A new concept for controlled drug release devices based on enzyme activity in wound fluids

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Introduction

The aim of this study is the development of a controlled drug release device for wound treatment of infected or chronic wounds.

Controlled drug release occurs when a polymer is combined with a drug or other active agents in such a way that the active agent is released from the material in a pre-designed way (Brannon-Peppas et al.1997). Existing antimicrobial wound dressings suffer from the drawback that the release of the antimicrobial agent is relatively unresponsive to the degree of infection of the wound requiring extensive release of drugs potentially causing side-effects and/or ultimately promoting the problem of resistances. The first step of our work is a fundamental understanding of the complex and dynamic process of normal wound healing or a disordered healing behavior in case of infection. In this study we identified bio-molecules secreted by microorganism or the human immune response system which can be used as triggers for a controlled drug release.

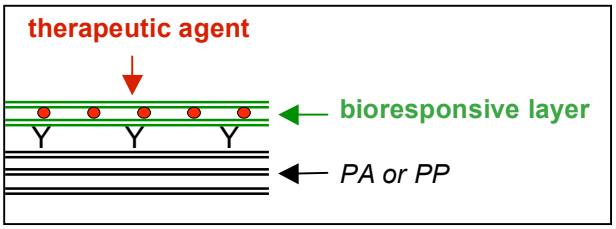

Figure 1: Schematic description of drug release device

For the realization of a controlled drug release device, 2 different layers are needed: a carrier layer which is needed as scaffold and a bio-responsive layer which is responsible for the interaction with the wound environment. The schematic description of the system is shown in Figure 1.

The wound contacting layer or so called “bioresponsive layer” consists of different proteins or polysaccharides, for example peptidoglycan or collagen, comprising incorporated therapeutic agents. Only in case of microbial infection, the drug delivery system is stimulated by bio-molecules present in wounds. As a consequence, the structure of the bioresponsive layer is modified resulting in a release of the therapeutic agent.

As a scaffold layer for the bio-responsive material, functionalized polypropylene and polyamide was used. For the construction of this system, 2 main steps were required: an activation (see Figure 2) and a coating step (see Figure 3). Activation was carried out to incorporate functional anchor-groups into the fiber. To achieve this high amount of functional groups, enzymatic surface hydrolysis in case of polyamide-fibers and hydroxylation in case of polypropylene-fibers was carried out.

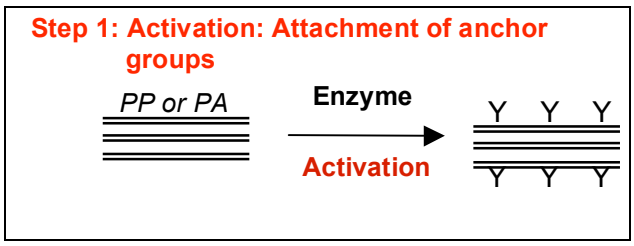

Figure 2: Enzymatic surface hydrolysis or hydroxylation for incorporation of anchor groups

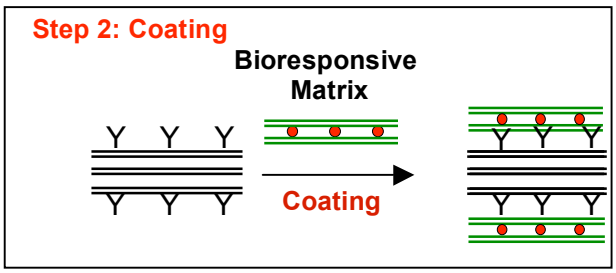

Figure 3: Coating of the carrier layer

To adapt the bio-responsive layer to real conditions in wounds (focused on Decubitus Ulcer wounds) different biochemical analysis were carried out. As most promising enzymes in wounds, Gelatinase and Cathespin G activities were measured (Yager et al. 1999). The drug release system was then constructed using substrates of these two enzymes.

Materials and Methods

Carrier layer

For the functionalisation of PP, monooxygenases were used while PA films were enzymatically hydrolysed with amidases on the surface. As we have previously described (Guebitz and Cavaco-Paulo et al. 2008), these treatments result in a substantial increase of surface hydrophilicity allowing the coating with proteins.

Enzyme Screening

Collagenase activity was measured using EnzCheck gelatinase/collagenase assay kit (E-12055, Molecular Probes). Cathepsin G activity in wounds was determined using an assays already described in literature (Trengove et al. 1999).

Additionally, zymogramm and western blot analysis was carried out to verify Collagenase activity.

Bioresponsive layer

The bio-responsive layer was composed of two different parts. The upper part was a blend of agarose and gelatine while the lower part comprised agarose with a substrate of Cathepsin G. The idea behind this set up was the analysis of a possible influence of Collagenase on the diffusion of Cathepsin G through the matrix. With the aid of Collagenase which is able to degrade the bio-responsive layer, we hoped to find an influence on the diffusion of Cathepsin G through the upper layer. This was verified by measuring the conversion of the used Cathespin G substrate.

Top layer

The upper layer consisted of an agarose-gelatine blend. For this layer 70 µL of a 3% agarose-solution (in collagenase buffer) was mixed with 30µL of a 25% gelatine-solution (in Collagenase buffer).

Bottom layer

In the layer below, Succinyl-Ala-Ala-Pro-Phe-p-Nitroanilide (SAAPPN) a substrate for Cathepsin G was selected and immobilized with agarose. For this layer 60 µL of a 3% agarose-solution (in HEPES buffer) were mixed with 5 µL of SAAPPN (43 mM in DMSO + HEPES-buffer).

For the experiment, three different enzyme ratios were used. The experiment was carried out in triplicate.

Results and Discussion

Carrier layer

Successful introduction of hydroxyl-groups in PP-fibers was affirmed with XPS measurement (see Figure 4). Therefore 3 different samples were measured. Sample B was normally treated with monooxygenase-solution, sample CO was inhibited with carbon monoxide and a reference fiber R was not treated with enzyme. In Figure 4 the received chromatogram of the different samples is illustrated. A clear change can be observed between non-and treated samples. In particular (Table 2), the reference fiber contains mainly aliphatic hydrocarbon with a binding energy of 258 eV as expected for PP. The enzymatically modified fiber has the smallest portion on aliphatic hydrocarbons. After enzyme treatment, two more species appear. Their higher binding energy is typical for binding states with higher oxidation. The signal at around 286.6 eV can be assigned to carbonyl-carbon and the signal at 288.4 eV to carbon from carboxylic acids, esters or amides.

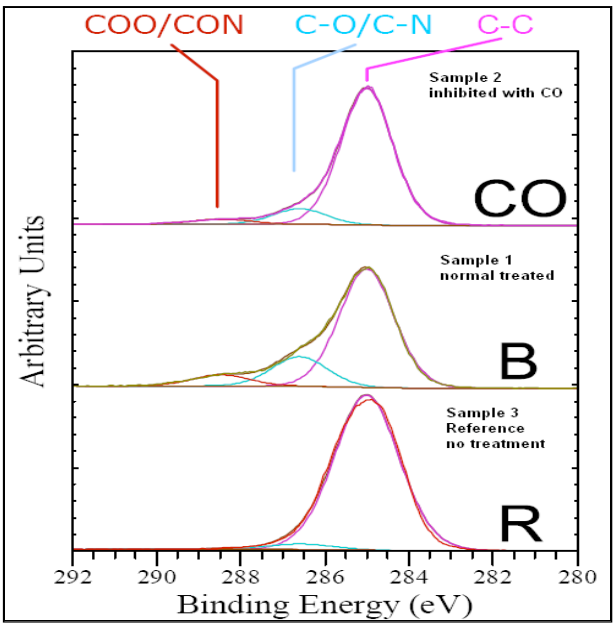

Figure 4: XPS chromatogram

Sample	C 1s%		
	ali	CO/CN	COO/CON
	%	%	%
Blank	96.2	3.4	0.4
Enzyme	74.0	18.6	7.3
Enzyme +CO	86.5	9.9	3.6

Table 2: Detailed results for XPS measurement, concerning aliphatic C-atoms, CO/CN and COO/CON

Bioresponsive layer

The degradation of the gelatine by the Collagenase clearly improved the transfer of Cathepsin G through layer 1 and resulted in a much better turnover of the substrate, resulting in a colour reaction. As shown in Table 1, addition of different concentrations of Collagenase (10U and 250 U) significantly (5 fold) improved the degradation of SAAPPN by Cathepsin G compared to the reaction without any Collagenase. The results presented in Table 2 were calculated as degradation rates ([ΔAbs/h]).

Sample mixture.	Used enzymes	
	Cathepsin	Collagenase
	U/mL	U/mL
Blank	0.00	0
Preparation 1	1.25	0
Preparation 2	1.25	250
Preparation 3	1.25	10

Table 1: Used enzyme ratios

Sample mixture	Degradation rate	
	ΔAbs/h	ΔAbs/h
	Copolymer	Agarose
Blank	0,000	0,000
Preparation 1	0,010	0,035
Preparation 2	**0,064**	**0,038**
Preparation 3	0,029	0,033

Table 2: Results of diffusion experiment

Conclusion and Outlook

Here we have presented a new model system for a controlled drug release device consisting of an enzymatically activated carrier layer and a bioresponsice polymer. It is well known that Collagenases play a crucial role in the complex process of wound healing while Cathepsin G is essential for an appropriate immune response. In case of disordered wound healing or infection, elevated levels of these two enzymes are described. For this reason we focused our work on specific biopolymers, being applicable substrates for exact these enzymes. Therefore we have chosen gelatine and a p-nitroanilide incorporated into an agarose matrix. Using different amounts and ratios of these two enzymes, we investigated the effect of Collagenase in this drug release system. The presence of Collagenase resulted in a much better turnover rate of the Cathepsin G substrate, which can be explained with an eased diffusion of Cathepsin G through the matrix.

In a further step we will try to adapt the sensitivity of our model system to "real" conditions in wounds concerning enzyme levels and possible interactions. Additionally we will replace the Cathepsin G substrate by real drugs, for example PHMB and Octenidin.

References

D. R. Yager et al. (1999) *The proteolytic environment of chronic wounds.* Wound Repair and Regeneration 7(6), 433-41

L. Brannon-Peppas et al. (1997) *Polymers in Controlled Drug Delivery.* Medical Plastics and Biomaterials Nov 1997 34-45

G. M. Guebitz and A. Cavaco-Paulo et al. 2008. *Enzymes go big: surface hydrolysis and functionalisation of synthetic polymers*. Trends in Biotechnology 26, 32-38.

Trengove et al. 1999 *Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors.* Wound Repair and Regneration 7(6) 442-452