C:H:N:O plasma polymers films for storage/release of antibiotics

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Abstract: The body implant surface is an ideal carrier for antibacterial agents followed by their gradual release with motivation to locally prevent bacterial infections. The plasma polymer coatings are perfect materials for antibiotics immobilization. It is shown in this study that the process parameters both during the film deposition and impregnation steps influence the amount of released antibiotics and thus allow to tailor the antibacterial effect of the resulting coatings.

Keywords: Antibiotics, Antibacterial, Plasma, Polymer, Implant

1. Introduction

Thin films capable to immobilize and gradually release antibacterial agents find application in biomedicine, e.g. as antibacterial coatings on the implants or medical tools. If used in implant surgery, the local treatment allows usage of low amounts of antibacterial agents, which leads in comparison with conventional systemic treatment to the reduction of possible side effects. Methods for immobilization of antibacterial agents often use porous materials like triphosphate ceramics [1] loaded with antibacterial substances, eventually covered by the diffusion barriers used to prolong the release of bioactive substances. As an alternative, an approach that utilizes sputtered plasma polymers for antibiotics immobilization was developed in our group, recently [2]. It was shown that just micrometer thick sputtered C:H:N:O film is able to immobilize the effective dose of ampicillin, even though that this antibiotic has quite high minimum inhibitory concentration. We estimated the release kinetics as Fickian diffusion according to Korsmeyer–Peppas equation [3]:

$$\frac{M_t}{M_\infty} = kt^n, \qquad (1)$$

where M_t is the cumulative release in time t, M_∞ is the total released antibiotics (in infinite time), k is the geometric and structural constant of the sample and finally n is the variable determining the release type. Furthermore, we showed that the amount of released ampicillin is proportional to the plasma polymer thickness. The possibility to introduce the diffusion barrier and regulate the release of antibacterial agents was also successfully tested.

In spite of these results that showed great potential of plasma polymers for effective antibiotics immobilization and their gradual release, certain questions remain still open - how the amount of antibiotics depends on the chemical composition of the film, how the concentration used for impregnation influences the total immobilized amount or if the amount of released antibiotics is dependent on the used substrate material. The main aim of this study is to find answers to these questions.

2. Methods

The fabrication method consists of three subsequent steps followed by the release measurements as it is schematically shown in Fig. 1.

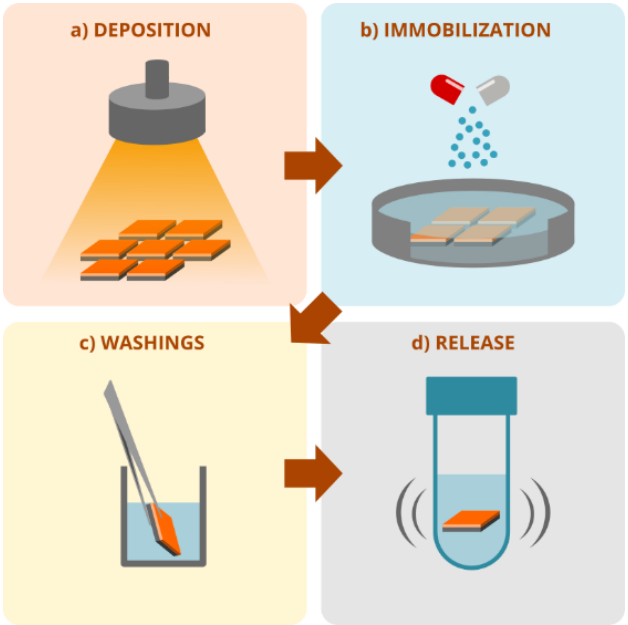

Fig. 1. Experimental method: a) Plasma polymer deposition, b) antibiotics immobilization c) washing steps d) antibiotics release followed by LC-MS measurements.

These steps are a) deposition of plasma polymer films by means of magnetron sputtering of nylon 6,6 target, b) their impregnation by antibiotics (ampicillin, ciprofloxacin in

this study) and c) washing of leftover antibiotics sediments – so that the antibiotics remain only inside the film. Then d) the samples were put into purified water and measurements by LC-MS/MS chromatography were performed in given times in order to quantify the time evolution of antibiotics release from the coatings. The release of antibiotics was optimally calibrated by a row of decimally diluted ampicillin and by using umbelliferone as an internal standard. If calibrated, the absolute amounts of released antibiotics are shown, if not the relative scale normed on the highest value is used.

The experimental conditions fixed in this study are given in Table 1. These deposition parameters represent the optimized conditions with respect to the film stability and experiment repeatability. In addition, it is worth noting that as shown in the previous work [2] the amount of immobilized antibiotics is proportional to the film thickness over 3 orders of magnitude. Because of this, the film thickness is not considered as key parameter in this study. Substrate lateral sizes were square 1.5x1.5 cm.

Table 1. The fixed experimental conditions

Variable	Value	Note
Target material	nylon 6,6	3-inch, 3 mm thick
Working gas	Argon	99.999% purity
Pressure	3 Pa	10^{-2} Pa base pressure
Substrate Distance	6 cm	chamber diameter 0.5m
Power	40 W	RF power 13,56 MHz
Impreg. temperature	25°C	room temperature
Washing steps	6x beaker	10 s shaking in 25 ml beaker

Fig. 2. Finding the optimum number of washing steps by methylene blue impregnation and set of washings.

The important step in the samples preparation is their washing that assures the complete removal of antibiotics dried on the surface of the coatings. The number of washing steps was determined by the experiment in which the substrates were impregnated with very strong dye (methylene blue) as shown in Fig. 2. Then the series of 10 s long washes by sample shaking in the beaker with isopropanol were performed. This was repeated with 10 substrates and the colorimetry of liquid was performed. It is found that the complete removal of the dye was achieved after 5 washing steps and thus for all further experiments 5+1 washing cycles were done.

Table 2. The variable experimental conditions. Diversions from default values are written in graph captions.

Variable	Default	Variation motivation
Substrate	Si	Substrate independency of process
Working gas	Argon	Forming functional groups
ATB Concentration	1 g/l	Tailoring amount of released ATB
Impregnation time	30 min	Tailoring amount of released ATB
Washing liquid	Isopropanol	Reproducibility enhancement

The variables chosen in the frame of this study are shown in Table 2. Each fabrication step in Fig.1 (a,b,c) adds free parameters of the experiment that needs to be optimized, therefore the results are divided into three sections.

3. Results and Discussion

3.1 Tailoring the deposition

The growth of plasma polymer depends on the working conditions of PECVD. It can be presumed that the antibiotics immobilization is the effect of the film, not substrate. In order to validate this assumption, we have used two different nonporous substrates - Si wafer and PEEK. We have deposited 1.8 µm thick films simultaneously onto both substrates. As shown in Fig. 3 the amount of antibiotics released from C:H:N:O films, as well as the release kinetics of stored antibiotics, were within the standard deviation the same independently of the substrate. In other words, these data show the possibility to use the method for virtually any substrate material.

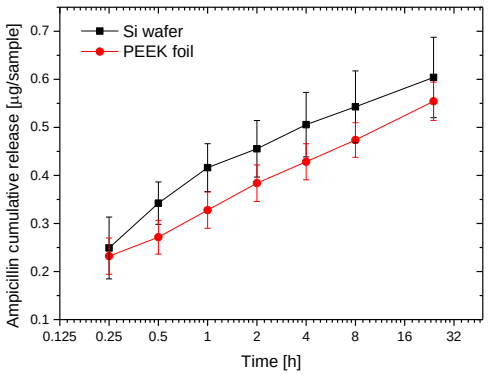

Fig. 3. Ampicillin cumulative release from 1.8 µm thick film related to the used substrate for deposition.

Since the used antibiotics (ampicillin, ciprofloxacin) possess the functional amino and/or carboxy groups, the very interesting parameter to tailor is the chemical composition of the produced films and in this way to control the immobilization process. In order to evaluate the impact of the chemical composition on the ability of produced coatings to store and release the antibiotics, experiments with different argon-based working gas mixtures were performed. The corresponding measured release curves are presented in Fig. 4. It can be seen that the amount of released antibiotics indeed strongly depends on the presence of a reactive gas during the magnetron sputtering of nylon 6,6 target. It is found that only a small addition of nitrogen significantly increases the amount of released ciprofloxacin. In contrast, the presence of hydrogen in the working gas mixture reliably spoils the

soaking ability of produced plasma polymer. This effect is probably caused by the termination of plasma polymer radicals during the polymerization by hydrogen radicals. Nevertheless, it is clear, that the varying of the chemical composition of C:H:N:O films can lead to a significant (5x) change in the released antibiotics mass and with it connected bactericidal potency of the coatings.

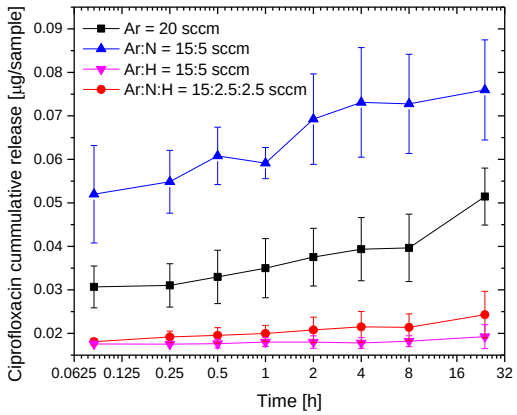

Fig. 4. The release of ciprofloxacin from the coatings prepared in different working gas mixtures. Ciprofloxacin concentration 2 g/l was used for 200 nm thick films impregnation.

3.2 Tailoring the impregnation

There are three basic free parameters with respect to the impregnation step: i) solution temperature ii) immersion time and iii) concentration of antibiotics in the solution used for the impregnation of plasma polymer films. The temperature of the solution was not varied in this study.

Concerning the immersion time, the results presented in Fig. 5 show that the amount of released antibiotics does not significantly change with the impregnation time if changed from 10 up to 30 minutes. This result is in the perfect match with our previous study [2] where the ellipsometry measurements showed that the major swelling of the films in a water base solution is realized within the first 10 minutes. Any longer immobilization time makes no difference.

The dependence of the amount of released antibiotics on the antibiotic concentration in the solution used for the impregnation of C:H:N:O films can point out to the mechanism of antibiotics immobilization that may proceed either via diffusion or by the functional group attachment. Expectedly, the antibiotics amount was found to rise with the used concentration of antibiotics in the solution. However, as can be seen in Fig. 6, the increase of the amount of released antibiotics is not linearly proportional to the antibiotic concentration used for the samples impregnation. The rise of bath concentration from 1 g/l to 5 g/l increases the amount of released ampicillin only twice. The reduction from 1 g/l to 0.2 g/l then reduces the amount of released antibiotics only 3 times. Based, on these results, it is possible to state that the immobilization of antibiotics in the C:H:N:O plasma polymer films is a mix between diffusion (the release would be proportional to the concentration) and functional group attachment (the release would be constant for all concentrations).

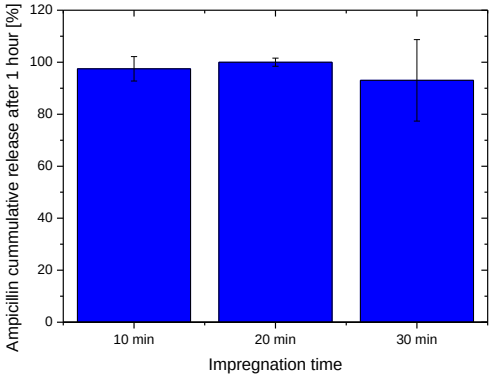

Fig. 5. Ampicillin relative release from the magnetron sputtered C:H:N:O 200 nm thick films in the dependence on impregnation time.

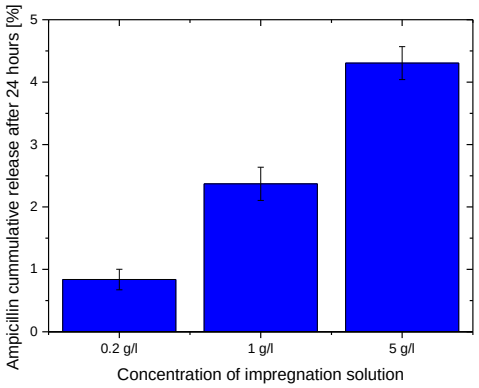

Fig. 6. Dependence of ampicillin release on the ampicillin concentrations used for the impregnation of 200 nm thick films.

3.3 Tailoring the washing

As already mentioned, the washing of the impregnated samples is the last and crucial preparation step, because it ensures that only antibiotics stored in the film volume will remain in the sample. Three liquids were compared in order to wash ciprofloxacin sediments. In the case of (i) water and (ii) 5 millimolar concentrated HCl water-based solution, almost no antibiotics were released – the concentrations are on the level of controls (ctrl), i.e. substrates without C:H:N:O films. The drying of samples was done in this particular experiments by using soaking paper, so probably antibiotics were dragged out from the film by capillary forces. Therefore, it is necessary during the impregnation step to use the liquid with low surface tension like alcohols. This can be seen in Fig. 7 showing that only isopropyl alcohol used for washing can remove sediments from the surface of the coatings, but has no impact on the antibiotics stored in the plasma polymer bulk.

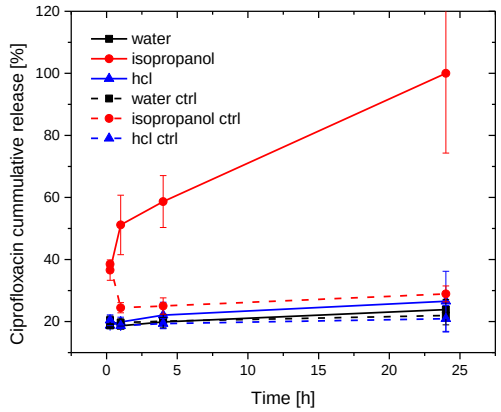

Fig. 7. Impact of the different liquids used for samples washing on the release of ciprofloxacin. The 200 nm thick films, prepared in 50% of Ar and 50% of N process gas, were impregnated by 5 g/l concentrated ciprofloxacin solution.

4. Conclusion

At recent papers [2,4-6] we demonstrated the ability of plasma polymers to immobilize either the antibiotics and/or metallic nanoparticles. Those papers together with this short contribution give the complete manual for the immobilization of the drugs and nanoparticles into the C:H:N:O plasma polymers. If those will be combined, the dual phase antibacterial effect would be obtained.

Finally, it is worth noting that in this contribution only two different antibiotics - ampicillin and ciprofloxacin – were immobilized into C:H:N:O plasma polymers with different chemical composition. The results show, that the question of ideal combination drug-plasma polymer is still opened and worth for further investigation.

5. Acknowledgment

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6. References

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