Ultrasonic-assisted 3D bioprinting a composite of alginate and particles/cells

M H Shao¹,², B Cui¹,²*, T F Zheng¹,², C H Wang¹,²

¹ School of Mechanical Engineering, Xi’an Jiaotong University, Xi’an 710049, China
² State Key Laboratory for Manufacturing and Systems Engineering, Xi’an 710049, China
* Corresponding author e-mail: cuibin@xjtu.edu.cn

Abstract. Extrusion-based printing technology has a promising development in the field of tissue engineering scaffold. Researchers package cells/particles in biomaterials and then extrude them through a nozzle and solidify to get a scaffold. This method can achieve uniform distribution of cells/particles in biomaterials or the addition of particles in biomaterials to enhance the mechanical properties of the scaffold. Sodium alginate is a kind of polysaccharide biomaterial which has a wide range of applications in the field of bioprinting. However, the diameter of the nozzle determines the accuracy of the cells/particles position in the alginate scaffold. A method of ultrasonic-assisted 3D bioprinting a composite of alginate and particles is proposed. The acoustic pressure can concentrate cells/particles to the center of the nozzle, which can achieve a more precise positioning than the diameter of the nozzle, and facilitate the purpose of depositing specific cells/particles to the determined location of the biomaterial.

1. Introduction
The concept of biofabrication was proposed by Mironov in 2009[1]. The innovative point of this concept is that proposed cells as bioprinting materials for scaffold, which can accurately position the printed cells and biomaterials during the printing process[2]. Chen R et al. prepared carbon particles/polylactic acid (PLA) composites by extrusion-based 3D printing. The tensile strength and modulus of the enhanced composite scaffold with a particle content of 30wt% increased by 53% and 438%, respectively[3]. Afterwards, the scaffold is usually inoculated and cultured with fibroblasts and keratinocytes extracted from the skin sheet, and the dense tissue obtained by the culture[4, 5]. This type of printer includes a three-axis motion platform, a temperature control system and a feeding system. In recent years, researchers have devoted themselves to developing ink materials that are more suitable for bioprinting. Among them, sodium alginate is widely used in this field because of its good biology, low cost, and degradability.

Sodium alginate is a natural polysaccharide copolymer that can be dissolved in water at room temperature. It is generally formed by the residues of guluronic acid (G) and mannuronic acid (M) are combined in a ratio of 1:4, and with a ratio of G to M the change of alginic acid will lead to changes in the physical and chemical properties of alginic acid, such as viscosity, ion selectivity, gelation properties, film-forming properties, etc[6]. The viscosity of alginate solution is determined by its molecular structure and can be cross-linked with divalent or multivalent cations to form insoluble alginate scaffold, which can not only improve its stability in aqueous media but also its mechanical properties. Among many cations, Ca²⁺ is often used to cross-link the sodium alginate solution to form a stable structure.
similar to the "egg box" structure, thereby improving the stability of sodium alginate[7, 8]. Therefore, the combination of calcium chloride and sodium alginate bioink can not only be cured as an excellent scaffold, but also the mechanical properties after forming can be determined by adjusting the concentration. Sodium alginate has several excellent properties, firstly, it can increase the viscosity of the aqueous solution when it is dissolved, which is useful for preparing extrudable materials for 3D printing[9]. Secondly, it has the ability to form a gel by adding calcium salt to the aqueous solution of alginate under mild conditions[4, 10]. Calcium ions replace sodium ions in sodium alginate to bind long-chain alginate molecules together to form Hydrogel, this property is based on the solid free-form technology based on extrusion and inkjet printing.

During the process of extruding the material from the nozzle to the printing platform through air pressure, mechanical or electromagnetic induction, calcium chloride is used as the crosslinking agent to make it Solidified into calcium alginate. However, the printing of sodium alginate/cell hybrid ink in the form of extrusion also has certain disadvantages[11]. Most bioprinters are expensive and the nozzles are easily clogged by particles and the cell activity is affected by shear and pressure, and solidification speed is difficult to control. Here, we propose a method of acoustic-assisted printing of sodium alginate and build a low-cost printing device. The results show that this solution has the potential to solve the problem of high cost and easy clogging in bioprinting.

2. Construction of ultrasonic-assisted bioprinting device

The work of extrusion-based bioprinting is putting biomaterial into a container, extruding and cooling for shaping on the bottom plate, and the material is formed layer by layer according to the specified trajectory and stacked layer by layer[12]. A general extrusion bioprinting device is mainly composed of three parts: a three-dimensional motion platform, a feeding system and a temperature control system. It is worth noting that sodium alginate gelation process can be formed as long as the corresponding crosslinking reaction is carried out at a temperature lower than 40°C[1]. Therefore, this device will not be equipped with a temperature control device. The device is composed of a motion system, a feeding system, and an ultrasonic-assisted system. The diagram of our device is shown in figure 1, and the actual installation is shown in figure 2.

![Figure 1](image_url)

Figure 1. The diagram of ultrasonic-assisted 3D bioprinting device. In this figure, No.1 represents a syringe pump; No.2 is the syringe; No.3 is the glass nozzle; No. 4 is the motion system; No.5 is the ceramic piezo; No.6 is the Signal generator; No.7 is the power amplifier.
Figure 2. The actual ultrasonic-assisted 3D bioprinting device.

Sodium alginate with particles is extruded into nozzle by syringe pump, the nozzle is driven by the PZT attached to it to produce vibration, and the PZT’s drive signal is generated by the signal generator and power amplifier. The nozzle resonates with the PZT, an acoustic standing wave is generated in the cylindrical tube. Particles/cells will gather at the acoustic pressure node, which further improves the deposition accuracy of particles/cells in the scaffold.

3. Experiment

3.1. Materials and equipment

Sodium alginate with polyethylene particles was printed by the device in Figure 2 to prove the effectiveness of our proposed acoustic-assisted printing method. The experimental setup is as follows: Preparation of sodium alginate (AR, 98%, Shanghai, Yuanye) solution with concentration of 0.5%, 1%, 2% and 3% w/w. Polyethylene particles solution (100μm, 1% w/w) is mixed with sodium alginate solution in a volume ratio of 1:5. The PZT (p 8, 20×10×2 mm) is glued to the glass nozzle that its inner diameter is 5.6mm, outer diameter is 7.8mm. The nozzle is fixed on the 3D printing platform (lab-made) with a special fixture, and the PZT is driven by the signals generated by Signal generator (American Tektronix AFG3251C) and power amplifier (Amplifier Research 75A).

The sine signal frequency of the signal generator is set to 492 KHz, the voltage is 3V, and the power is set to 0.75W. Absorbent paper soaked with calcium chloride solution is placed on the printing platform for cross-linking of the gel scaffold. The concentration of calcium ions affects the degree of cross-linking of sodium alginate. Excessive calcium ions or too long time cross-linking will cause the aging and degradation of the hydrogels. These factors have been thoroughly studied by the predecessors, and the secondary crosslinking effect of 2% w/w calcium chloride solution is better[13]. The sample printed by this device is shown in figure 3.
3.2. Results and discussion

The first thing to pay attention to is to print the diameter of sodium alginate. The factors that affect the diameter of sodium alginate include the concentration of sodium alginate, the concentration of cross-linking agent calcium chloride, the speed of ink extrusion \(c\), and the speed of movement of the nozzle \(v\). We conducted experiments and found that the concentration of sodium alginate and calcium chloride, which can guarantee the printing quality (complete curing and ensure self-supporting mechanical strength) are 1% \(w/w\) and 2% \(w/w\) respectively, and the printing speed is fixed 0.06m/s is more appropriate. When the above parameters are determined, the concentration of the sodium alginate is the critical factor for the diameter of the polyethylene particle line in the nozzle. The smaller the particle line diameter, the higher the positioning accuracy in the stent, and vice versa. Figure 4 shows the variation of particle line diameter with the concentration of sodium alginate under the conditions of 2% \(w/w\) calcium chloride concentration, 0.06m/s printing speed, and 1ml/s extrusion speed.
Figure 4. (a) Particles gathered at the acoustic pressure node in the nozzle (b) The variation of particle line diameter with the concentration of sodium alginate.

The left and right sides of the figure 4(a) show the shape of the particle line in the nozzle when the sodium alginate concentration is 2.0% \(w/w\) and 0.5% \(w/w\), respectively. Analyzing figure 4(b), we can know that the diameter of the particle line decreases as the concentration of sodium alginate decreases. When the concentration of sodium alginate is 0.5%, the minimum diameter of the particle line is about 500 \(\mu m\). The smaller the diameter, the higher the positioning accuracy of the particles in the biomaterial, and the better the performance of the scaffold.

4. Conclusions
Sodium alginate is a kind of polysaccharide biomaterial which has a wide range of applications in the field of bioprinting. Extrusion-based 3D bioprinting technology usually uses composite composed of biomaterials and cells/particles as the printing bioink. Encapsulating cells in the material can increase the survival rate and the addition of particles can enhance the mechanical properties of bioink. However, the diameter of the nozzle determines the accuracy of the cells/particles position in the scaffold. Here, we proposed a method of acoustic-assisted printing of sodium alginate and build a low-cost printing device. The acoustic pressure node where the particles/cells will gather in the nozzle can improve the positioning accuracy of the particles/cells. The results indicate that this is a promising method that can achieve high-precision printing with a low-cost device.

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