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The Technological Parameter Optimization of Rapid Freeze Prototyping for 3D Tissue Scaffold Fabrication

***Abstract**. When using the Rapid freeze prototype (RFP) process to fabricate 3D tissue bone scaffold, the selecting of technological parameters directly affects lap ratio, which is the foundation of continuous fabrication and scaffold quality. Through analyzing the force of non-solidified fiber, Velocity ratio, liquid temperature, solid content, nozzle diameter and row spacing have been chosen as the influence factors and the L16 (45) orthogonal experiments have been done The experiments result is that material viscosity and row spacing is the most important factors for lap ratio influence. Optimal parameters combination has been got by range analysis. Finally, the mixed solution of gelatin-chitosan experiment with the optimal parameters has successfully produced multilayer tissue bone scaffold, including multiple scale structure.*

***Streszczenie.** W artykule zaprezentowano process wytwarzania materiałów trójwymiarowych metoda gwałtownego oziębiania – RFP (rapid freeze prototype). Materiałem wyjściowym jest mieszanina żelatyny i chitozanu. Na proces tworzenia struktury ma wpływ wiele czynników, takich jak: temperatura cieczy, zawartość składnika stałego, rozmiary węzłów, średnica i odstępy między rzędami. (**Optymalizacja parametrów technologicznych przy wytwarzaniu struktur trójwymiarowych metoda RFP**)*

Keywords: Rapid freeze prototype; Lap ratio; Orthogonal experiment; Optimal parameters.
Słowa kluczowe: szybkie oziębianie, struktury trójwymiarowe

1 Introduction

Rapid Freeze Prototyping(RFP)is a new kind of rapid prototyping technology. From the analysis of forming principle, it is still based on the discrete/overlap principle. From the aspect of material and process conditions, it is a completely new technology. It deposits water or water-soluble material [1] to the table by a continuous nozzle or discrete one orderly [2-4]. At the same time, the phase of liquid material is changed rapidly (from liquid into solid) by a low environmental temperature (-15 ~ -30°C) [1] [5-7]. The specific forming temperature is determined by material's thermal properties, the way of extrusion and nozzle size. Comparing with FDM, RFP is more conducive to ensure material's biological activities [8-9]. In addition, RFP is widely used by its highly flexible characteristics, and it can accurately control the shape of micropore in the tissue engineering field [10-11].

It usually combines RP and vacuum freeze drying technology to forming 3-D bone scaffolds with multi-scale pores: the bone scaffolds' outlines and macro pore structure(10^{-1}~1mm) is fabricated from RP technology, while the micro pore structure(10^{-3}~10^{-1}mm) is gotten from lyophilization method [5].Cong used RPF method and 4% chitosan solution to forming tissue engineering scaffolds, his research focus on software development and path planning [12].WANG used RPF technology to forming liver cells/gelatin scaffold. Cell adhesion and reproduction works well. The quality of the scaffold is very poorly [13, 14]. Materials must quickly be solidified after certain extrusion velocity, and its deformation also needs to be analyzed when its phase change from liquid to solid.

To sum up, RPF has been got a great deal of attention in the field of bone scaffolds manufacture. Researchers have done a large number of experiments to get bone scaffolds with certain biological material, but neither involved the influence of process parameters to structure, nor the optimization method.

2 Lap ratio

In the process of RFP，choosing different technological parameter can get three different results: incomplete lap (Fig.1a), normal lap (Fig.1b), redundant lap (Fig.2).Incomplete lap means that the N+1 layer fiber shows the discrete state based on the N layer. And it will lead to next layer loss the hosted platform that induces forming suspension. The redundant lap can get the continuing deposited fiber, but the cross-sectional size of fiber is too large. There is no enough space for positioning the N+2 layer, which means there is interference between the nozzle and the N+1 layer, so the Manufacturing process will be interrupted. To solve the problem of loss lap and redundant lap is the guarantee of successful completion of RFP. According to the breakpoint statistics, the lap ratio is defined as W_r=1-breakpoint number/100, while the ratio of redundant lap was defined 110%.

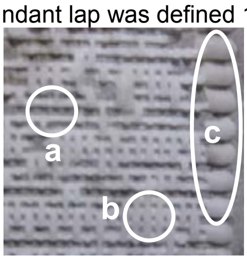

Fig.1.Incomplete lap and over accumulation


Fig.2.Redundant lap

3 Experimental

3.1 Experimental material

Material used in the experiment is the mixture solution of gelatin and chitosan. Gelatin should be mixed at 65°C hot water. Then Prepare of 5% chitosan solution. Fully mix two solutions to get the mixture of gelatin and chitosan. Because gelatin can be dissolved easily, solute content is regulated by the content of gelatin.

3.2 Experimental platform

Due to squeezing out water-soluble biological materials in the low temperature, RFP's principle is similar to FDM. Experimental platform is shown in Fig.3, including feeding system, motion control system and temperature system. Comparing with FDM process, the mainly different points lie in their feeding systems and the environment temperature control systems. RFP's material is the liquid with a certain viscosity at room temperature. After being extruded from the nozzle, it will be frozen at -15°C~-30°C. Because maintain material have certain rheological properties before it be extruded, it is necessary to heat up the nozzle, the specific structure is shown in the Fig.4. In order to solve the problem of over-accumulation at the corner (Fig.1c), the most effective way is to block the supply of material when the nozzle reaches the corner. The solenoid valve is used to link nozzle and material room, and the start-stop control of feeding can be achieved by the open and close to solenoid valve. In addition, in order to achieve a simple nozzle structure, reduce the inertia during the movement of the nozzle and improve the accuracy of forming, pneumatic feeding system is selected. As shown in Fig.5, Due to its small size, the prototype system can be directly placed in the industry refrigerator where the temperature can be controlled.

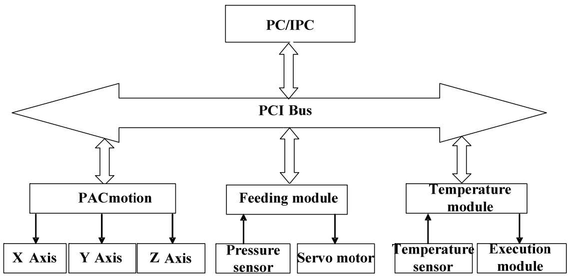

Fig 3.The frame of control system

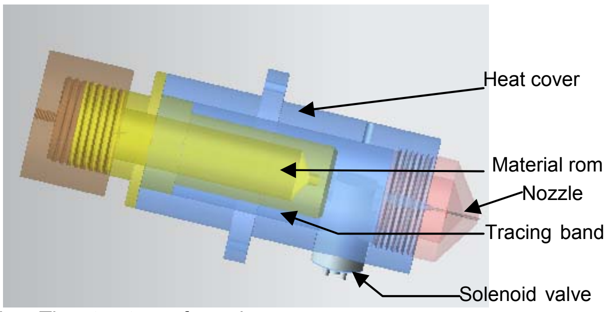

Fig.4.The structure of nozzle

Fig.5.The experimental device

3.3 Experimental scheme

Breaking force balance before fiber is solidified is the primary cause of the incomplete lap. As shown in figure 6, before the deposited material completely solidified, it will endure the gravity G, the traction force F drag from the nozzle and the tension F ξ the material initial has. When the resultant force of the two later forces F is balance with G, fiber will not have deformation; when G is greater than F, material will have the tendency of the downward deformation. Along with the increase of the deformation, the tension will increase. Finally, tension and deformation will reach a certain balance. If they can't reach balance all along, it will cause the fiber disconnect. At the same time, if Fdrag is greater than the resultant force of F ξ and G, the fiber will be stretched and even to be ruptured. In the controllable processing parameters, velocity ratio *i* (P/V) is an important factor to influence traction Fdrag. Material temperature T_f(°C) and material solid content *w*(%)have a big influence on viscosity, which will have an indirect influence on tension. Nozzle diameter *D*(mm)and row spacing *L*(mm)directly determine G.

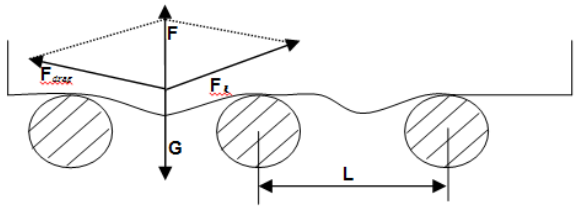

Fig.6.The force analysis of deposited material

The influence factors of lapping ratio is studied for optimizing of process parameters through the L16(4^5) orthogonal experiment. As it is shown in table 1, we choose *i*, T_f, *w*, *D*, *L* as the main factors, each factor is chosen in four levels. Where *i* is the velocity ratio of material feeding to nozzle movement, we use the ratio of nozzle pressure P and nozzle movement velocity V to replace. In addition, the environment temperature is set at -20°C.

Table1. The factors and levels selected for orthogonal experiment

Factor		Velocity ratio (*P/V*)	Material temperature(℃)	Solid content *(%)*	Nozzle diameter(mm)	Row spacing (mm)
Symbol		*i*	T_f	*w*	*D*	*L*
Level	1	1.1	60	15%	0.3	0.8
	2	1.2	65	20%	0.4	0.9
	3	1.3	70	25%	0.5	1.0
	4	1.4	75	30%	0.6	1.1

Table2. The result and analysis of orthogonal experiment

Factor	1	2	3	4	5	Lap ratio
Series No	Level of *i*	Level of T_f	Level of *w*	Level of *D*	Level of *L*	*Wr*
1	1	1	1	1	1	62
2	1	2	2	2	2	90
3	1	3	3	3	3	85
4	1	4	4	4	4	72
5	2	1	2	3	4	65
6	2	2	1	4	3	75
7	2	3	4	1	2	100
8	2	4	3	2	1	82
9	3	1	3	4	2	95
10	3	2	4	3	1	110
11	3	3	1	2	4	54
12	3	4	2	1	3	70
13	4	1	4	2	3	100
14	4	2	3	1	4	85
15	4	3	2	4	1	110
16	4	4	1	3	2	78
Mean value 1	77.250	80.500	67.250	79.250	91.000	
Mean value 2	80.500	90.000	83.750	81.500	90.750	
Mean value 3	82.250	87.250	86.750	84.500	82.500	
Mean value 4	93.250	75.500	95.500	88.000	69.000	
Range	17.000	14.250	39.500	2.250	11.500	
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Results and Discussion

From the analysis of range in Table 2, the importance of factor that decide whether material can deposit smoothly, can be ordered as follow: solid content w(39.5)> velocity ratio *i*(17)> Temperature T_f(14.25)> Row spacing *L*(11.5)> Nozzle diameter *D*(2.25). According to experiment result, the relationship between test factor and Wr was plotted such as figure 7.

Velocity ratio: *i* is the important impact factor whether lap smoothly. And it is proportional to Wr. The reason is, when *i* is lower, the fiber squeezed out nozzle bears more tension, and the fiber diameter becomes smaller. And if the tension is bigger than material's viscous force, the textile fiber will be broken. When *i* rise, the tension of material is reduced; the only external force of material is gravity. Therefore, it is not easy to be broken. However, when *i* is big enough, the extrusion swelling phenomenon will be obvious, which can largen the fiber diameter, and increase the phenomenon of redundant lap.

Material temperature: Because of the experimental material contains the higher composition of gelatin, whose viscosity is sensitive to temperature. The optimum temperature condition can guarantee the material has better viscosity and fluidity, which are important factors to decide whether fiber lap smoothly. When the temperature rises to 65°C, W_r is the highest, when the temperature further rises, W_r decrease instead. The reason is, when the material temperature is 65°C, its viscosity and the fluidity has the best matching. If the temperature is too low, material fluidity will be insufficient. In contrary, if the temperature is too high, material may obtain a better fluidity, but the viscous force will drop at the same time.

Solid content: From the experimental result of 15%~30% solutions we can see, along with *w* grows, W_r also elevates. The reason is that the solid content of solution directly decides the material viscosity which has the most important influence on the formation result.

Nozzle diameter: From the experiment we can find, *D* has certain influence to W_r, along with *D* increase, Wr will also increase. The reason is that in the case of keeping other invariable technological parameters, *D* directly infects the fiber section size. What's more, through range analysis, we can find that compares other factors, *D* has little influence on W_r.

Row spacing: *L* is proportional to W_r. because when *L* is bigger; the material gravity between the rows is bigger. When the gravity is big enough, it will break the constraint of its own viscous force.

From Fig.7, we can see that selecting the parameters combination (i_1, T_{f2}, w_3, D_4, L_1) may achieve a better Wr result. However, we can't ignore that some higher parameter values may bring the redundant lap, which affect pore size and scaffold's porosity. For instance, when *i* and the *D* are oversize or the row spacing is too small, it will appear the question of redundant lap. Above all, we choose (i_2, T_{f2}, w_4, D_3, L_2) as the technological parameter combination.

Using the optimizing parameter combination(i_2, T_{f2}, w_4, D_3, L_2), and setting solenoid valve's closed lead as 0.3s, the delay time 1.1s, the macroscopic pore structure has been manufactured. After dried in a vacuum freeze dryer with 12 hours, multiscale pore bone scaffold (Fig.8a 90°crossover Fig.8b 60°crossover) has obtained. As shown in fig.8c, the macroscopic pore size is 349μm, and as shown in fig.8d the micro-pore size is between 5 to 20μm. Its porosity is 85% measured by drainage method [12]. Moreover, macroscopic, microscopic porosity can be adjusted through the change of row spacing and Solid content. With the optimized technological parameter, the question of over-accumulation was effectively worked out at the small curvature turning-place, and Wr is very well.

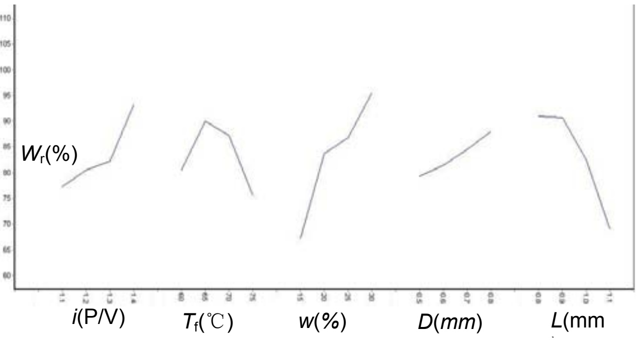
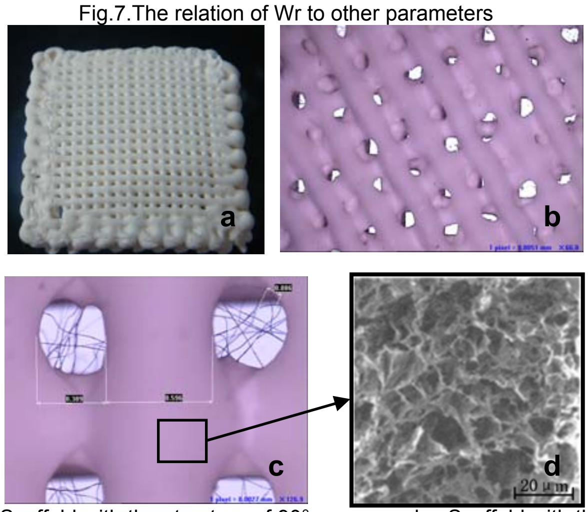


Fig.7.The relation of Wr to other parameters



a. Scaffold with the structure of 90°crossover ,b. Scaffold with the structure of 60°crossover, c. Macro pore d. Micro pore
Fig.8. 3D bone scaffold with multiscale pores

5 Conclusions

Lap ratio (W_r) is the main factor of the question, whether the 3D tissue bone scaffold can be fabricated by RFP. The influence of technological parameters on Wr has been studied by the Orthogonal experiment $L16(4^5)$ with the mixed solution of gelatin- chitosan. The research indicated that the important order of the influencing factors on Wr was: concentration *w*(39.5)>velocity ratio *i*(17)> temperature T_f(14.25)> row spacing *L*(11.5)> nozzle diameter *D*(2.25). With the optimized process parameters, the multi-layer deposition is realized smoothly. And after freeze drying, three-dimensional bone scaffolds with multiscale pore was fabricated.

The next step is to synthesize theory and experiment, research the technological parameter that can build the bigger inner pore, fabricate gradient pore, and study the effect of different structure on the mechanical property and degradation of scaffold.

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