Highly Concentrated Hydroxyapatite Suspension for DLP Printing

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Abstract: Due to the lack of commercially available high-quality hydroxyapatite (HA) powder, it is still rarely reported by the literature concerning the development of UV resin for digital light processing 3D printing. The previous studies still had the problem of delamination and also poor sintering performance led by low solid-load slurry. Here low viscosity and high solid-load hydroxyapatite (HA) UV resin suspensions were developed for digital light processing 3D printing. In this study, the effect of the type of dispersant, the dose levels of dispersant and the solid load of HA powder on the rheology properties were thoroughly investigated to obtain a flowable highly-concentrated HA UV resin suspension. Finally, a 40vol% slurry with viscosity of 3.7Pa·s at a shear rate of 10 s⁻¹ was successfully developed. After 3D printing and sintering, a dense ceramic with relative density of 95.85% can be obtained at a sintering temperature of 1300°C.

1. Introduction
Bones are the hard organs that make up the inner structure of human and their function is support and protect the body, which play a vital role in kinetic system. Bones are basically composed of organic and inorganic components, of which the inorganic constituent accounts for practically 70%. Calcium phosphate is the main inorganic, which accounts for nearly 95%, besides, magnesium, sodium, potassium, and some trace elements also contained [1-3]. Millions of people suffer every year from bone defects and fractures, tumors surgery, congenital malformation, arthritis, osteoporosis, infection, and other bone related disease result from ageing [4]. Bone transplantation, refers to the procedure of replacing losing or damaged bones with materials from either the patients themselves (autograft) or donors (allograft), which firstly established about two centuries ago [5-6]. Apparently, autograft is considered as optimal replantation, since the bone obtained from the patients themselves contains living cells and growth factors. However, shortage of bone resources and secondary procedures are great limitation for autograft in application. In addition, there are still exist risk regarding
immunological reaction, disease transmission between the patient and the donor bone lead to the unpredictability during healing in some cases [7-8].

Due to the above drawbacks, there is an urgently demand for appropriate bone substitutes, which are extricate from the limitations of bone supply, rejection and disease [9]. Tissue engineering is a serviceable tool for the reconstruction of massive bone defects in medical research [10]. One of the most significative research achievement of tissue engineering is the development of porous bioceramic scaffolds for bone regeneration [11]. Among the various forms of calcium phosphate ceramics, hydroxyapatite (HA, Ca10(PO4)6(OH)2) have attracted widely attention owing to their excellent osteoconductive and bioactive properties [12]. HA is thermodynamically the most stable phase in physiological conditions and has the ability for straight to chemical bonding to the bone in bone tissue engineering. It shows excellent performance as an artificial substitute material for bone, which do a great deal favor for cells adhering, proliferating, and mineralizing on the surface of materials [13-14]. Some ideal properties are needed for the HA bone tissue engineering scaffold such as appropriate pore size, morphology and controllable porosity, which put forward higher requirements for fabricating technology [15-17].

Traditional scaffold fabrication technologies show many challenges in the creation and hard to get ideal bone substitute, including complex mold manufacturing process, long fabrication times, serious waste of materials, and inaccuracy and uncontrollable of pattern. Additive manufacturing (AM) is also called 3D printing or free forming technology, in which printing is done by adding materials layer by layer into any form of pattern, is regarded as a promising tool for bone tissue engineering manufacturing revolution.

Compared with the traditional technology, 3D printing technology which is free from the limitation of making or processing mold technology overcome the difficulty of forming problem of the complex structural part, and greatly reduces the processing procedure and shortens the processing period. Furthermore, the more complicated structure has, the more obvious advantages shows. 3D printing mainly contains establish a model by CAD software firstly [18] and then import model into a computer linking with 3D printing machine. At present 3D printing technology is mainly classified into several descriptions: selective laser sintering (SLS) selective laser melting (SLM), stereolithography (SLA),3D printing (3DP), direct inkjet writing (DIW) and digital light processing (DLP) [19-20]. Among these 3D printing approaches, the light-curable resin used in DLP technology is particularly attractive, which is in fact a mask-based SL that a single layer is shined to the photopolymerisable slurry surface by exposing the ultraviolet light source once only. DLP makes it possible for optimizing the ultimate properties of the printed sample by simply changing the photocurable slurry resin formula which is particularly attractive.

In spite of these advantages, the 3D printing of high quality HA ceramics is still rarely known by the published results: the microstructure defects of pores and delamination resulted from low solid-load slurry were still quite problematic. To be used in DLP, the ceramic slurry should have a low viscosity to make sure the scraper of a DLP machine can spread ceramic suspensions evenly and slurry can self-level nicely before each exposure. In this work, the effect of the type of dispersant, the appropriate dose of dispersant and the solid load of HA powder on the rheology properties were systematically investigated to obtain a usable highly-concentrated HA UV resin suspension.

2. Experimental

2.1. Materials

In order to reduce the viscosity of ceramic suspensions, polyacrylate dispersant (CPM-D-1) and alkanolamine salt dispersants (CPM-D-2) was used to modify the surface of HA powders, which has an obvious influence on rheological property but a minimal impact on curing behavior.

Small amount of surfactant has an obvious influence on rheological property but little effect on curing behavior. A HA ceramic powder (CPM-P-HA) with particle sizes of D50=3.97μm was used as the ceramic filler, and three types resin with one, two and three amounts of functional group
respectively. All materials mentioned above were kindly provided by Jiaxing CeramPlus Tech. Ltd. (Zhejiang Province, China).

2.2. HA suspension preparation

Generally speaking, UV curable ceramic suspensions used for DLP printing contain following components: resin solvent, ceramic powder, photoinitiator and some additives such as dispersant, defoamer. Firstly, three types of acrylic resins with different functional group numbers were mixed to form the resin mixture, and then TPO were added into the mixture as the photoinitiator. Then a testing HA ceramic powder and two dispersants with different volume ratio were added to mix a pre-experimental slurry to obtain the best ratio of two types dispersant. The next work was designed experiments with different contents (3, 4, 5 and 6wt%) of dispersant and added HA ceramic powder partly and partly to make sure the most appropriate content of dispersant, preparation process of HA suspension is showed in Figure. 1.

![Figure 1. The preparation and dispersion of HA slurry.](image)

2.3. Fabrication and subsequent processing

CeramPlus DLP-50 (Jiaxing CeramPlus Tech. Ltd., China) as shown in Figure 2 a high resolution (50μm) equipped with the DLP printing machine. In the first, a 3D model is established by using computer three-dimensional design software such as CAD or 3D reconstruction technology of medical image and then the model is exported to an STL (stereolithography) file format. Then, the STL file is imported into printing software and the appropriate printing parameters are set as following: exposure intensity was100mw/cm2, burn-in exposure time was 5000ms and single-layer exposure time was
1000ms. Hereafter, the suspension is loaded into a tank, and the printing of the slurry is controlled by a computer file linked with DLP printing machine.

To achieve a strong and safety HA ceramic part apply to bone tissue engineering and remove the toxic and harmful substances for tissue cells, it must to carry on subsequent processing including rinsing, drying, debinding and sintering to the green body. The as-printed green samples were cleaned with anhydrous ethanol and enhance complete solidification by a secondary curing furnace using a UV-light curing furnace.

Conventional pressureless sintering was had started for fabricated HA samples that debinded with a low heating rate of 1°C/min up to 600 °C and holding there for 2 h to debinding in order to avoid cracking and deformation of ceramic specimen. Then, the samples were sintered at 1300 °C for 2 h with a higher heating rate of 5 °C/min, and finally naturally cooled till room temperature.

2.4. Characterization
Rheological behavior of the photosensitive HA suspensions was characterized using a stress-controlled rotational rheometer ((Kinexus pro, Malvern, UK) with a cone with a diameter of 20 mm by applying shear rates in a shear rate range from 1 to 100 s⁻¹ at 25°C. To investigate the relationship between the size of the HA particle and the rheological property of the suspension, the dynamic laser light scattering (Zetasizer Nano ZS, Malvern, UK) was used to determine the HA particle size after surface modification. Microstructural analysis of sintered sample was done using a scanning electron microscope (SEM, Phenom Pro, Phenom World, Netherlands).

Bulk density of as-sintered part was measured according to the water immersion method based on Archimedes principle. The theoretical density of hydroxyapatite was taken as 3.16 g·cm⁻³.

3. Results and discussion

3.1. Effects of dispersant proportion
Wetting dispersing agent plays an important role in dispersing powders in a light-cured resin base system, and it is a very important experimental addition reagent. Based on amounts of our previous experiments on the desperation of HA powders, we found the effect of mixed use with polyacrylate dispersant (CPM-D-1) and alkanolamine salt dispersants (CPM-D-2) were much better compared with using one of them as dispersant.

![The rheological curves of HA suspension with different proportion (CPM-D-1:CPM-D-2) of two dispersants in pre-experiment.](image)
To get dense ceramic compacts, suspensions should contain at least 40 vol% ceramic powder [21], so we selected 40vol% solid loading provisionally. Figure 3 was the rheological property of 40 vol% solid loading HA suspension in pre-experiment which using a generally available HA powder with different proportion of two dispersants (D06 and D80). It is obviously that all HA suspensions show shear thinning tendency with the increase of shear rate and shear viscosity of suspension with 1:1 proportion is the lowest while shear rate at 10 s⁻¹, which means dispersant successfully modified the HA powder, which relieves power agglomeration. Due to the decisive factor of proportion for dispersant is chemical property, so the proportion at 1:1 was chosen to conduct the further investigation of HA slurry.

3.2. Effects of dispersant content
According to the result of pre-experiment, 40vol% solid loading HA slurry with two types dispersant with proportion of 1:1 using a uniform particle size HA powder were apply to test the best content of dispersant. Dosage of dispersant great depend on the particles size and surface areas. HA powders have high surface energy and are easy to agglomerate to form larger particles in a liquid environment. Dispersant is a high molecular polymer that can enwrap ceramic powder particles, the capillary forces generated come from the polymerization of powder particles can be reduces as well as the increase of the distance between the powders, which effectively prevent the settlement and condensation of particles. As we can see in Figure 4, shear viscosity was reduced with the raise of content of dispersant at a shear rate of 10 s⁻¹ until it reach 5wt% and the viscosity reached 3.7Pa·s. Further increase with the dispersant dosage resulted in an increase of viscosity. With a proper amount of dispersant, the suspension dispersed more uniformly, which attributed to better fluidity and lower viscosity. When the concentration of dispersant content was over 5%, the adsorption capacity of the HA particle reached a saturation point. As the result, we will select 5wt% as the dispersant dosage for further formulation of the HA ceramic suspension. More solid loads were investigated for reducing the viscosity.

![Figure 4. Shear viscosity of HA slurry with different dispersant content.](image)

For an acceptable printing performance with the stereolithography method, it is widely accepted that a viscosity of < 5 Pa·s at shear rate of 30 s⁻¹ is necessary. The rheological curve of final HA ceramic suspension is exhibited in figure 5, curve is quite smooth over the wide shear rate range and this expressed a well dispersed and stable suspension. The viscosity of this developed slurry is better
than necessary to be used for DLP printing. The viscosity at 10 s⁻¹ and 30 s⁻¹ are both less than 3 Pa·s.

Then the particle size of HA suspension was also investigated after dilution and the particle size
distribution was shown in figure 6. HA particle size distribute uniformly with a nice symmetric
distribution, which represent the dispersant adsorbed on the surface of HA particles uniformly form
adsorption layers. Therefore, the HA ceramic suspension apply to DLP printing can promise to
achieve ideal structure.

![Figure 5. Viscosity vs. shear rate property of HA slurry.](image)

![Figure 6. Particle distribution for HA slurry.](image)

The photograph of the sintered sample with truss structure is shown in Figure 7A, no cracks can be
observed in the whole structure by naked eyes and the relative density measured by Archimedes
drainage method was 95.85%. Moreover, the microstructure of the fracture surface of the sample was
shown in Figure 7B, there is no obvious crack either but few tiny pores which may be caused by a
respectively rapid heating rate after 600°C. The high relative density of the sample sintered with no
pressure will guarantee a respectively high mechanical property. Nevertheless, as we all know,
Mechanical properties of ceramics are strongly influenced by morphology, porosity and grain size
[22,23]. Thus, to test the mechanical properties and explore a better sintering process are necessary to
our next step.
4. Conclusions

In this paper, a HA ceramic suspension was developed for DLP applied to bone tissue engineering. The dispersants (CPM-D-1 and CPM-D-2) play an important role in dispersing HA in resin liquid environment by improving stability and decreasing viscosity of suspension. The effect of proportion of two type dispersants as well as the content of dispersant on the dispersion behavior and rheology property was researched to determine a formulation of the suspension.

The result of rheology property with different ratio dispersant indicated that the dispersant has good dispersion ability to the HA ceramic slurry and we draw a conclusion that 1:1 is the best proportion to reduce shear viscosity at shear rate of 10 s⁻¹. And shear viscosity achieved the lowest of 3.7Pa·s when content of dispersant is 5% of the quality of the HA powder. Therefore, a 40vol% solid loading HA ceramic suspension with 5% content dispersant was successfully applied for DLP and a dense HA ceramic part with relative density of 95.85% was obtained after sintering at 1300°C.

Combining all advantage of DLP in fabricating complex bodies and the excellent biological performance of HA bioceramic, the stable and liquidity HA suspension for DLP applied to bone tissue engineering was developed. In addition, more attention will be paid to the study of the various HA bone tissue engineering scaffolds using this suspension fabricated by DLP in further work.

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