12 MPa. Finally, the printed linear diameter was measured and compared with the set diameter to evaluate the printing accuracy.

2.3. Three-dimensional printing and characterization of heart model

A heart model was designed and imported into the printer for 3D bio-printing test. The printing air pressure is set to 0.08 kPa, and the printing speed is 8 mm/s. The heart was printed in the beaker at 20 °C, and during the printing process, a 405 nm wavelength light was given to cure the PEGDA materials of the ink. Finally, the gel suspension was set to 5 °C to regain its fluidity, and the printed heart was removed and washed with 5 °C PBS solution for three times to remove the F-127 materials. The printed heart was stored in sterile PBS solution.

L929 cells were harvested and seeded on the surface of TCP (tissue culture plate, control) and 3D printed heart to investigate their biocompatibility. Cell viability was characterized by live/dead staining using the Calcein AM/PI kit, and the cell morphology was observed under fluorescence microscope.

3. Results and discussion

The fluidity and support of the Pluronic F-127 gel were measured to verify its printability. The viscosity of different concentrations of Pluronic F-127 gel under different temperature conditions was determined. The results in Fig. 1A showed that the viscosity of the gel was increased with the temperature. As a temperature-sensitive polymer, Pluronic F-127 was easy to dissolve at low temperature and easy to cure at high temperature. However, even when the temperature rises to 40 °C, the viscosity at concentrations below 15% was still very low and insufficient for printing. However, when the concentration exceeded 30%, it became excessively viscous and interfered with the flow of the medium. It was found that the viscosity of Pluronic F-127 gel medium slowly increases at 20 °C when the concentration is 25%, and we believe that the gel medium at this time has both good support ability and fluidity at this time.

Furthermore, the printing accuracy of the gel suspension system was verified on the bioprinter with the bio-ink material (Fig. 1B). We adjusted the printing speed and extrusion air pressure of the bio-ink, respectively, and characterized the printing accuracy. The results are shown in Fig. 1D, 1E. It was found that when the printing speed was 8

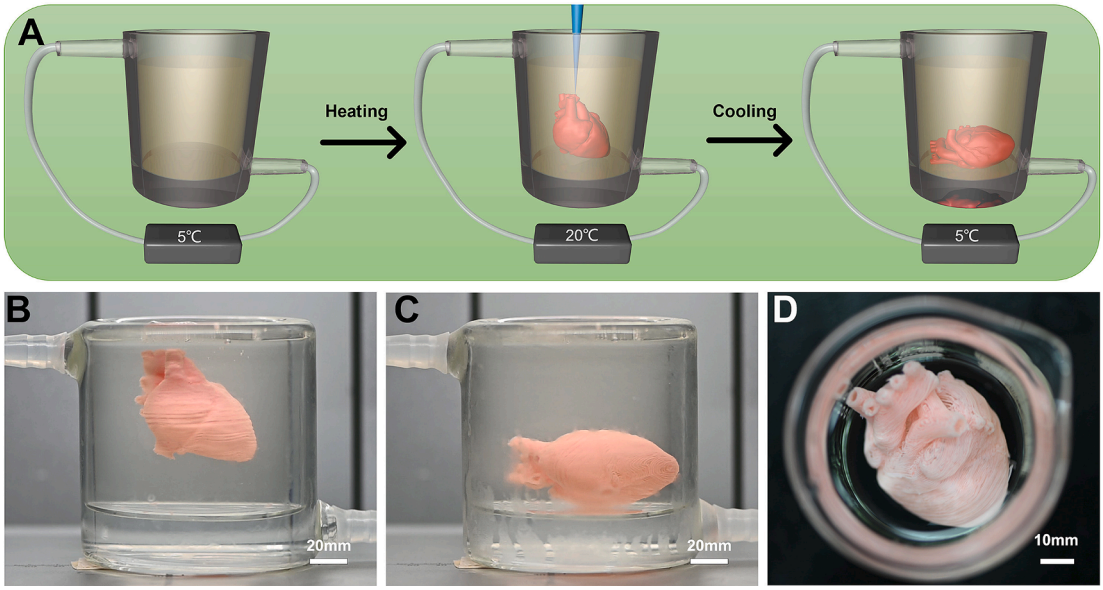

Fig. 2. (A) Schematic illustration of 3D printed heart in gel suspension printing system. General photos of 3D printed heart (B) suspended in gel at 20 °C, (C) precipitated in solution at 5 °C, and (D) stored in PBS solution.

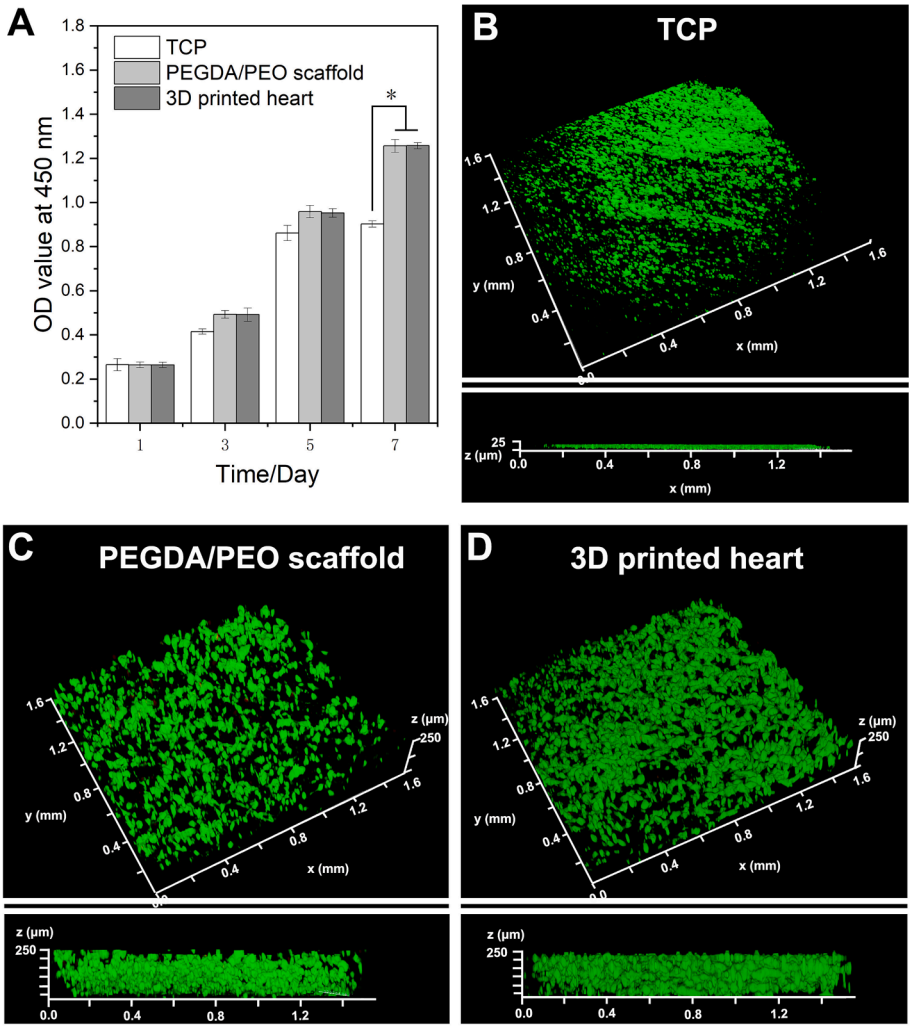

Fig. 3. (A) CCK-8 assay and 3D fluorescence results of L929 cells cultured on (B) TCP, (C) PEGDA/PEO scaffold and (D) 3D printed heart for 7 days.

mm/s and the extrusion air pressure was 0.08 MPa, the whole gel suspension printing system achieved the best printing accuracy. The printed scaffold was shown in Fig. 1C, and its printing accuracy can reach 0.1 mm. It demonstrated that the printing system has the potential to print soft tissue scaffolds with high precision.

The abovementioned gel suspension bioprinting system was used to prepare a heart model to verify its ability to print soft tissue organs. Specifically, according to the previous gel medium configuration and printing parameters, circulating water was used to control the temperature of the entire gel medium, and the bioink was used to print the heart organ model (Fig. 2A). The digital photos showed that the 3D printed heart structure was relatively complete, and the corresponding blood vessels and cavities were clearly visible, which fully verified the feasibility of the printing system (Fig. 2B–D). In addition, the use of Pluronic F-127 gel temperature-sensitive material as the gel suspension medium in this study was innovative. By controlling the temperature of the circulating water in the outer layer of the printing beaker, printing of the scaffold and removal of the gel can be easily achieved. In general, the gel suspension medium was inexpensive and easy to use.

The biocompatibility of the 3D printed heart was characterized (Fig. 3). By seeding L929 cells on the surface, the CCK-8 results show a good proliferation behavior of the cells on both the 3D printed heart and the PEGDA/PEO hydrogel, indicating that the F-127 gel medium does not affect the biocompatibility of the whole 3D printed heart. In addition, the 3D fluorescence images showed that it has good three-dimensional cell growth. It was suggested that the soft tissues produced by the 3D bio-printing system have good biocompatibility and clinical application potential.

4. Conclusions

The gel medium was prepared by controlling the concentration of Pluronic F-127 and the solution temperature, and a gel suspension 3D printing system was established to achieve high-precision unassisted successful printing of heart models. The *in vitro* cell experiments proved that the printed model has good biocompatibility and cell growth in three dimensions. It is concluded that the gel suspension printing system has the potential to be used for printing soft tissues and organs.

CRediT authorship contribution statement

Zhe Chen: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft. **Yu Han:** Methodology, Formal analysis, Investigation. **Pengfei Cai:** Methodology, Formal analysis. **Xiumei Mo:** Conceptualization, Writing - review & editing. **Yunlong Zhang:** Resources, Formal analysis. **Jinglei Wu:** Resources, Writing - review & editing. **Binbin Sun:** Conceptualization, Validation, Investigation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matlet.2023.134235.

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