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A study on degradation behavior of 3D printed gellan gum scaffolds

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Abstract

Gellan gum (GG) is one of the natural hydrogels showing potential for tissue engineering. In this study, we investigate GG for wound dressing and cartilage applications through 3D printing which allows for the creation of complex structures and scaffolds with different porosities. Degradation of two different GG scaffold designs and one solid sample were performed using both simulated body fluid and phosphate buffered saline. It was found that the scaffolds with a higher surface area to mass ratio have a higher degradation rate, and that the compressive modulus and strength increase after degradation in simulated body fluid.

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1. Introduction

Gellan gum (GG) is one type of hydrogel that has been used as a biomaterial for tissue engineering purposes. GG was discovered in 1978 and became available in the commercial market by C.P. Kelco in the U.S. and Japan [1]. GG is a natural polymer with repeating units consisting of α-L-rhamnose, β-D-glucose and β-D-glucoronate with molar ratios of 1:2:1 [2, 3]. The molecular form of GG is a threefold double-helical structure and under appropriate aqueous conditions, an aggregation of its double-helical structure causes a gel network leading to gelling [4]. It is known that different types of cations can influence gelation behavior in GG. Some examples are Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} [5, 6]. GG was originally used as a food thickener, but now is being used as a biomaterial in many applications such as ophthalmic formulations [11] and oral drug delivery [12].

Biomaterials for tissue engineering require biocompatibility, biodegradability, adequate porosity, sufficient water absorption, and suitable mechanical strength tailored to its application [7]. GG has great potential for many tissue engineering applications because of its easily tunable properties degradation rates and mechanical properties [6] as well as its heat and acid stability [8]. Researchers have been studying GG for tissue engineering applications such as wound dressings [8,12-14,19], artificial cartilage application [8-10, 16], and bone osteogenesis [17]. GG has the desirable properties for wound dressings including soft texture and tunable mechanical properties [18]. GG can crosslink with other chemicals and encapsulate drugs for wound dressing [19]. GG has also been considered as a potential solution for cartilage regeneration, but this application remains a challenge topic because of cartilage tissue's poor self-repair capability [17].

Manufacturing of GG for wound dressing or cartilage applications is difficult because complex structures are needed for these purposes, such as for patient-specific geometries or organ mimicking designs. This difficulty is because of GG's soft texture and temperature sensitive properties. 3D printing has become a solution to this manufacturing problem, and the printability of GG has been established by previous research [14]. 3D printing not only makes manufacturing of GG more feasible but it also allows for the control of porosity. Being able to control the porosity is beneficial for wound dressing applications because the drug delivery rate as well as the degradation rate can be changed by different porosities or surface areas and customized for individual patients [20]. Previous research demonstrates how degradation rate is

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affected by changing the concentration of GG or its crosslinking method, however, the effects of changing porosity on degradation rate have not yet been studied. Chemical reactions between the GG and chemicals within the body fluid during degradation may change the mechanical properties of material because GG is sensitive to the ions that are naturally in the human body [2]. Knowing how the hydrogel will respond to the body is important because it could exhibit different properties over time. Whether the structure becomes stronger or weaker *in vivo* compared to the original design, can be critical for both wound dressing and cartilage replacement purposes. [6, 10]. For example, if artificial cartilage degrades faster than expected, it will not provide the needed mechanical properties.

The purpose of this study is to determine how 3D printed GG can be applied to wound dressing or cartilage replacement applications. In this study, we will perform degradation tests with phosphate buffered saline (PBS) and simulated body fluid (SBF) which are intended for wound dressing and cartilage replacement applications, respectively. Degradation rates of scaffolds with different porosities will be measured, and compression tests will also be performed in order to determine how the mechanical properties change throughout degradation.

2. Materials and Methods

2.1. Material

In this research, the gellan gum powder, Gelrite (G1910, Sigma-Aldrich, St. Louis, MO, USA), was used to fabricate the hydrogel. The powder was used as it was received. Ultra-pure water (18.2MΩ) purified by Barnestead Water Purification System (Dubuque, Iowa, USA) was used for all solutions in this study. Two mediums were used for the degradation tests, PBS and SBF.

To prepare PBS, a PBS tablet (P4417, Sigma-Aldrich) was dissolved in ultra-pure water. The solution's pH value (pH 7.4 at 25 °C) was checked for concentration. SBF was prepared following the protocol by Tas [21]. The chemicals for producing SBF, NaCl (99.5%), $NaHCO_3$ (99.5%), KCl (99.0%), Na_2SO_4 (99.0%), $Na_2HPO_4 \cdot 2H_2O$ (99.5%), $MgCl_2 \cdot 6H_2O$(99.0%),$CaCl_2 \cdot H_2O$(99.0%), $(CH_2OH)_3CNH_2$ (99.5%), and HCl (37 vol%) were all acquired from Sigma-Aldrich.

2.2. Preparation of hydrogel

The GG powder was mixed with ultra-pure water (2% w/v), and heated in a water bath (Digital constant temperature tank, HH-2) at 90°C for 30 min while stirring occasionally. For the solid samples, the heated GG solution was poured directly into a mold and cooled to room temperature. The solid samples had a 5-mm thickness and a diameter of 12 mm on the top and 10 mm on the bottom. The bottom of the sample was made smaller for easier demolding. For 3D printing, the hydrogel was poured into a syringe (3 cc) maintained at 53°C in the water bath before the printing process.

2.3. 3D Printing process

The GG scaffold was printed with the Inkredible Bioprinter (Cellink, Gothenburg, Sweden). A custom heating system, as shown in Fig.1, was added to the printhead to maintain the hydrogel solution at the desired temperature for printing. The heating system includes a 5W heating film, a conductive copper sleeve, a thermistor, a temperature controller (Arduino UNO R3 Mega 2560, Glendora, CA, USA), and a DC power supply. The thermistor was attached on the surface of the conductive copper sleeve. The nozzle tip used was a 21-gauge needle. The power supply was also directly connected to the nozzle tip for heating to prevent the hydrogel solution from solidifying inside the nozzle.

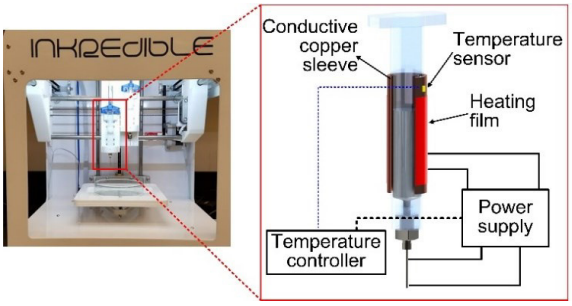

Fig. 1. 3D printing setup with modified printhead

The Inkredible Bioprinter uses a pneumatic extrusion system and a pressure of 7 to 11 kPa was used to print the hydrogel scaffolds. Two scaffold designs were fabricated. The two designs had either 3 mm or 4 mm distances between two adjacent tool paths. The size of the scaffolds was 24 mm by 24 mm with a total of 12 layers (total thickness of 2 mm). The tool path of the 3 mm design is illustrated in Fig.2. The feed rate used was 800 mm/s (solid lines in Fig. 2) for the longer toolpath and a faster feed rate 1800 mm/s (dash lines in Fig.2) at each turning point when the toolpath overlapped with next layer, to prevent excessive material deposition at the shorter edges and corners. The scaffolds were directly printed onto glass petri dishes that were then used for the degradation test.

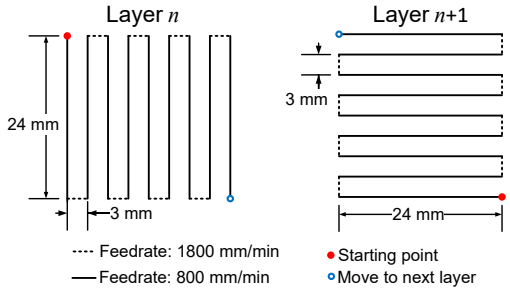

Fig. 2. Printing tool path of the scaffold.