Effect of cross-linking agent concentration on mechanical properties of Collagen-CaP nanostructured composite

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Abstrakt

An infection is responsible for many complications accompanying orthopedic surgery. Osteomyelitis may lead to total implant failure due to loosening of the prosthesis. Such a situation frequently results in necessity of re-implantation. To avoid potential post-surgery complications, various solutions are available. Our study is devoted to optimization of preparation methods for collagen-CaP nanostructured composite aimed to function as a drug carrier. The collagen-CaP composite will be used as implant surface coating. In this paper, the effect of crosslinking conditions on mechanical properties and structural stability of collagen-CaP is studied. Three different concentrations of an ethanol solution of N-(3-dimethylaminopropyl)-N0-ethylcarbodiimide hydrochloride/N-hydroxy-succinimide were used to cross-link electrospinned collagen-CaP layer for 24 hours. The mechanical properties were evaluated by means of uniaxial tensile tests and structural stability was assessed via SEM and degradation test. The results suggest that crosslinking improves all studied properties.

Keywords: nanoparticles, degradation products, polymeric drug delivery systems, pharmacokinetics, anti-infectives

1. Introduction

The infection of implanted endoprostheses represents a serious problem as far as orthopedic and trauma surgery are concerned [1]. One of the ways in which to increase the efficacy of the therapy is to use a local antibiotic delivery system. The local antibiotic treatment of prosthetic joint infection, as opposed to the use of systemic antibiotics, enjoys the advantage of achieving high antibiotic concentrations, which exceed the minimum inhibitory concentration (MIC) without increasing the level of systemic toxicity [2]. Local carriers of antibiotics used in the field of orthopedics are classified according to composition as synthetic and natural polymers, ceramics, composites, and bone grafts. It is expected that such a local carrier will be used particularly in the case of known prosthetic joint infections or as a preventative procedure regarding primary joint replacement in a potentially infected site [3]. The aim of our project is a development of composite nanostructured layer that will provide a bone/implant (titanium alloy) bioactive interface, which will enhance the physiological healing process, will be capable of filling bone defects, and will act as a powerful antibacterial agent against those microorganisms susceptible to vancomycin. The layer is made of collagen (type I, isolated from calf skin) and vancomycin hydrochloride. The application of collagen in the field of tissue engineering is limited because of its poor mechanical properties, high rate of swelling, low structural stability, and low resistivity against the enzymatic degradation of its untreated form.

The aim of the experiment described in this study was to verify the effect of different cross-linking conditions on mechanical and structural properties of electrospinned collagen. Three different concentrations of an ethanol solution of N-(3-dimethylaminopropyl)-N0-ethylcarbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) were chosen to determine the optimal cross-linking conditions. The structural properties and degradation rate were investigated in detail and subsequently compared to determine the optimal cross-linking conditions. Furthermore, mechanical properties were evaluated by means of the conducting of uniaxial tensile tests.

2. Materials and Methods

The layers were prepared by needle electrospinning (4Spin, Contipro a.s., CR) of 8 wt% solution of collagen (VUP Medical, CR), in PBS/EtOH, to which was added polyethylene oxide (PEO) (Mn 900,000, Sigma-Aldrich, Germany) in an amount of 8 wt% on the weight of collagen alone. Finally, vancomycin (Mylan S.A.S., France) was added to a solution, as a 10% by weight collagen and PEO. Cross-linking of collagen was carried out by means of a 95 %wt ethanol solution of EDC/NHS (4/1, wt/wt). The theoretical concentration of cross-linking agents was determined based on representation of each amino acid with free functional groups for EDC and previous experiments. The theoretical concentration (II) of EDC/collagen was of 5/8 (wt/wt). Furthermore, a double concentration (III) and half concentration (IV) of EDC/NHS were applied.

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Figure 1: *Biaxial testing machine for soft tissue and elastomer mechanical testing*

Mechanical properties were evaluated by means of the conducting of uniaxial tensile tests of rectangular strips of the layers (Zwick/Roell, Germany). During the test procedure, the strain at failure, the ultimate tensile strength, and the modulus of elasticity were determined. By using contrasting marks on the surface of samples (n=5), the video extensometer automatically determined the reference length and elongation of the samples. Tensile experiments were conducted at a constant clamp velocity of 0.1 mm/s. The loading force was measured by a U9B (±250N, HBM, Germany) force transducer.

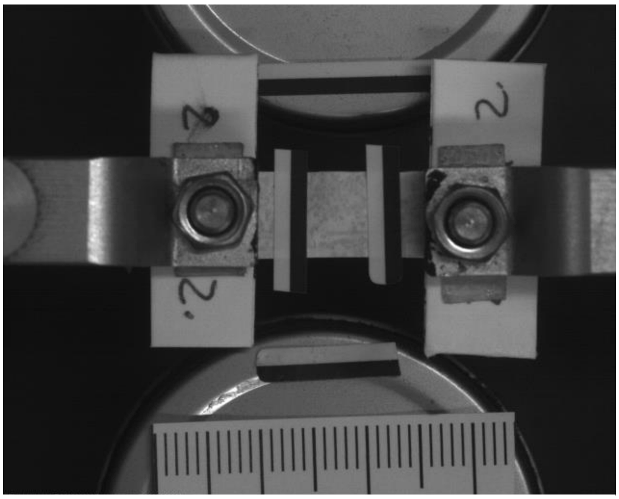

Figure 2: *Uniaxial tensile test setup*

For degradation tests, ultraviolet-visible spectrophotometry was used for the quantification of the free amino groups released during the degradation of the samples (n=3) immersed in the PBS (37°C, pH 7.4) and 2,4,6-trinitrobenzenesulphonic acid (TNBS, Sigma-Aldrich, Germany) solution. PBS was collected after 2, 6, and 24 h and after 4, 10, 15, and 30 days. The evaluation of the samples was conducted using calibration curve methodology in which the L-lysine (Sigma-Aldrich) solution was used as the standard solution. Scanning electron microscope Quanta 450 (FEI, USA) was used for an image analysis of samples before and after cross-linking.

Statistically significant differences were investigated principally by means of nonparametric methods (STATGRAPHICS Centurion XV, StatPoint, USA) because of the problematical nature of the verification of the normality of the assessed data (Shapiro-Wilk W tests) or the violation of homoscedasticity (Bartlett's test). The Kruskal-Wallis and the Mann-Whitney W test were used for the conducting of post hoc analysis. All the analyses of variance were performed at a 95% confidence level (p values < 0.05 were considered to be significant).

3. Results and Discussion

The stress-strain relationships obtained through uniaxial tensile testing are depicted in Figure 3. All the cross-linked samples exhibited a statistically significant increase in initial young's modulus and ultimate tensile strength (Fig. 4 and 5). The most statistically significant increases in ultimate tensile strength and initial modulus of elasticity were determined in the case of samples cross-linked by a half concentration (IV). This group also showed the highest variance. On the other hand, group II (theoretical concentration) showed only slightly dispersed results of initial Young's modulus and ultimate tensile strength as well as statistically significant increase in both, modulus and strength.

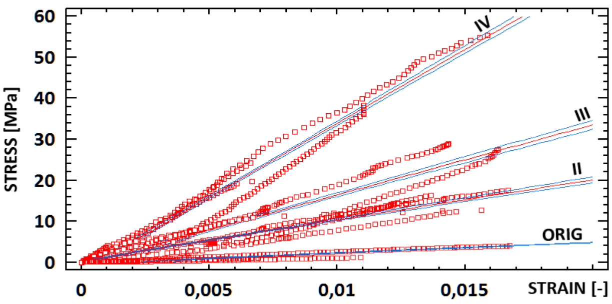

Figure 3: *Stress-strain relationships obtained via uniaxial tensile testing together with confidence intervals for the regression line (at 95% confidence level).*

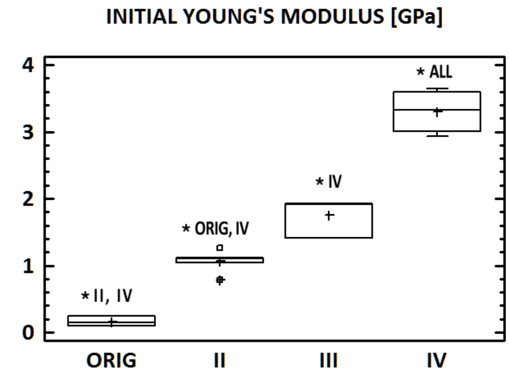

Figure 4: *Box plots compare initial Young's modulus. The sign "*" denotes statistically significant differences (Kruskal-Wallis, 0.05; Duncan's MRT was used to find significant differences in between groups).*

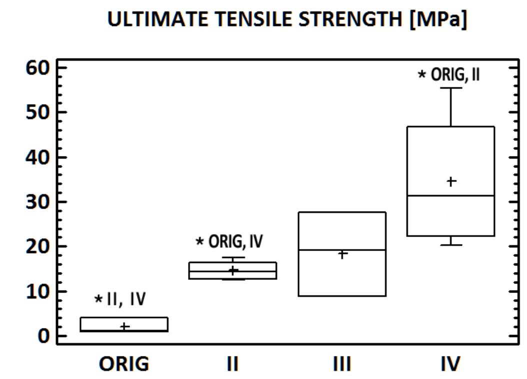

Figure 5: *Box plots comparing ultimate tensile strength. The sign "*" denotes statistically significant differences (Kruskal-Wallis, 0.05; Duncan's MRT was used to find significant differences in between groups)*

Expected accelerated mass loss (Fig. 6) was observed in the non-cross-linked samples (ORIG). Based on the relative comparison of released amino acids, the materials studied can be divided into 2 different groups. Non-cross-linked samples and the EDC/NHS cross-linked samples.

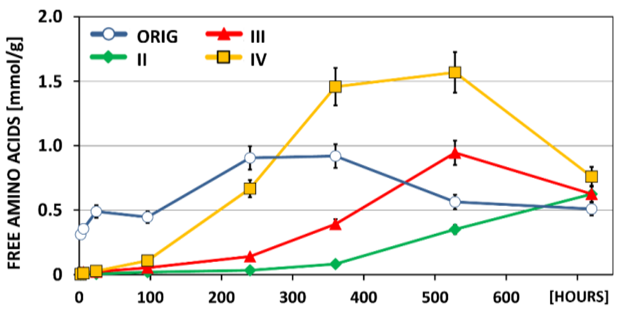

Figure 6: *The concentration of amino acids released from non-cross-linked and cross-linked samples (arithmetical average, SD).*

Non-cross-linked showed the lowest level of resistance to decomposition under physiological conditions. All cross-linked samples showed greater immunity to degradative environment. The highest resistance to degradation was seen in group II.

SEM images were taken in order to analyze a possible effect of cross-linking on the inner structure of the samples (Fig. 7). Images show that non-cross-linked samples has fibrous architecture with no visible homogeneous parts of structure. In contrast to non-cross-linked samples, homogenization can be observed on the edges of samples cross-linked by theoretical and half concentration of EDC/NHS (II and IV). Samples cross-linked by double concentration (III) are mostly homogenous except the surface where some fibers can be observed. The loss of porous structure caused by cross-linking procedure can be an explanation of the increase in ultimate tensile strength and initial modulus.

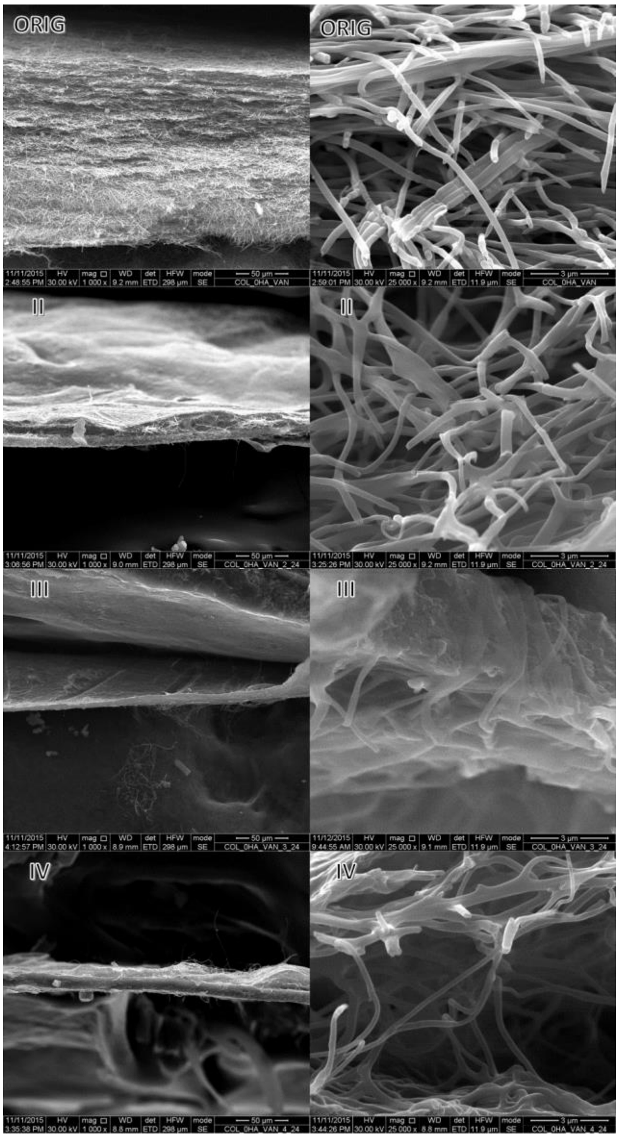

Figure 7: *SEM images of samples before and after cross-linking (mag.1000x and 25,000x).*

4. Conclusion

During the experiment, all of the cross-linked samples showed increase in ultimate tensile strength and initial modulus compared to non-cross-linked samples. In structural stability, cross-linked samples showed a higher resistance to degradation than non-cross-linked, except samples cross-linked by half concentration of EDC/NHS. Samples cross-linked by double concentration of EDC/NHS evinced the loss of inner fibrous structure which would limit the elution of antibiotics. The theoretical concentration of EDC/NHS appears to be more suitable than other concentrations applied in this study

Acknowledgements

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