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Biocompatible Polymers made of Lignocellulosic Biomass that have been treated with Glycerol it can be used for Tissue Regeneration

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Abstract

The emerging research field known as 3D bioprinting has emerged as a result of the deep cross-fertilization of 3D printing technology with numerous fields like mechanics, materials, and biomedicine.Extrusion 3D bioprinting, the most widely used technology for 3D bioprinting, can print biomaterials with a wide range of applicability and viscosities. In this review, we set up a composite hydrogel with glycerol as a multifunctional co-dissolvable and gelatin-oxidized nanocellulose as the lattice, as well as the ideal structure of the not entirely settled by material depiction.The hydrogel's microstructure was examined with scanning electron microscopy (SEM), which revealed a three-dimensional porous network structure with microporous pore sizes between 200 and 300 micrometers.According to infrared spectra, the addition of glycerol increased the hydrogel's properties without affecting the gelatin-oxidized nanocellulose.In the meantime, the printed area is clear and structurally stable, and the composite hydrogel is suitable for extrusion-based 3D bioprinting due to its obvious shear-thinning and good mechanical properties.According to a number of findings, the hydrogel's good pore structure, mechanical properties, and printable performance make it suitable for extrusion-based 3D bioprinting.A novel concept and material for 3D bioprinting are presented by this gelatin-oxidized nanocellulose hydrogel, which also expands the material's application range.

Keywords: 3D bioprinting; Oxidized nanocellulose; Gelatin; Glycerol; Hydrogel; Printability

Introduction

3D printing is one sort of added substance fabricating (AM). Without the need for costly and time-consuming molds, it enables the creation of custom or intricate structures.It is frequently used in architecture, process design, education, medicine, biology, and aerospace [1]. 3D printing is best known for stacking layers to create three-dimensional solids.By achieving spatially oriented manipulation of biological materials, growth factors, cells, and controlled stacking, it overcomes the challenges of traditional tissue engineering. This is the ideal blend of biomedicine, tissue engineering, regenerative medicine, and 3D printing technology.Biological 3D printing is a new field of study that combines biomedicine and 3D printing technology [2]. It overcomes the limitations of conventional tissue engineering to create scaffolds with a high degree of structural complexity and design adaptability [3].This has significant research significance as well as the potential for widespread application.

There are four categories of 3D bioprinting technologies based on the forming principle and printing materials: The earliest 3D bioprinting technique is thought to have been inkjet 3D bioprinting, in which bioinks are dispensed into a series of micro-droplets printed in layers to shape cell-containing 3D structures using printheads that are either thermally or piezoelectrically driven [4]. Light-curing, extrusion printing, inkjet printing, and laser direct writing are additional printing techniques. Inkjet printing, which allows the establishment of various printheads and gives quicker printing speeds, is the most affordable strategy for 3D bioprinting.However, it cannot print materials with a high viscosity or bioinks in high concentrations, and it may mechanically or thermally harm cells while printing [5]. Even though laser direct printing is more expensive than inkjet printing, it can print a wider range of materials and high-viscosity bioinks. Moreover, it forestalls the bioink from coming into direct contact with the handling gadget, which guarantees high cell action.Light-cured printing uses light to selectively cross-link bioinks in layers to form three-dimensional structures, just like laser direct printing [6]. Despite the fact that UV light and photoinitiators can harm cells, light-cured printing has the advantages of high printing efficiency and accuracy as well as being a straightforward device that is simple to control.The most prevalent 3D bioprinting technology is extrusion printing, which was developed from inkjet printing and extrudes continuous fibrous filaments.It can print tissue structures with good structural strength, bioinks of varying viscosities and cell concentrations, and a lot of different material applications.However, no mature printing equipment has been reported due to the high requirements for printing materials, printing processes, and molding equipment [7]. The majority of inkjet, laser direct writing, and light-curing 3D bioprinting printing equipment is built independently and is still in the laboratory research stage.In this study, the effectiveness of hydrogel printing was investigated using the extrusion 3D bioprinting technique [8].

Three requirements must be met before a bioink can be used in extrusion-based 3D bioprinting.First, the properties that reduce shear. Second, the printed structure's stability is guaranteed by its superior mechanical properties.Thirdly, excellent biocompatibility Hydrogels, a distinct class of 3D polymer network "soft" materials, make extruded 3D bioprinting of bioinks, which are widely used in agriculture, industry, and the biomedical field, possible [9]. This is a capability for rapid sol-gel transition (the deposition platform can quickly shape smooth extrusion from the nozzle).They are strong mechanically, pliable and flexible, transparent optically, biodegradable, biocompatible, and tough.Due to their biocompatibility and good shear-thinning properties, sodium

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alginate (SA) and gelatin (GEL) are the hydrogel compositions that are used the most frequently in research on extrusion 3D bioprinting. By partially hydrolyzing collagen, GEL is a protein with excellent cell adhesion and biocompatibility properties.The temperature-controlled cross-linking property of GEL and the divalent cation cross-linking property of SA can be effectively utilized in extrusion-based biological 3D printing by combining them.But there are two problems with this hydrogel:low dry matter content, which causes print collapse and has a significant impact on shape fidelity, and high viscosity, which can harm cells when exposed to excessive air pressure during the printing process [10].

SA has high viscosity and thickening properties at low concentrations. In this study, the only material with shear-thinning properties was GEL, which decreased the hydrogel's viscosity and printing air pressure. The appearance of shear-thinning properties and material stiffness are two examples of the physical and chemical properties that can be significantly altered by adding nanoparticles to hydrogels, according to studies.Due to their distinctive nanostructures, excellent mechanical properties, and high biocompatibility, nanocellulose-based hydrogels have received a lot of attention in the field of tissue engineering over the past few years.In a few recent articles, hydrogel formulations made by adding nanocellulose to alginate have also been suggested for use in biomedical applications.However, only the formulation's composition was described without conducting a thorough investigation.We used TEMPO system oxidized nanocellulose (T-CNF) in this study because it effectively prevents nanocellulose from agglomerating, improves its dispersion and stability in water, and improves the mechanical properties and stability of gelatin-based hydrogels [11].

Bio-based hydrogels frequently fail due to their low dry matter content.In this study, we used glycerol (GLY) rather than water to reduce the volume and proportion of non-volatile components, reduce excessive shrinkage, and permit the samples to maintain their shape after curing in order to prevent structural collapse following printing. When producing gel compositions with elasticity and toughness, it was demonstrated that the addition of food-grade additives like glycerol, sugar, and citric acid to gelatin does not affect the material's biocompatibility or safety.The hydrogel's stability and water retention are enhanced by the addition of GLY, but its durability as a bioprinting material is decreased.This is because rapid drying of the gelatin-based hydrogel can cause it to harden in the air [12].

For the purpose of 3D bioprinting, this study produced a biocompatible composite hydrogel based on GEL, T-CNF, and GLY.The hydrogel's viscosity and mechanical properties can be altered by adding T-CNF and GLY.The best proportion of each hydrogel component was determined through morphological observation, chemical structure characterization, a rheological property test, a mechanical property test, and a swelling performance test.Last but not least, the hydrogel proved to be more stable at room temperature, printable, and faithful to shape when it came to extrusion bioprinting [13].

Materials and Methods

Materials

Shanghai Sinopharm Chemical Reagent Co., Ltd., which supplied the deionized water for all of the experiments, In Shanghai, there is McLean Biochemical Technology Co., Ltd.Tianjin Wood Spirit Biotechnology Co., Ltd., located in Tianjin, China, is the company that manufactures T-CNF, or TEMPO system oxidized nanocellulose. also, Shanghai Aladdin Biochemical Innovation Co., Ltd. in Shanghai, China, glutaraldehyde (GA).All reagents were utilized straight out of the bundle.

How to Make Hydrogels

Composite hydrogel matrices made of gelatin and oxidized nanocellulose were used to make various kinds of hydrogels. The multifunctional co-solvent glycerol's content was changed to make different kinds of hydrogels.The initial objective of the experiment was to adjust the viscosity of hydrogels so that they could be extruded effortlessly through the print nozzle and keep their shape and structure on the deposition platform.Following the initial tests, four hydrogels of varying composition ratios were chosen for the subsequent evaluation.

Hydrogels' Morphological and Chemical PropertiesThe Microscopic Topography of Hydrogels

Hydrogels' Macroscopic Topography

In order to observe the materials' dispersion, the ultrasonic dispersion and magnetic stirring of the composite hydrogels were allowed to stand for an hour in a water bath at 37 °C.After that, to observe the hydrogel's macromorphology following low-temperature curing, the hydrogel was poured into a cylindrical mold with an inner diameter of 15 mm and a height of 10 mm [14]. The mold was then placed in the refrigerator for one hour at 4 °C.

Hydrogels were examined using a scanning electron microscope

A freeze dryer (ALPHA 2–4 LD plus, Marin Christ, Ltd., Osterode, Germany) with a cold trap temperature of 70 °C was used to cross-link the cryogenically cured cylindrical hydrogel samples after they were pre-frozen overnight at 20 °C.Using an ultra-high resolution field emission scanning electron microscope (SU8020, Hitachi, Ltd., Tokyo, Japan) and an accelerating voltage of 5 kV, the lyophilized hydrogels were cut horizontally and coated in gold [15].

Compound Primary Portrayal of Hydrogels

A Fourier transform infrared (FT-IR) spectrometer (Nicolet iS10, Thermo Fisher Scientific, Ltd., Waltham, MA, USA) and the compression method with KBr (Sinopharm Chemical Reagent, Ltd., Shanghai, China) were used to measure the hydrogels' functional group changes.The resolution was set to 4 cm1, and the scanning range was set to 500–4000 cm1.

Results and Discussion

Findings from Chemical and Morphological Characterization of Hydrogels

Hydrogel morphology analysis

The hydrogels remained uniform and stable after being dispersed with ultrasonics and stirred with magnets.There was no precipitation after a long period of rest, indicating that the hydrogels' components were evenly distributed and stable in nature.The cast hydrogel mold is simple to use and has a smooth surface after being chilled for an hour, indicating its durability and stability.Since the GEL particle is light yellow, the hydrogel likewise has a degree of light yellow that is equivalent to that of the GEL.The continuous expansion in air rises inside the hydrogel as the GLY content expanded might be owing to an expansion in the hydrogel's general consistency, which dials back the arrival of the air blended during the extensive blending process.After that, the hydrogel's air bubbles will be removed through an extraction

procedure.Other hydrogel performance tests won't be affected by air bubbles because of this.

Additionally, the hydrogel appears yellowish-milky white when the GLY content is zero.As the GLY content rises, the hydrogel gradually becomes clear and transparent.This could be because the polymer network, water, and glycerol have finally agreed on something.The addition of GLY stabilizes the hydrogel without affecting its properties, as determined by macromorphological analysis.

Analyses of Hydrogels' Findings from Scanning Electron Microscopy

The microstructural variations of composite hydrogels are primarily responsible for the significant differences in their properties. In this manner, the microstructure of hydrogels can be pictured by the checking electron microscopy (SEM) procedure, which can mirror the inward cross-connecting of hydrogels and notice the microporous structure morphology and piece of strong substances.

The SEM images of the freeze-dried hydrogel samples.The composite hydrogels created in this study have been shown to have a three-dimensional porous network structure with micropore sizes of 200–300 m.The pore sizes of GTG0 are unique and not consistently scattered;The micropores of the composite hydrogel gradually become uniform in size and distribution as the concentration of GLY increases. GTG30's uniform distribution, moderate number of micropores, and excellent structure and porosity make it possible to achieve a balance between the composite hydrogel's mechanical properties and material transport.The hydrogel's mechanical properties will be negatively impacted by GTG50's excessively dense micropores.

Analyses of Hydrogels' Rheological Properties

Hydrogels' shear-thinning properties are especially crucial for extrusion-based 3D bioprinting techniques.The shear-thinning property is a non-Newtonian fluid phenomenon in which, as the shear rate continues to rise, the viscosity decreases rather than increases. This is because the physical interaction between macromolecules and macromolecules under high shear stress is temporarily disrupted, causing polymer chains to be rearranged and segment entanglement to decrease.Viscosity recovery, on the other hand, occurs when the shear force is removed and the viscosity rises rapidly once more.The shear-diminishing property of the hydrogel permits the exceptionally gooey hydrogel to go through the spout without a hitch, and the consistency recuperation property guarantees that the hydrogel can have great shape constancy after statement on the stage.Thixotropy is another name for the "shear-thinning" and "viscosity recovery" effects that hydrogels undergo during 3D bioprinting.

Since hydrogels have unique thickness and flexibility, and the gel properties are delicate to the heap applied in the estimation cycle, the strategies utilized in rheological testing are supposed to absolutely affect the design and morphology of the gel, and getting the rheological information with great repeatability might be troublesome.To test the rheological properties of hydrogels, suitable rheometer conditions should be chosen, and the normal force load placed on hydrogels should be controlled.The normal force was recorded in real time whenever the parallel plate reached the 2 mm gap until it reached the 2 mm gap in order to eliminate the effect of the normal force on the rheological performance test.The maximum load during the process of realizing the set gap is up to 1.6 N, and even a small load will not significantly alter the hydrogel's structure.Allow the sample to relax the normal force and remove any excess material prior to officially commencing the rheological test.

Conclusion

By incorporating GLY as a multifunctional co-solvent into the gelatin-oxidized nanocellulose-based hydrogels, we were able to successfully prepare composite hydrogels with good pore structure and mechanical properties that are suitable for extrusion-based 3D bioprinting.Through material characterization techniques and 3D printing experiments, we also found the best hydrogel ratios.According to macromorphological analysis, the addition of GLY stabilized the hydrogels but had no effect on their properties.SEM results revealed that the composite hydrogels also had a lot of porosity, with GTG30 having a uniform distribution of microporous structure and uniform pore size.By using effective strength additives like T-CNF and increasing the proportion of non-volatile components, the composite hydrogels acquire good shear-thinning properties.Consequently, the system always possesses hydrogel-like solid properties, preventing printing collapse and improving shape fidelity and stability of printed structures.Also tested was the compressive strength of the four composite hydrogels. The results showed that GTG30 has the best mechanical properties, the strongest compressive strength, and better swelling.Tests have shown that the composite hydrogel GTG30 has the highest overall performance and the best ratio for extrusion-based 3D bioprinting.After that, a number of printing tests demonstrated that, in terms of printability, structural stability, and shape fidelity, GTG30 was superior to other hydrogel ratios.Based on the results of a number of tests, the composite hydrogel with gelatin-oxidized nanocellulose as the matrix and glycerol as the multifunctional co-solvent has the potential to provide new materials, concepts, and applications for 3D bioprinting.

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