

## Collagen Hydrogels Cross-Linked by Squaric Acid

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**Abstract :** Hydrogels are a class of materials widely used in medicine for many years. Proteins, such as collagen, due to the presence of a large number of functional groups are easily wettable by polar solvents and can create hydrogels. The supramolecular network capable to swelling is created by cross-linking of the biopolymers using various reagents. Many cross-linking agents has been tested for last years, however, researchers still are looking for a new, more secure reactants. Squaric acid, 3,4-dihydroxy 3-cyclobutene 1,2- dione, is a very strong acid, which possess flat and rigid structure. Due to the presence of two carboxyl groups the squaric acid willingly reacts with amino groups of collagen. The main purpose of this study was to investigate the influence of addition of squaric acid on the chemical, physical and biological properties of collagen materials. The collagen type I was extracted from rat tail tendons and 1% solution in 0.1M acetic acid was prepared. The samples were cross-linked by the addition of 5%, 10% and 20% of squaric acid. The mixtures of all reagents were incubated 30 min on magnetic stirrer and then dialyzed against deionized water. The FTIR spectra show that the collagen structure is not changed by cross-linking by squaric acid. Although the mechanical properties of the collagen material deteriorate, the temperature of thermal denaturation of collagen increases after cross-linking, what indicates that the protein network was created. The lyophilized collagen gels exhibit porous structure and the pore size decreases with the higher addition of squaric acid. Also the swelling ability is lower after the cross-linking. The in vitro study demonstrates that the materials are attractive for 3T3 cells. The addition of squaric acid causes formation of cross-link bonds in the collagen materials and the transparent, stiff hydrogels are obtained. The changes of physicochemical properties of the material are typical for cross-linking process, except mechanical properties - it requires further experiments. However, the results let us to conclude that squaric acid is a suitable cross-linker for protein materials for medicine and tissue engineering.

**Keywords :** collagen, squaric acid, cross-linking, hydrogel

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