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Use 3D printing technology to prepare polylactic acid bone scaffold material and test its physical and chemical properties

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Abstract

Objective: In this study, polylactic acid was used as raw material and 3D printing technology was used to make specimens that simulate the structure of bone tissue, and test its various physical and chemical properties, so as to provide experimental basis for clinical bone tissue repair. Method: Using computer-aided design (CAD) system software, the polylactic acid material was designed into 26 cube specimens with a length of 14mm, a width of 14mm, and a height of 7mm, and the porosity of the material was set to 70%. The designed three-dimensional model was inputted as the STL format into the 3D printer, adjust the printing path required to make the mesh complex, and execute the printing; and 26 are randomly divided into 4 groups (A, B, C, D),group A, B, C have eight of them are used for porosity testing, compressive strength testing, and extracting solution, and two of group D are used for optical microscope observation and electron microscope observation. Through porosity test, compressive strength and cell compatibility experiment to characterize the performance of each group of materials.

Keywords

3D printing technology; polylactic acid; bone tissue engineering, scaffold material.

1. Introduction

The current treatment methods for bone defects include autologous bone, allogeneic bone and artificial bone transplantation. Among them, the source of autologous bone is extremely limited, which will produce a second operation area and increase the patient's pain; allogeneic bone transplantation has the risk of virus infection and immune rejection, so the research of artificial bone implant materials in the field of biomedicine is becoming more and more in-depth [1]. In the medical field, the initial recognition of biocompatibility only requires that the material can coexist with tissues and organs without causing any adverse local or systemic reactions of the host. The later implanted materials need to have a positive interaction with the host to achieve the purpose of regulating the activity and function of host cells, tissues and organs.

The emerging 3D printing technology in recent years is also known as additive manufacturing. It is a technology that uses bondable materials to make object models based on digital model data and prints layer by layer, that is, 3D is formed by layering materials layer by layer to make entity model [2]. When 3D printing technology is used in vitro auxiliary medical care, the requirements for material biocompatibility are relatively low, and the materials are only required to achieve suitable mechanical strength and printability. However, when used to prepare tissue engineering scaffold materials, the materials are required to have good biological safety.

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2. Experimental method

2.1. Making a 3D bionic bone bracket

The main components of human bones are 70% calcium phosphate and 30% type I collagen. The loose and porous spatial structure constructed from this component retains the function of natural bone tissue conduction stimulation to the cellular level [3-4], It can withstand a certain intensity of pressure and has strong mechanical properties; the bone tissue is composed of bone and cancellous bone, cortical bone is highly compact, the cortical size is in the range of 10~500μm, and the porosity is 5~10%; and Cancellous bone is highly porous, with a diameter of about 50 to 300μm, and a porosity of 75 to 90% [5].

Use 3D modeling software to construct the internal space structure of the specimen and print it. According to the bone appearance structure, the corresponding loose and porous bone model is customized by combining the 3D reverse modeling of the outer contour and the internal space structure. The space structure is shown in Figure 1a. Relying on computer technology, the target product model can be accurately designed, and the 3D printing technology can better achieve the perfect fit between the scaffold and the shape of the tissue defect, while also ensuring that the pores inside the material are regular and connected, and the pore size is controllable [6].

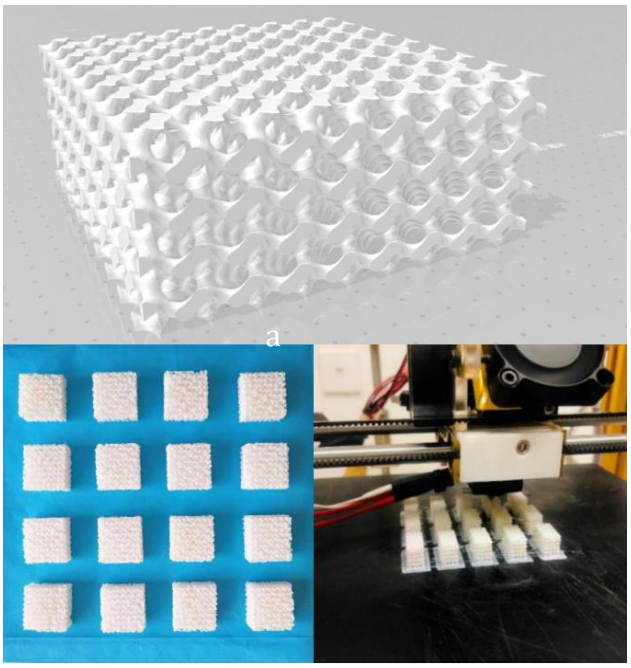

Figure1 3D bionic bone scaffold space structure (a), test piece (b) and production process (c)

*Using Fu*sed Deposition Modeling (FDM) layer stack 3D printer to print bone specimens, the specific printing parameters are set as follows: print nozzle diameter 0.2mm, print layer height 0.13mm, printer wire high-purity PLA polylactic acid material , The printing size is 14mm×14mm×7mm, the printing time is 40min, and the number of printing is 26.

2.2. Compressive strength test

Using a compressive strength tester (AGS-X Unit type, Japan SHIMDZU) to set the compression to 25%, test the compressive strength of 8 polylactic acid specimens, and calculate the mean and variance for data analysis.

2.3. Porosity and pore size detection of scaffold

The porosity of the test piece was tested using absolute ethanol and a beaker. The volume difference was measured several times, and the porosity calculation formula was imported, and the average porosity was calculated to be 76%.

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The proper porosity of the printed specimen has a greater impact on cell adhesion and is the key to the successful application of clinical implants [7]. According to the porosity formula, the porosity experiment is numerically calculated and simulated, and the image of the factors affecting the porosity value is obtained, as shown in Figure 2.

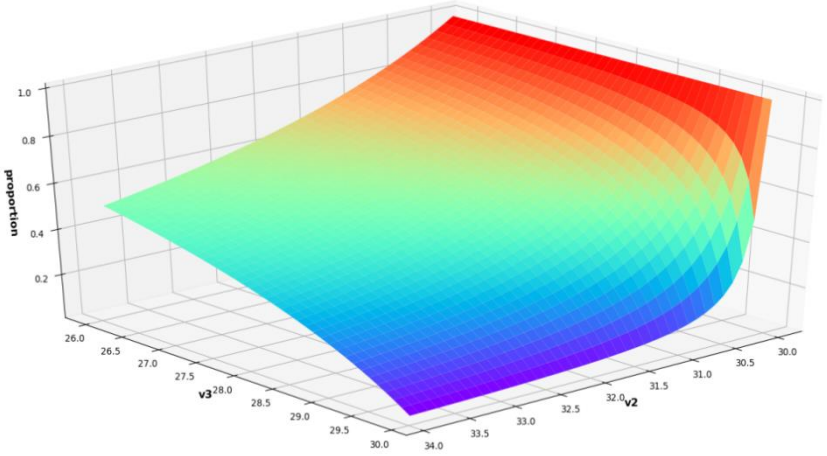

Figure 2 Numerical analysis of porosity experiment

The pore diameter of the specimen is detected by using the slice electron microscope ranging analysis method. The specimen slice can be observed roughly in a honeycomb shape under the microscope, and the hole pitch diameter and hole density can be roughly measured, which compensates for the errors of the 3D printer and the actual model production. Inconsistent circumstances. The actual measurement aperture is 1mm long diameter and 0.8mm short diameter.

Observed under a microscope (Figure 3d), the pores are clearly visible, six sides are transparent, and the cross section of the pores is round and rough. Observed under the electron microscope at 20 times (Figure 3e), the pores are clear and uniform in size. The scaffolds have traces of printing layer by layer, and there are filaments adhered between the scaffolds, which is the residual polylactic acid material during printing.

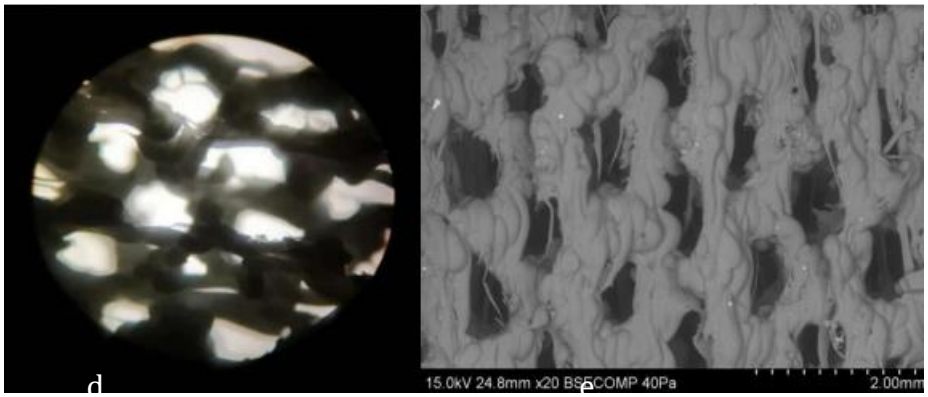

Figure 3 The pore microscope imaging (d) and electron microscope imaging (e) of the specimen

2.4. Cytotoxicity test

Experimental grouping: PLA extract group, complete medium as control group and blank group.

Extraction solution: soak the PLA scaffold material in alcohol for 24h, rinse with deionized water and dry naturally, grind it into a powder in an ultra-clean table and irradiate it with a UV lamp for 12h; put the bone scaffold material into a centrifuge tube at a ratio of 0.1g/mL,

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Extracted with complete medium in a constant temperature water bath at 37°C for 72 hours, filtered with a 0.22μm filter, and placed in a refrigerator at 4°C for later use.

Inoculate MC3T3-E1 cells in a 96-well plate as required, 2×103 cells/well, 3 multiple wells in each group. After the attachment is completed, add 100μL of extract to each well (it is a complete medium with 10% FBS) The soaked PLA scaffold material extract), cultured in a CO2 constant temperature incubator for 1d, 3d, and 5d, and the liquid was changed every other day. When testing, add 10μL of CCK-8 reagent to each well, and then incubate at 37°C for 3h in the dark. After removing the well plate, shake on a shaker for 5min to ensure that the solution is uniform. Aspirate 100μL of the test solution from each well and move it to the new side. In the well, by adjusting the wavelength of the microplate reader to make the wavelength at 490nm, measure the optical density (OD), this method can indirectly reflect the number of living cells [8].

Bring the measured data into the formula: Relative cell proliferation rate (RGR) = (PLA group absorbance value-blank absorbance value) / (control absorbance value-blank absorbance value) × 100% to calculate the relative cell proliferation rate, and according to RGR value, refer to "United States Pharmacopoeia" toxicity classification method [9] to evaluate cytotoxicity, the evaluation standard is: (CTS): 0 grade, RGR ≥ 100%; 1 grade, RGR is 75% to 99%; 2 grade, RGR is 50%~74%; Grade 3, RGR is 25%~49%; Grade 4, RGR is 1%~24%; Grade 5, RGR<1%; Grade 0 and 1 are qualified, and Grade 2 should be combined with cell morphology Evaluation, grades 3 to 5 are unqualified.

3. Experimental results

3.1. Compressive strength test results

When the stent is compressed to a yielding state, the maximum strength that the stent can withstand is 372.20±8.84 MPa.

3.1.1. Compressive strength analysis

The elastic modulus of cortical bone is 3~30 GPa, The main supporting role is [10] when the compressive strength is 130-225 MPa; The elastic modulus of cancellous bone is 1~2 GPa, Compressive strength 7-10 MPa[11]. According to Hu Huiqiang, 15 mm, Length 10 mm, width Height 3 mm, About 480-520μm, aperture size Bracket porosity (64.76±1.55)%, The maximum compression strength of the scaffold in the control group was (78.09±2.86) MPa[12], the Calculated, The compression strength of this experimental support should be at least (59.76±2.19 MPa. The actual measured value is 372.20±8.84 MPa,. Figure According to the stress standard of cancellous bone and cortical bone tissue.

3.2. Cell proliferation test results

The results of in vitro experiments show that MC3T3-E1 cells are stable after resuscitation and subculture. Observe the survival of the cells in the material extract to identify the cytotoxicity of the material.

Table 4 Comparison of OD values of three groups in three time periods (x±s, n=3)

Group	1d	3d	5d
PLA group	0.285±0.008ab	0.617±0.026ab	0.704±0.013ab
Complete medium group	0.272±0.028b	0.604±0.050b	0.694±0.008b
Blank group	0.090±0.001	0.092±0.001	0.103±0.003
Time factor	F=1466.982,P<0.001		
Group factors	F=520.286,P<0.001		

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Note :" compared with the complete medium group, the P>0.05 difference was not statistically significant ;" compared with the blank group, the P>0.05 difference was statistically significant 3 parallel tests (n=3), results are expressed as mean ± standard deviation. The results of cell proliferation in the PLA group, control group and blank group d、3d、5d 1 are shown in Table 4, The number of MC3T3-E1 osteoblasts increased with time in PLA group and control group, difference was statistically significant (P<0.05), The data in the analysis table show that, At the same culture time, The OD value of PLA group is greater than that of the other two groups, PLA group and control group were compared with the blank group, P<0.05 difference was statistically significant. Comparison between PLA and control groups, P>0.05 difference was not statistically significant.

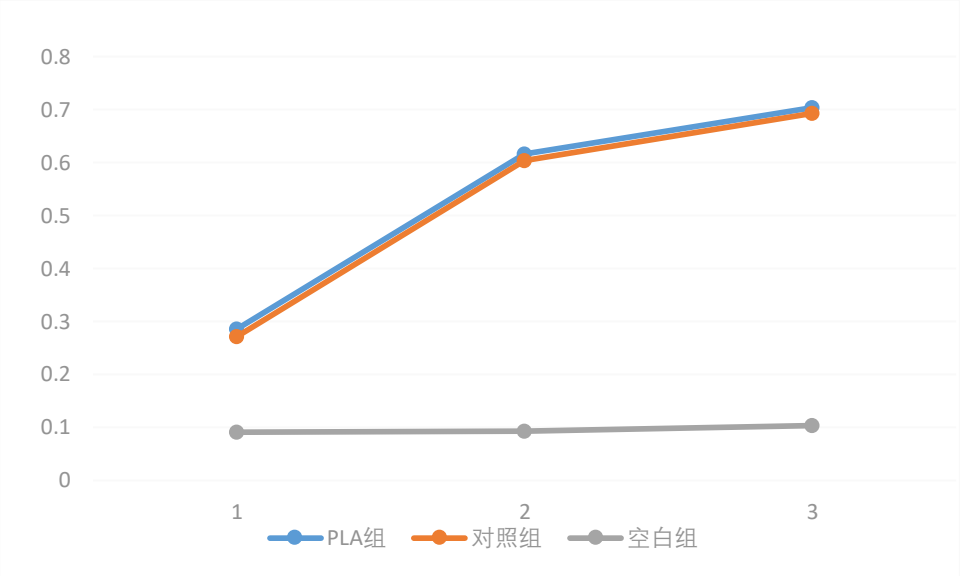

Figure 4 Three groups of three time periods OD value change trend

3.2.1. Biological toxicity analysis

The growth of the cells in the PLA group was good, indicating that the PLA material has good biological safety and no short-term potential cytotoxicity.

In this experiment, 3D printed polylactic acid specimens were used to culture MC3T3-E1 cells outside of the extraction liquid, which preliminarily showed that the polylactic acid material has no potential cytotoxicity in the in vitro toxicity test in the short term, and more comprehensive biological experimental research should be continued. Observe the biocompatibility of the material in detail.

4. Discuss

Tissue engineering is a discipline that combines cell biology and material science to construct tissues or organs in vitro or in vivo. the three elements are scaffolds, cells and suitable biochemical signals, respectively. bone scaffolds are the most basic elements in bone tissue engineering. scaffolds in the field of bone tissue engineering mainly include natural and synthetic materials. Among them, synthetic materials have excellent bone inductivity and sufficient strength. 3D the development of printing technology provides a broader space for the application of materials. Under the control of computer, the material can be accumulated by different printing methods, and the material with predetermined shape and characteristics can be obtained. Such materials often have good biocompatibility, good bone induction function and osteogenic characteristics. It has great application potential in the field of bone tissue engineering research.

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4.1. D Printed PLA specimens have excellent biocompatibility and mechanical properties

The research on 3D printing technology is being carried out in depth, which makes the potential of 3D printing technology in bone transplantation gradually prominent. Existing studies have shown that 3 D printing scaffolds play an important role in providing oxygen and nutrition, biomechanics, osteogenic differentiation of cells, scaffold degradation and so on. this experiment achieved the biomechanical level of the printed specimen by controlling the 3 D printing set pore size, porosity, size, and obtained the scaffold material which is conducive to the growth and differentiation of cells. the good proliferation results of PLA group MC3T3-E1 cells showed that 3D printing techniques were feasible in clinical application.

5. Application of Polylactic Acid Material in Medical Field

Polylactic acid is essentially a thermoplastic aliphatic polyester with a glass transition temperature of about 60℃ and a glass state at room temperature with a melting point of about 175℃. Often used in plastic products, non-woven fabric and fast food lunch boxes and other life areas. In addition, it is widely used in the field of biomedical fracture internal fixation materials, ophthalmic implant materials, tissue engineering stent materials and drug controlled release materials.

this experiment used polylactic acid as the raw material of the scaffold. the PLA scaffold materials prepared by 3 D printing were proved to have good physical and mechanical properties and biocompatibility by testing the compressive strength, porosity, cell proliferation and toxicity.

6. Reverse Modeling Technology

In this experiment ,3 D modeling software is used to model the bone tissue. In clinic, the shape of the missing bone can be recovered better by reverse modeling, so as to achieve better repair effect. For example, according to the computer tomography (CT) image data provided by the hospital ,160 CT tomography images were collected and arranged sequentially. The multi-layer bone structure images were arranged as shown in figure 5.

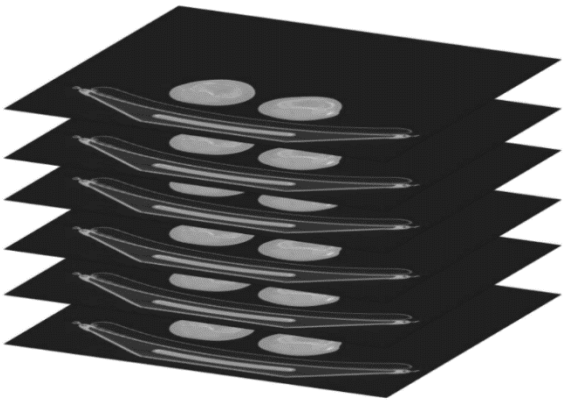

Figure 5 Image images scanned by CT tomography

3D modeling software was used to convert it into a 3d model. finally, according to the requirements of tissue engineering, the heterogeneous porous structure geometric description model (figure 6) was established to generate a 3d bionic scaffold model with internal microstructure suitable for bone cell growth for bone tissue defect repair.

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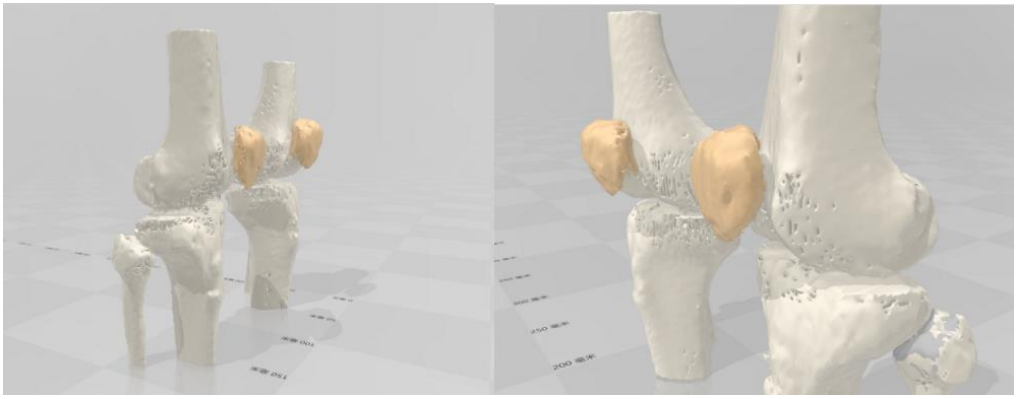

Figure 6 Visualization of 3D Reverse Modeling Skeleton Model

By combining polylactic acid materials with 3 D printing technology, good biological and mechanical properties were obtained. Combined with reverse modeling technology, bone defect can be repaired more accurately, and new ideas can be provided for bone tissue defect repair materials and preparation methods.

7. Conclusion

Using FDM layer-to-stack 3D printer can prepare bionic scaffold materials similar to cancellous bone tissue.

PLA3D printed scaffold has good compressive strength and porosity, good biocompatibility, cytotoxicity level 0, and can be used as a biomimetic bone tissue scaffold material to support the defect site.

Acknowledgements

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