Features of structures formation on the basis of chitosan derivatives by a prototype of 263 nm laser stereolithograph

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Abstract. We have developed technology of polysaccharides based matrices formation by laser stereolithography (SLA) method using UV range laser radiation. Experimental data on a laser parameters selection of single-layer structures polymerization for compositions based on unsaturated chitosan derivatives with different degree of substitution and with the addition of polyethylene glycol diacrylate is shown.

1. Introduction

Experimental development of 3D structures printing which compatible with the human body began in the early 21st century [1]. Currently, the technology of biological printing is experiencing of exponential growth. The first experiments in this direction showed the possibility of fragments creating of different tissues and organs. A defining stage in the development of technology for the recovery of the bodies was the opportunity to create biocompatible porous matrix "scaffold" that can be created a given three-dimensional model with the specified parameters of the structure at the micro level. The scaffolds are three-dimensional porous or fibrous matrix, one of the main functions of which is to provide a mechanical frame for cells [2]. The scaffolds should have the following properties: biocompatibility, absence of immunological rejection, intoxicity, biodegradation and some others.

Previously it was shown that on the basis of reactive chitosan by the method of two-photon femtosecond microstereolithography it is possible to form a three-dimensional matrix, which can be used as carriers for primary neuronal cultures [3]. The advantage of this method is the possibility of creating complex structures with high resolution, however, the performance of the method is quite low and the cost of the installation exploitation is quite high. Moreover, experience shows that such installations have a number of advantages, but also they have significant disadvantages: low printing speed due to high resolution, which greatly limits the possibility of rapid prototyping; high cost and large sizes of installations, and the complexity of their configuration and operation.

Developed stereolithograph will considerably simplify the process of printing, increase the speed at the several times without significant loss of quality. In addition, a large variety of biocompatible printing materials provides wide opportunities for its application. Furthermore, a significant advantage is the simple design and low cost of optics, mechanics, laser and other components.

The aim of this work was to develop a prototype a 3D printer based on SLA technology, using the source of laser radiation at a wavelength of 263 nm for compositions based on chitosan, as well as conducting experiments on selection of optimal parameters for these compositions.

2. Scheme of experimental setup, experimental technique.

Experimental setup is shown on figure 1. We used diode pumped ultraviolet solid state laser (263 nm, 25 mW, 263 TECH-Basic, the pulse duration 4 ns and frequency up to 4 kHz). We focused laser pulses by spherical lens (f=8 cm), which made it possible to focus light to a spot about 100 microns in diameter. To move the laser radiation across the sample, was used the two-mirror galvanoscanner LScanH-10 (Ateko, Russia) with the basic parameters: the scan range of ± 6°; maximum scanning speed – up to 480°/s; field processing (in the current experiment) 11 x 11 mm. The positioning accuracy of the focused beam in the current configuration was about 15 μm. The laser light was got from the top perpendicular to the surface of the photopolymer. As software we used the program LDesiner 5.0.
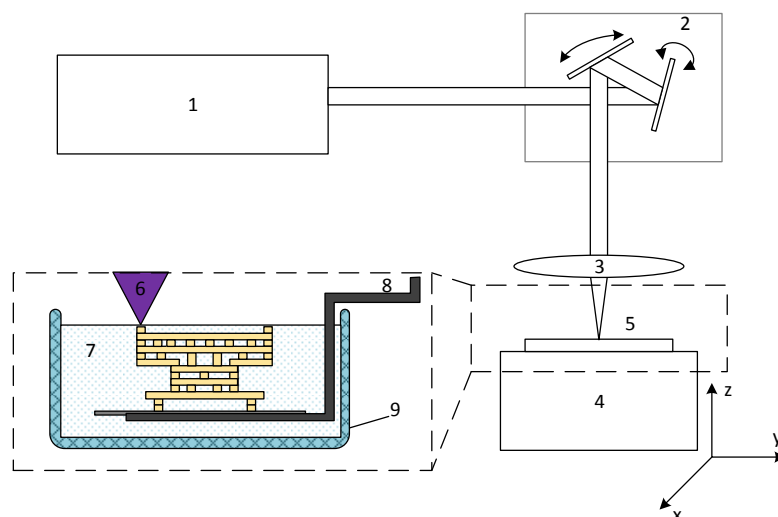

Figure 1. A schematic diagram of a 3D printer running on a single-photon photopolymerization: 1 – laser; 2 – two-mirror galvanoscanner; 3 – lens; 4 – three-coordinate table; 5 – substrate; 6 – focused laser beam; 7 - the photosensitive composition;8 – z-coordinate axis translator; 9 - quartz cuvette.

The following laser parameters were chosen for the experiment: pulse repetition rate 1.6 kHz, the spot diameter on the sample of 100 microns, the intensity of 0.2 W/cm^2. As a test figure was selected, a circle with a diameter of 6 mm, the area of which is filled by individual lines in laser scanning process.

3. Materials

One of the materials used to create tissue-engineering scaffolds is a natural polysaccharide chitosan and its derivatives. The main advantage of the selected polysaccharide is its non-toxicity, availability, and ability to form hydrogel system similar in mechanical characteristics to the soft body tissues [3]. Cleavage of the chitin N-acetyl-D-glucosamine an acetyl group or carrying deacetylation reaction underlies of chitosan forming. Chitosan and derivatives as a cellulose possess fibre- and film-forming [4–6] properties.

It was to perform a solid-state synthesis of unsaturated ether of chitosan – allyl chitosan – for the construction of 3D structures [7,8]. Number of allyl groups per chitosan's unit varies depending conditions and the ratio of reactants in which the synthesis was conducted. The greater the degree of substitution, the more unsaturated groups per unit volume of the composition, and hence more likely to be formed three-dimensional crosslinking during the photopolymerization process. Three type polymer compositions with different percentages of unsaturated groups were synthesized: CT-A1 (0.08-0.10)[7]., CT-A2 (0.25), CT-A3 (0.50).

To increase the number of unsaturated groups (double bonds) per unit volume, which can be the spatial crosslinking of the polymer composition and for varying the mechanical properties of the obtained matrix, swelling properties and biodegradation of [9] in the composition administered polyethylene glycol diacrylate (PEG-DA) with a molecular weight 2000 Da.
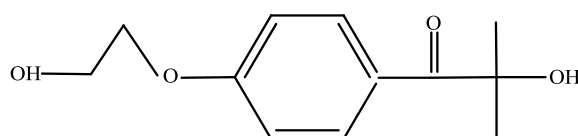

Figure 2. Irgacure 2959

In this study we used a biocompatible [10] photoinitiator Irgacure 2959 (Ciba), which upon irradiation of short-wave UV range (~ 263nm) decomposes to form benzoyl and ketyl fragments [11], which in turn all start the process of three-dimensional cross-linking.

4. Experimental results and Discussion

For polymer compositions with different degree of substitution and the addition of PEG-DA performed selection of optimal parameters. Fill density and the scan speed of the laser beam was varied. According to the results of test experiments, the optimal density of laser scan lines per millimeter was found - 15 lines/mm. At the test experiments the width of a represented single polymerized line is 60 μm, and it above parameters of the laser radiation. Most likely this is caused by the Gaussian profile at the laser light spot. This number of lines is provided complete filling of the area of the test figure in the form of polymerized areas with little lines overlap.
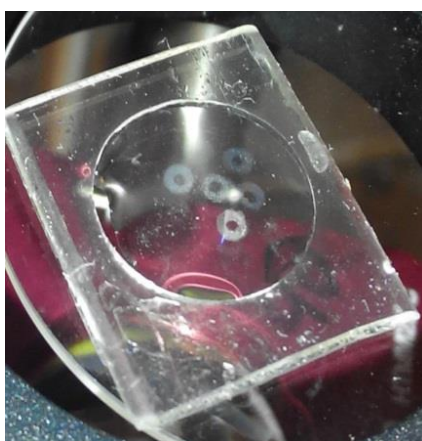

Figure 3. Photo of the test structure of the composition CT-A3.
Printed with a different speed in the silicone spacer. Diameter of cylinders 3 mm.

The most useful parameter for varying scan was chosen - speed of the laser radiation, because it affects the amount of energy that absorbs the sample. Scan range of printed speed was selected - from 2 to 20 mm/sec, from the results of test experiments.

4.1 A series of experiments for the samples CT-A1, CT-A2, CT-A3 *with 1% Irgacure 2959.*
After printing the sample were observed bright white spots. Then, the samples were placed in a petri dish filled with distilled water, the purpose of laundering the samples obtained from the glass substrate and unpolymerized material which dissolves in water completely. Best results were obtained with the composition CT-A2 and CT-A3 at speeds of 2, 4, 6 mm / sec. Samples polymerized films do not dissolve in water, retains its shape, the size of the increase was not observed.

For CT-A1 compositions was observed sharp "disintegration" of the polymerized film into fragments in the form of filaments, immediately after placing a sample in water. It is assumed that this is due to an insufficient number of double bonds which affect the degree of crosslinking and the strength of the sample.

4.2 A series of experiments for the samples CT-A1, CT-A2, CT-A3 *with 1% Irgacure 2959 and addition of PEG-DA.*
For all samples, the transparent areas of polymerized material were observed with a clear boundary. After 30 minutes, after placing all the samples in the CT-A3 water, we observed increase of a film size approximately 2-fold. After 60 minutes, samples printed with speed of 2, 4, 6, 8, 10 mm /sec is still observed in the water. For printing speed of 20 mm/sec were observed samples only in the form of separate fragments of the individual fibers.

After placing irradiated CT-A2 samples in the water, there was a gradual increase size of the film approximately 2-fold. After 60 minutes, samples irradiated at 2 and 4 mm / sec was observed in water as very transparencies film. Samples, printed with speed of 6, 8, 10, 20 mm/sec after 20 minutes in distilled water were not detected.

To the photosensitive compositions of CT-A1 observed much more rapid increase of sample size (relatively CT-A2 and CT-A3) when placed in water, that can be linked to less count of chemical links thru a less count of unsaturated groups at the sample. After 60 minutes in the water were observed samples printed at 2 and 4 mm/sec. The sample printed at a speed of 4 mm/sec significantly deformed and curled in the "roll". The samples were irradiated at higher speeds, dissolved after 10 minutes.

5. Conclusion
3D printer prototype was assembled using a UV laser radiation and galvo scanner. We investigated the original biocompatible compositions based on chitosan with different number of unsaturated groups per unit volume (with different degree of substitution) and with the addition of a copolymerizable component PEG-DA. Selection of parameters for of polymer compositions based on allyl chitosan with different degree of substitution was done - the density filling lines and scan speed.
In the first series of experiments without addition copolymerizable component PEG-DA for CT-A3 resistant film samples were obtained at speeds less than 6 mm / sec. In the case of compositions with less unsaturated bonds (CT-A2) resistant film were obtained at speeds of up to 4 mm/sec. For compositions CT-A1 resistant films failed.
In a series of experiments with addition copolymerizable component PEG-DA was observed increase size of samples approximately 2-fold by placing the samples in a petri dish filled with distilled water. Stable film for CT-A3 were obtained at speeds of 10 mm/sec. For CT-A2 and CT-A1 films were obtained at speeds up to 4 mm/sec, and a day later dissolved.
It should be noted that the CT-A3 compositions without addition copolymerizable component PEG-DA and with it resistant films were obtained which remained intact after soaking in distilled water for one week.

6. References

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