THE BACTERICIDAL DEGRADATION EFFECTIVENESS OF TANNIC ACID-BASED THIN FILMS FOR WOUND CARE

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Introduction

Chitosan and tannic acid have been reported as active compounds with antimicrobial properties. The aim of the experimental study was to detect a potential synergy of antibacterial properties of chitosan and tannic acid against biofilm formation. The concentration of tannic acid released from material after immersion in SBF, SGF (simulated gastric fluid), SIF (simulated intestinal fluid) was determined by spectrophotometric method. Also, microbiological studies were carried out as an inhibition of bacteria growth test and bacteria adhesion observation. The obtained results allow for the selection of the optimal composition of the chitosan and tannic acid films, which will ensure adequate properties and bactericidal effectiveness.

Materials and Methods

Chitosan and tannic acid were purchased from Sigma-Aldrich company (Germany). Chitosan (CTS; DD=78%, Mv=1.8 × 10^6, shrimp derived) and tannic acid (TA; Mv=1701.2 g/mol) were dissolved in 0.1M acetic acid, separately, at a concentration of 2%. Complexes of chitosan and tannic acid were prepared in the weight ratios of 80/20 and 50/50, based on the previous research [1]. Thin films were prepared by solvent evaporation.

Tannic acid release

Tannic acid release was carried out in three different types of conditions - simulated body fluid (SBF; pH=7.4), simulated gastric fluid (SGF; pH=1.2) and simulated intestinal fluid (SIF, pH=6.8) which contained corresponding digestive enzymes. Selected solutions were prepared as traditional media and reference the appropriate conditions for film testing [2]. The total content of polyphenols was determined by the Folin-Ciocalteu method.

Inhibition of bacterial growth

Inhibition of bacterial growth was evaluated by measuring the turbidity of cultured bacterial broth with the tested materials according to McFarland standards [3]. The optical density was measured using The DensiCHEK Plus (BioMerieux, USA) and the readings were made after: 0.5, 2, 4 and 6h. The maximum measuring range of this device is 4 McFarland index /MSi/.

Adhesion of bacteria to the surface

Evaluation of bacterial adhesion to the film surfaces was performed by immersing the specimens in a bacterial solution, drying, covering them by gold, and then assessing with the use of scanning electron microscope (LEO Electron Microscopy Ltd, England). The Staphylococcus aureus strain (ATCC 29213) with the initial concentration of 1x108 CFU/ml was added to 30 ml of the Tryptic Soy Bulion (Merck, Poland) and incubated with specimens at 37°C for 14 days. Before the tests, the films (n=3) were soaked in 70% EtOH for 1 h and then washed in a sterile phosphate buffer solution.

Results and Discussion

The concentration of tannic acid released was detected after films' immersion in three different media – simulated body fluid (SBF; pH=7.4), simulated gastric fluid (SGF; pH=1.2) and simulated intestinal fluid (SIF, pH=6.8). The released concentration was calculated per 1mg of film. Tannic acid was released firstly from the material surface and then after 4h, as a result of material swelling, more tannic acid was released. Higher concentration of tannic acid was noticed for materials based on chitosan and tannic acid in 50/50 ratio than for 80/20, which is similar to the film composition. Both types of films showed constant tannic acid release for 24-72h immersion time in SBF and SIF. In SGF, after 72h, maximum concentration of TA was noticed as a result of total material dissolution. In SBF and SIF conditions the films remained in a solid form. Thereby, the obtained materials are proposed to be applied in contact with body fluids or in intestinal parts (pH around 7). In stomach-like conditions, the proposed materials would totally dissolve, which may be beneficial for drug delivery purposes. The results showed that the released tannic acid concentration depends on the medium's pH as well as on time of contact.

The prepared films were immersed in the bacterial solution of *Staphylococcus aureus* and their effect on the multiplication of bacteria was evaluated (table below).

Time:	*Staphylococcus aureus* stain (ATCC 29213)			
	K	The films composition		
		100CTS	80CTS/20TA	50CTS/50TA
0h	1.5			
2h	2.93	2.14#*	2.51#*	2.85#*
4h	>4	3.47#*	3.86#*	>4
6h	>4	>4	>4	>4

*Statistical analysis was performed between groups and control after 24h and the group, where the statistically significant difference occurred was marked.
max. SD ± 0.05

The prepared films were immersed in a bacterial solution and the adhesion of bacteria to their surface was evaluated (FIG. 1).

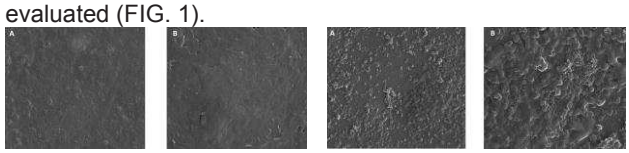

FIG. 1. The SEM images of films based on chitosan (left) and chitosan and tannic acid mixed in 50/50 ratio (right) with bacteria in magnification a) 10 000x b) 25 000x

Conclusions

Higher chitosan content resulted in the increase of bacteria growth inhibition. The burst effect of tannic acid release was noticed, which suggests that the obtained films may be beneficial for the pharmaceutical application. Based on the results, we believe that these chitosan/tannic acid films could be potentially used as wound dressing materials.

References

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