DOUBLE CROSSLINKING OF CHITOSAN/VANILLIN AS A BASIS TO MECHANICALLY STRONG GRADIENT HYDROGEL SCAFFOLDS FOR CARTILAGE TISSUE ENGINEERING

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Introduction

Natural polymers have significant advantages over synthetic ones, e.g. biocompatibility, nontoxicity, and similarity to many biological structures what can be especially useful in tissue engineering [1]. Chitosan (CS) is one of the most abundant natural polysaccharides, its structure and properties are similar to those of glycosaminoglycans (GAGs) – natural components of the extracellular matrix (ECM). CS combined with an appropriate crosslinking agent can create chemically and mechanically stable hydrogels [2, 3]. Moreover, they can be easily modified to obtain materials with a range of various properties and hierarchical structures. This can be exploited in osteochondral tissue engineering, in which mainly the biochemical gradient has been reproduced so far, and new material solutions are still needed [4, 5]. This study aimed to evaluate the influence of double crosslinking of CS scaffolds with vanillin or tripolyphosphate on the hydrogel properties.

Materials and Methods

Chitosan (CS; Acros Organics, MW=100,000-300,000), and Avantor Performance Materials Poland S.A. reagents: acetic acid (AAc), vanillin (VAN), sodium tripolyphosphate (TPP) and ethanol (EtOH) were used as received. First, CS was dissolved in 2% AAc and vanillin was dissolved in ethanol were. Then, the VAN solutions were added dropwise to the CS and homogenised by sonication (10min). The final concentration of CS solution was controlled at 5% w/v. The mass ratios of CS:VAN were 1:0.8, 1:1, 1:1.2, 1:1.4, 1:1.6 and denoted as 0.8van, 1van, 1.2van, 1.4van, 1.6van, respectively. was mixed for 24h. The obtained hydrogels were maintained for 6 days at room temperature for effective crosslinking. After this time, 6x6 mm cubic samples of 1van, 1.2van, 1.4van were cut and immersed in vanillin solution (5% in ethanol, denoted as 1van_V, 1.2van_V, 1.4van_V) and TPP solution (5% in distilled water, denoted as 1van_T, 1.2van_T, 1.4van_T) for 24h to improve the crosslinking process. Some of the samples were frozen at -80°C and freeze-dried for further analysis.

Microstructural (digital microscope, SEM), structural (FTIR-ATR), mechanical (compression test), surface (wettability) and biological (biodegradability in PBS, bioactivity in SBF) properties of the obtained materials were evaluated.

Results and Discussion

Test results allowed to assess the influence of the amount and type of the crosslinking agent, the time of the crosslinking process and single vs double crosslinking process on the properties of the obtained scaffolds. Compression strength of the single (0.8van, 1van, 1.2van, 1.4van, 1.6van) and double (1.2van_V, 1.2van_T) crosslinked samples is shown in FIG. 1.

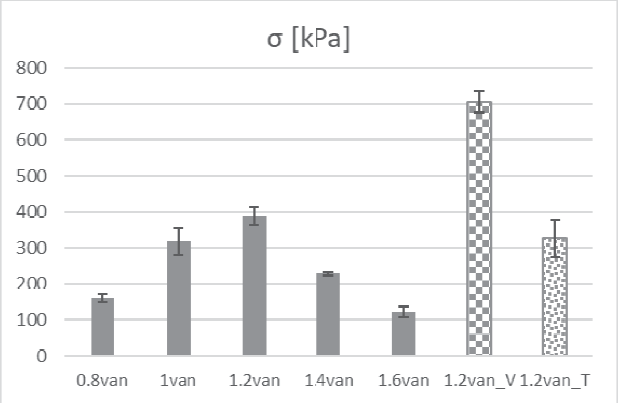

FIG. 1. Compression strength of the obtained hydrogel after single and double crosslinking.

For the single crosslinked samples, the highest compression strength was observed in the case of 1.2van. Double VAN-VAN crosslinking caused twofold increase of the compression strenght. Also, increase of the chemical stability was visible in the double-crosslinked, VAN-VAN samples, as well as the decrease of the swelling ratio. Double crosslinking with a TPP solution as a second crosslinker did not cause such differences.

Conclusions

The double-crosslinked CS hydrogels with higher mechanical properties and chemical stability were obtained in this study with the use of vanillin as a natural crosslinker. In the next step, they will be modified with additives, such as hydroxyapatite or graphene oxide to obtain hierarchical structures for cartilage tissue engineering.

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