Chitosan-based hydrogel biomaterials: *in vitro* investigation

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Introduction:

Hydrogel materials are three-dimensional networks of hydrophylic polymer chains that can swell without dissolving in aqueous media and hold large amounts of water. Their porosity, high swelling ability,and hydrophilic nature make them perfect materials as carriers of hydrophilic biologically active compounds. Moreover they can mimic biomechanical characteristics of extracellular matrix (ECM). Due to their unique properties hydrogel materials have enormous potential in many biomedical areas. The aim of this study was synthesis and *in vitro* investigation of hydrogel materials based on chitosan (CS) and pectin (PC) crosslinked with 2,3,4-trihydroxybenzaldehyde (THBA), with the addition of sol-gel-derived bioactive glass (BG) and rosmarinic acid (RA). Swelling and degradation was investigated by incubating the materials in phosphate buffered saline (PBS), while the antioxidant activity was evaluated using ABTS and DPPH free radical scavenging assays and ferric reducing antioxidant power (FRAP). The release of THBA and RA from hydrogels to PBS was evaluated using HPLC. Moreover the *in vitro* mineralization in simulated body fluid (SBF) and cell studies on human normal skin fibroblasts (BJ) and human colon cancer epithelial cells (HT-29) were held.

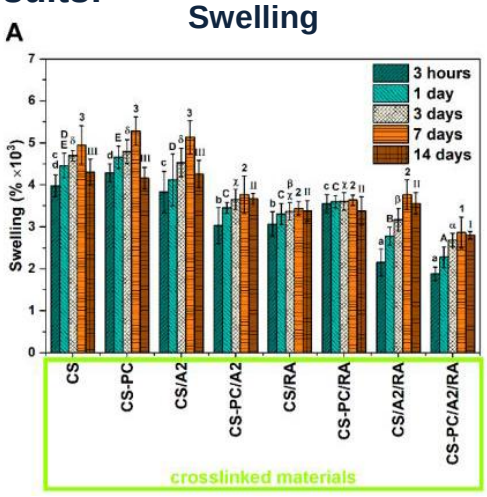
Materials:

Hydrogel materials were produced based on two biopolymers - chitosan (CS) and pectin (PC), crosslinked with 2,3,4-trihydroxybenzaldehyde (THBA). Also rosmarinic acid (RA) and a bioactive glass (BG) were used as functional components.

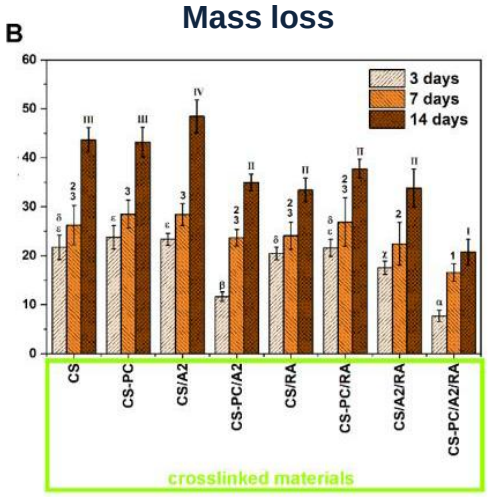
Material	CS (w/w%)	PC (w/w%)	THBA (w/w%)	RA (w/w%)	A2 BG (w/w%)	Material	CS (w/w%)	PC (w/w%)	THBA (w/w%)	RA (w/w%)	A2 BG (w/w%)
Uncrosslinked materials						Crosslinked materials					
CS	100	-	-	-	-	CS	100	-	2	-	-
CS-PC	70	30	-	-	-	CS-PC	70	30	2	-	-
CS/A2	100	-	-	-	5	CS/A2	100	-	2	-	5
CS-PC/A2	70	30	-	-	5	CS-PC/A2	70	30	2	-	5
CS/RA	100	-	-	2	-	CS/RA	100	-	2	2	-
CS-PC/RA	70	30	-	2	-	CS-PC/RA	70	30	2	2	-
CS/A2/RA	100	-	-	2	5	CS/A2/RA	100	-	2	2	5
CS-PC/A2/RA	70	30	-	2	5	CS-PC/A2/RA	70	30	2	2	5

Results:

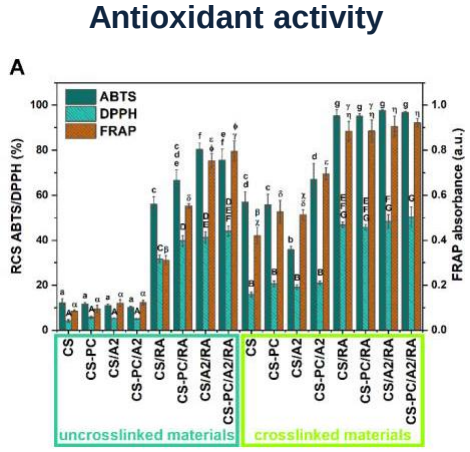
Swelling



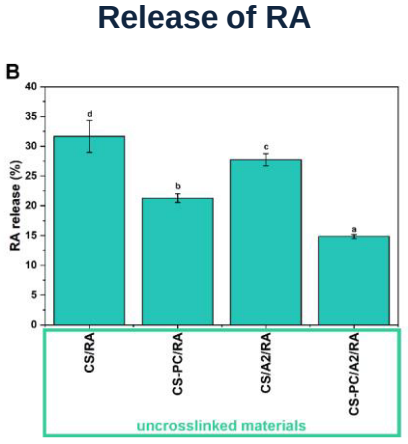
Mass loss



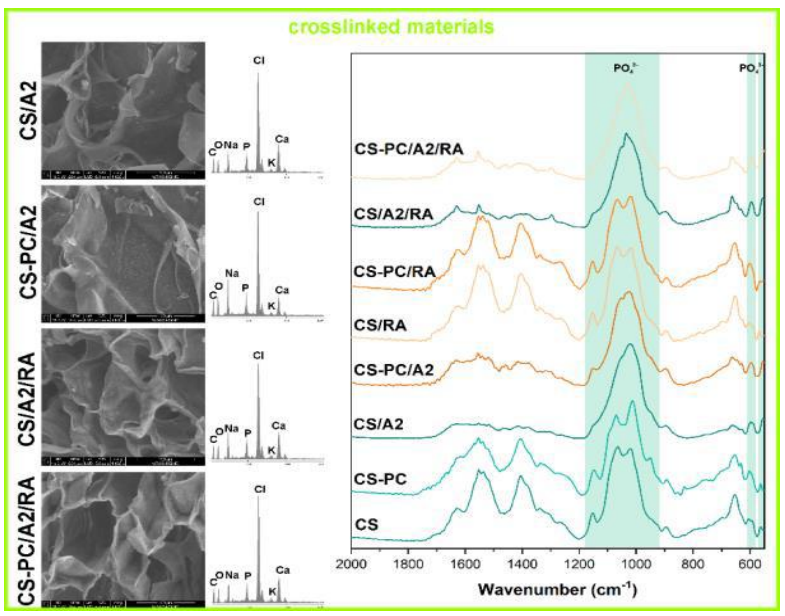
Antioxidant activity



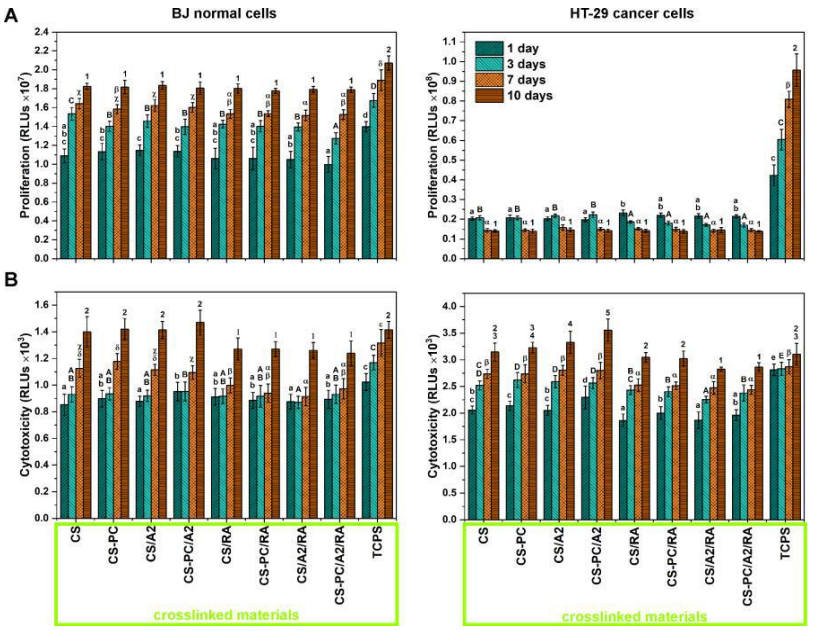
Release of RA



SEM images, EDX and ATR-FTIR spectra



In vitro cell studies



Conclusions:

The conducted studies showed that crosslinking process resulted in high swelling capacity and delayed degradation of hydrogels. Moreover THBA provided high antioxidant activity and a selective antiproliferative effect on cancer cells, PC altered swelling and degradation behaviours, BG exhibited the ability to mineralize in SBF, while the addition of RA enhanced antioxidant and anticancer activities. The obtained results indicated that this hydrogels represent promising multifunctional biomaterials with a wide range of tunable biological properties with great potential for use in tissue engineering.

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