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Polyalkylcyanoacrylates as *in situ* formed diffusion barriers in multimaterial drug carriers

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Abstract

Polymeric hydrogels typically release their drug payload rapidly due to their high water content and the diffusivity for drug molecules. This study proposes a multimaterial system to sustain the release by covering the hydrogel with a poly(alkyl-2-cyanoacrylate) [PACA]-based film, which should be formed by an *in situ* polymerization on the hydrogel surface initiated upon contact with water. A series of PACA-hydrogel hybrid systems with increasing PACA side chain hydrophobicity was prepared using physically crosslinked alginate films and hydrophilic diclofenac sodium as model hydrogel/drug system. Successful synthesis of PACA at the hydrogel surface was confirmed and the PACA layer was identified to be most homogeneous for poly(*n*-butyl-2-cyanoacrylate) on both the micro- and nanolevel. At the same time, the diclofenac release from the hybrid systems was substantially sustained from ~ 1 d for unmodified hydrogels up to >14 d depending on the type of

PACA employed as diffusion barrier. Overall, *in situ* polymerized PACA films on hydrogels may be widely applicable to various hydrogel matrices, different matrix sizes as well as more complex shaped hydrogel carriers.

Keywords: multimaterial drug carrier, poly(alkyl-2-cyanoacrylate), alginate, hydrogel, polymer coating

Introduction

For sustained drug delivery, high demands are to be made on carriers in order to ideally fulfill the envisioned tasks and provide the required carrier functionalities such as control of drug release rates or degradability. It is generally accepted that due to the variety of possible polymer properties depending, e.g., on their composition, architecture, and morphology [1], polymers may be the most feasible and thus most commonly employed class of materials to embed and release drugs. In order to establish combinations of properties and functions that fit the application [2], the tools of macromolecular chemistry can be used to tailor for instance their rate of hydrolysis in aqueous environment in case of degradable materials [3], their mechanical properties, or their capability to interact with water. Still, it may be hardly possible to integrate extremely different features such hydrophobicity and hydrophilicity in a homogeneous material, if no phase separation into microdomains is desired [4]. Therefore, multimaterial systems may be employed, where different functionalities are provided by the different materials that are combined e.g., by dispersion [5] or layering.

In addition to the capability to release embedded substances in a sustained manner, an important prerequisite for drug carriers is the stability of bioactive molecules in the polymer, especially when it comes to highly sensitive hydrophilic molecules such as proteins [6]. Hydrogels, in a simplified description, may be mimicking a physiological environment for such hydrophilic molecules due to

their high water content and may additionally match the mechanical properties of soft tissue. Therefore, hydrogels based on purely synthetic polymers [6] or on modified biopolymers like polysaccharides [7] [8] [9] or proteins [10] are of steadily rising relevance in drug delivery, cell delivery, and tissue engineering. While a high water content may support the stability of sensitive drugs compared to more hydrophobic polymer matrices, its drawback may be high drug diffusivity. This may result in short drug release periods [11] if the hydrodynamic diameter of the drug is not larger than the mesh sizes of the hydrogel polymer network or if no additional mechanisms that limit drug diffusion such as covalent/non-covalent binding are implemented in the system.

Covalent binding effectively excludes undesired drug diffusion, while the release may be enabled by hydrolytically or enzymatically cleavable linkers [12]. In addition to the complex design and preparation of such systems, the drug coupling to the hydrogel demands suitable functional groups. Covalent functionalization may disadvantageously be associated with residual coupling reagents, immunotoxicological concerns if bound drug acts as repetitive epitopes [13], and the need to determine the fate and toxicity of drug-hydrogel conjugates and their numerous degradation products. Non-covalent binding may be, e.g., realized by host guest complexes based on cyclodextrins [14]. Additionally, highly specific antigen-antibody [15] or protein-ligand [16] interactions were employed to build additional netpoints in covalently crosslinked hydrogels in order to reversibly reduce the mesh sizes and entrap large hydrophilic components until an excess soluble antigen or ligand serves as release stimulus. Ionic interactions with low specificity [17], preferentially as multivalent binding to increased binding strength, can be realized only for substances that bear suitable charged groups.

Since diffusivity inside the hydrogels does not account for drug release, an alternative concept may be to focus only on the hydrogel surface and provide it with a physical barrier that limits drug diffusion out of the carrier [11], i.e., to establish a multimaterial system. For polyanionic hydrogel particles, multivalent adsorption of polycations reduced the pore sizes and decreased the release

rates at least for large substances [18] [19] [20]. Besides ionic interactions, also multivalent hydrophobic interaction as for silk fibroin upon induced formation of hydrophobic crystalline domains sustained the release of large hydrophilic molecules [21]. Still, based on the relative hydrophilicity of these coating materials, they sustain drug release by acting as a molecular mesh, which may not be effective if the molecular weight of the drug is reduced.

In order to control also the release of small hydrophilic molecules, their diffusion needs to be inhibited by applying a homogeneous and more hydrophobic diffusion barrier, as has been realized for hydrogel particles with poly[(methacrylic acid)-*co*-(methyl methacrylate)] by spray drying [22] or with poly[(*rac*-lactide)-*co*-glycolide] or poly(*rac*-lactide)-*b*-poly(ethylene glycol) by multi-step emulsion processes [23] [24]. Such processes were limited to systems of particulate shape and in some cases involved intermediate drying, i.e., conditions possibly resulting in a loss of some of the proposed advantageous features of hydrogels in terms of drug stability.

Therefore, a multimaterial-based strategy to sustain the release of hydrophilic components from hydrogels should be provided that in principle may be applicable to various hydrogel shapes and neither demands intermediate drying nor prior covalent functionalization of drugs or matrix polymers. This concept should base on a hydrophobic polymer that is placed as a layer on top of the hydrogel, limits water penetration, and efficiently acts as diffusion barrier for hydrophilic drugs. For being in principle applicable to different hydrogel matrices and shapes, the deposition/formation of the coating should be instantly triggered at the hydrogel surface. Alkylcyanoacrylates are known to undergo rapid anionic polymerization to polyalkylcyanoacrylates (PACA) upon contact with water [25], have excellent film forming properties [26], and are biomedically used as glues for wound closure. Additionally, PACA were explored as nanoparticulate drug carriers [25] and recently studied for their thermal and mechanical properties depending on the PACA side chain type [26] and the interaction with hydrophilic polymers [27] [28]. PACA are referred to be biocompatible and degradable with a number of different proposed pathways, out of which the hydrolysis of the ester

side chains appears to be most prominent *in vivo* [25]. In addition to nanoparticulate drug carrier systems [25], cyanoacrylates have e.g. been used to introduce drug as powder into implantable, dry-state biopolymer foams and sustain its release over several weeks [29].

It was hypothesized that cyanoacrylate polymerization to PACA may be selectively initiated *in situ* by water at the surface of hydrogels and subsequently acting as an efficient diffusion barrier particularly for small molecules. Therefore, the general capability to form the proposed multimaterial system should be evaluated including the effects of cyanoacrylate side chain type on the coating properties and the release of a highly diffusive small molecule, diclofenac sodium, as a model drug. As hydrogel, alginate with physical crosslinking by calcium ions should be explored as an example of a well-established material for drug carriers.

Materials and methods

Materials

Sodium alginate (#72138, Sigma, Taufkirchen, Germany) and anhydrous calcium chloride (Scharlau, Barcelona, Spain) were used for hydrogel preparation. Alkyl-2-cyanoacrylate monomer compositions used to synthesize the respective polymers were Loctite 491 for poly(methyl-2-cyanoacrylate) [PMCA], Sicomet 40 for poly(ethyl-2-cyanoacrylate) [PECA], Loctite 4081 for poly(methoxyethyl-2-cyanoacrylate) [PMECA], and Sicomet 6000 for poly(*n*-butyl-2-cyanoacrylate) [PBCA] (all Henkel, Düsseldorf, Germany). Solvents were Miglyol 812 (Triglycerides of caprylic/capric acid; Sasol, Hamburg, Germany), *n*-hexane (Promochem, Wesel, Germany), and *n*-heptane (Sigma) for cyanoacrylates as well as methanol and acetonitrile (LiChrosolv, Merck, Darmstadt, Germany) for HPLC.

Preparation of alginate hydrogel films

Sodium alginate was dissolved at 3 wt.% in water. Alginate films of defined sample volume and geometry were prepared using molds that consisted of i) bottom glass plates, ii) filter paper as support for crosslinking and subsequent sample handling (No. 292, Sartorius, Göttingen, Germany), iii) teflon spacers of 0.25 mm or 1 mm thickness with circular cavities that were filled with the alginate solution (∅ 14 mm), iv) top glass plates, and v) clamps for assembling the device. The device was placed in a closed container in such a way that only the filter paper was immersed into a 10 wt.% $CaCl_2$ solution. By support of the filter paper, calcium ions were allowed to diffuse into the mold at room temperature for 24 h and resulted in physical crosslinking of alginate hydrogel films. After disassembly of the mold, the ∅ 14 mm samples on the paper support were separated by concentrically cutting the filter paper with a ∅ 18 mm punch cutter.

Creation of multimaterial system with PACA layer

Ten microliters of the respective cyanoacrylate monomer were dissolved in 3 mL of *n*-hexane, *n*-heptane, or Miglyol 812 and used to synthesize PACA by covering the alginate samples at room temperature for 6 h. In order to avoid alterations of the cyanoacrylate concentration by solvent evaporation and additionally ensure defined exposure of the top surface, coating was performed in inverted screw cap vials as well sealed reaction vessels (Suppl. Fig. 1). The selected vessels had orifice diameters that corresponded to the diameter of the gel samples, while the paper support was clamped in the lids of the vials and hold the gel film in place. For recovering the multimaterial system, the vials were turned so that the coating solution was removed from the sample, followed by several washing steps with 1 mL of the respective pure solvent, and rapid evaporation of the washing medium before further use. Intermediate storage of coated and uncoated samples (not in case of drug-loaded samples) before analysis was performed in 1 wt.% $CaCl_2$ solution.

ATR-FTIR

For detection of PACA formation, Fourier transform infrared spectroscopy (FTIR) was performed in the Attenuated total reflectance (ATR) mode on a Nicolet Magna IR 550 spectrometer (Thermo Scientific, Dreieich, Germany) by placing the hydrogels into the sample holder of a horizontal single-reflection diamond ATR unit (DuraSampl IR, Resultec, Illerkirchberg, Germany).

Characterization of surface properties

The contact angle with aqueous medium was determined for uncoated, PACA coated, and only solvent treated samples at room temperature using droplet shape analysis in the captive bubble mode with a DSA 100 instrument (Krüss, Hamburg, Germany). Samples were inverted and placed into 1 wt.% $CaCl_2$ solution as measurement medium while being contacted with an air bubble, which was either decreased in size (advancing contact angle, ACA) or increased in size (receding contact angle, RCA). The difference between ACA and RCA was determined as hysteresis. Artifacts due to surface active impurities were excluded by measuring the surface tension of the medium before and after analysis by a K12 Processor Tensiometer (Kruess).

Optical profilometry was additionally employed to analyze the surface topology of hydrogels according to DIN EN ISO 13565 on a MicroProf instrument (FRT, Bergisch Gladbach, Germany). In order to limit the formation of artifacts by drying of samples during analysis at ambient conditions, a 1 wt.% $CaCl_2$ solution was placed around the hydrogel films while keeping the surface free of this solution. Virtual optical sections of the surface were acquired using the FRT Aquire and FRT Mark III 3.7 software.

For atomic force microscopy (AFM) on a VeccoDi Multimode V instrument (Veeco, Tucson, AZ, USA) in the tapping mode at room temperature with 5x5 µm scan sizes, samples of 1 mm thickness were used. The sample support was fixed in the chamber, which was subsequently filled with 1 wt.% $CaCl_2$. The root mean square average roughness (R_q) was calculated from the deviation of the height at a specific sample point Z_i compared to the mean surface height $\bar{Z}$ for all data points i of the sample area according to equation 1.

$$R_q = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Z_i - \bar{Z})^2} \quad (1)$$

Scanning electron microscopy (SEM) analysis of PACA required prior removal of the hydrogel by dissolution at room temperature in 15 mL of calcium-free aqueous medium on a horizontal shaker at 85 rpm for 48 h. The thin PACA films were isolated from the obtained alginate solution, freeze dried, sputtered with Pt/Pd, and analyzed with a Zeiss Gemini Supra 40VP (Carl Zeiss NTS, Oberkochen, Germany).

Drug loading and quantification of release

Diclofenac sodium was loaded in the hydrogels by dissolving sodium alginate in a 10 $mg \cdot ml^{-1}$ aqueous drug solution and preparing hydrogel films as reported above. Uncoated and coated hydrogels as well as drug powder were evaluated for diclofenac release/dissolution in 5 mL phosphate buffer saline (PBS; 5.8 mM Na_2HPO_4, 5.8 mM NaH_2PO_4, 150 mM NaCl; adjusted with NaOH to pH 7.4) at 37 °C in a horizontal shaker (60 rpm, Certomat IS, Sartorius Stedim Systems, Guxhagen, Germany). In the linear tubular polypropylene test vessels with two openings, samples were mounted at the bottom with their coated side facing the vessel lumen using screw caps. Samples were subsequently covered with PBS and sampling was performed from the top opening of the vessel by withdrawing 1 mL of release medium and replacing it with fresh PBS.

Diclofenac quantification by high performance liquid chromatography (HPLC) analysis was performed with a 125-4 RP-18 column (LiChroCART® 125-4, LiChrospher® 100, 5 µm; Merck, Darmstadt, Germany) on an Agilent 1200 series HPLC (Agilent Tech¬nologies Deutschland GmbH, Böblingen, Germany) with UV detection at 275 nm. In an isocratic method, the mobile phase consisted of 10:35:55 acetonitrile/ methanol/ 20 mM sodium acetate buffer pH 5.6 and was used at a flow rate of 1.2 $mL \cdot min^{-1}$.

Results and discussion

Creation of a multimaterial system with an *in situ* polymerized PACA layer

Since cyanoacrylates rapidly polymerize in the presence of traces of water and have excellent film forming properties, the concept of the proposed multimaterial system bases on using water as generally present in hydrogels to selectively initiate the PACA synthesis at the hydrogel surface (Fig. 1). Nucleophilic groups of the hydrogel-forming polymer, in this case hydroxyl moieties, may theoretically also initiate cyanoacrylate polymerization, but this appears unlikely based on the much lower content and reactivity compared to excessive water. At the surface, a PACA film of uniform thickness may be formed that could also adapt to possible curvatures of the hydrogel, which may not be similarly achieved for other coating techniques. Here water molecules or, more specifically, hydroxide ions from the dissociation of water are the required component to initiate anionic polymerization, thus ideally neither the type of polymer serving as hydrogel matrix nor the hydrogel shape should be of major influence on the cyanoacrylate polymerization. Therefore, for a systematic study, a model system was selected that consisted of planar samples as best suited for several techniques of surface characterization to proof the concept. Sodium alginate served as gel-forming matrix polymer, since it is well established as drug carrier material and contains physical crosslinks, which on the one hand enabled isolation of the coating for experimental characterization and on the

other hand represents a typical example of hydrogel systems that are faced with short release periods [30].

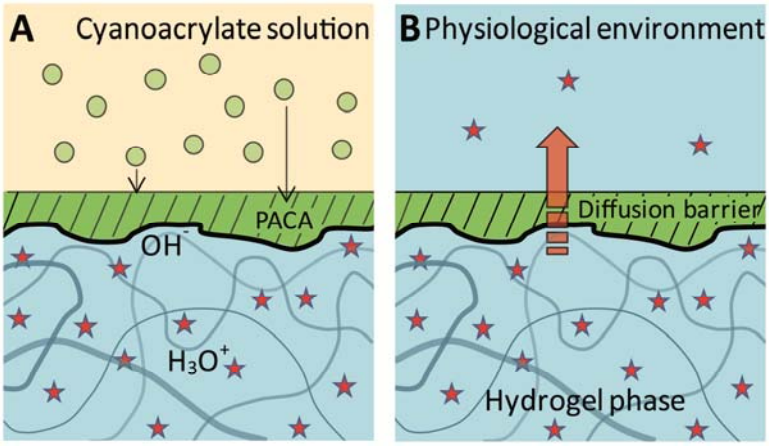

Fig. 1: Creation of multimaterial drug release system. (A) *In situ* anionic polymerization of cyanoacrylate monomers (green dots) to PACA at the hydrogel surface as initiated at the interface of hydrogel and coating solution by water from the hydrogel. (B) After transfer in physiological environment, the PACA layer acts as diffusion barrier to drug molecules (red stars) embedded in the hydrogel.

The consumption and extraction of water from the hydrogel for initiation of cyanoacrylate polymerization is related to the extent of parallel initiation of their anionic polymerization. In order to reduce massive parallel initiation, a dilution of cyanoacrylate monomers was required and the employed solvent was expected to be an important experimental parameter to affect the morphology of the PACA layer [26]. Without suitable dilution, massive nucleation would result in strong water extraction and perturbation of the hydrogel as observed in preliminary studies for high cyanoacrylate concentrations (25 vol.% *n*-butyl-2-cyanoacrylate in hexane; Suppl. Fig. 2). A number of preconditions had to be fulfilled by solvents to be used for *in situ* PACA synthesis, namely, i) free miscibility with the cyanoacrylate monomers, ii) no induction of cyanoacrylate polymerization, iii) immiscibility with water to exclude solvent-mediated water extraction from the hydrogel causing hydrogel shrinkage and cyanoacrylate polymerization in the medium rather than at

the hydrogel surface, and iv) poor solvent strength for PACA to enable PACA deposition at the hydrogel. Based on these requirements, hexane, heptane, and Miglyol 812 (caprylic/capric acid triglyceride with clinical use as carrier in intravenous injections) were selected as candidate solvents [31] [32] [26].

Since an uncontrolled alteration of the hydrogel shape during synthesis of the PACA layer was undesired, the cyanoacrylate monomer concentration has been stepwise reduced until perturbations of the hydrogel shape were no longer visually detectable, which was the case at cyanoacrylate concentrations $\leq$ 1 vol.% in the cyanoacrylate solution. Under such conditions, it first had to be qualitatively shown that PACA synthesis at all occurred at the hydrogel surface. Therefore, hydrogel samples have been analyzed by ATR-FTIR, where IR light has a penetration depth of only few micrometers thus making it possible to detect also thin layers of PACA on the hydrogel bulk. When exemplarily comparing spectra from hydrogels before and after PBCA coating with those of pure PBCA (Fig. 2), a perfect agreement of the characteristic peaks of PBCA such as the carbonyl (C=O) stretching absorption at ~1750 cm^{-1} or the ester (C-O) band at ~1250 cm^{-1} were obvious for pure PBCA and the PBCA coated hydrogel. Thus, the proposed concept of coating by *in situ* polymerization at the hydrogel surface could be qualitatively proven. However, it should be noted that the peak at 1640 cm^{-1} corresponding to the bending vibration of water was also present in the spectrum of the coated hydrogels, suggesting that the PACA layer was either very thin so that the underlying hydrogel bulk was detected or that the PACA film was imperfect or broke when pressing the sample in the ATR unit. Therefore, the surface properties of the coated hydrogels needed to be characterized in more detail.

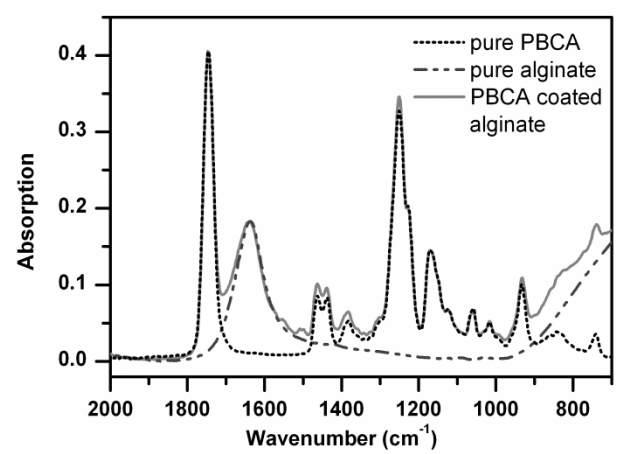

Fig. 2: Proof of PACA deposition on the hydrogel surface by ATR-FTIR analysis as exemplarily shown for PBCA coated samples.

Solvent effects on coating properties

In order to efficiently act as a diffusion barrier in future drug release applications, the PACA layer on top of hydrogels should ideally be free of voids. When recalling the requirements for solvents properties and considering potentially competitive contributions to different processes, the effects of a specific solvent on the properties of formed PACA layers appear hardly predictable. For instance, in addition to the solubility and miscability, also the viscosity of the solvent is much higher for Miglyol 812 compared to hexane and heptane (~30 mPa·s vs. ~0.3-0.4 mPa·s at 20 °C), and may affect polymerization kinetics by the diffusivity and thus the availability of monomers for reaction at the phase boundary. Due to the hydrophobic nature of PACA, the deposition at the hydrogel surface should result in an alteration of surface properties, which were determined by contact angle measurements. As illustrated in Table 1, a clear shift from low angles for uncoated hydrogels (hydrophilic surface) to larger angles for PBCA coated hydrogels (hydrophobic surface) was observed. In control experiments, the exposure to pure solvents could be excluded as potential alternative causes of increased contact angles by water extraction or morphological rearrangements of the hydrogel surface. Despite washing of Miglyol 812 based samples with hexane after PBCA

polymerization, the oil remained entrapped in the coating as also visible by very high contact angles. Therefore, this medium was not considered any further in the study. When comparing hexane and heptane based PBCA coatings, the larger advancing contact angles of 70.3° ± 8.8° for heptane as cyanoacrylate solvent might be a hint for more perfectly coated surfaces. Interestingly, this values for PBCA/heptane was very close to contact angle of 68.9° ± 10.3° as reported before for PBCA films [33].

Tab. 1: Surface properties of PBCA-hydrogel multimaterial systems depending on the cyanoacrylate solvent as determined by contact angle measurements[1].

Contact angle of films	Uncoated	*n*-Hexane		*n*-Heptane		Miglyol 812	
		Solvent treated	PBCA coated gel	Solvent treated	PBCA coated gel	Solvent treated	PBCA coated gel
Advancing [°]	35.7 ± 2.4	25.1 ± 3.7	61.8 ± 9.4	33.4 ± 3.9	70.3 ± 8.8	27.7 ± 1.9	99.7 ± 4.7
Receding [°]	35.4 ± 2.4	26.1 ± 2.6	44.2 ± 2.4	30.9 ± 2.5	39.5 ± 1.8	28.0±1.7	51.3 ± 5.9
Hysteresis [°]	0.3	1.0	17.6	2.5	30.7	0.3	48.4

[1] n ≥ 9, mean, ± standard deviation

Since massive parallel initiation of polymerization and the associated water extraction from hydrogels is undesired, advantageous cyanoacrylate solvents will favor the propagation of polymer chain growth over initiation in order to obtaining relevant degrees of polymerization and thus stable coatings. Although the employed monomer solvents exhibit poor dielectric properties as had been discussed to support propagation of PACA chain growth [34], this may not be relevant here since initiation of anionic polymerization by hydroxide ions does not require solvent-assisted stabilization of betaine-like initiation complexes as for instance in case of amines as initiators. In contrast, it may be relevant that the very limited polarity of hexane and heptane results in extremely low solubility of water in the solvent (~0.01%). Accordingly, since the presence of hydroxide ions from water dissociation in the cyanoacrylate solvent can be neglected, only the limited interfacial area of the hydrogel is available for initiation. Together with the high dilution of monomers, i.e., their required diffusion to the interface, this may lead to a spatially controlled preference of polymer propagation.

At least, recent data evidence that high PBCA molecular weights > 100 kDa with low polydispersities (1.2-1.4) can be obtained in a comparable set-up at the interface of water with such solvents [26].

Theoretically, the high hysteresis in contact angle measurements, i.e., the difference between wetting and dewetting of samples (Table 1) as observed for both hexane and heptane based PACA synthesis might theoretically be caused by a macroscopic phase separation. A clustering of hydrophilic groups such as hydroxyl endgroups of PBCA may result in such phase separation; however, based on the low relative numbers of endgroups at high PBCA molecular weight, such phenomena may be excluded as also confirmed by SEM and AFM (see below). Instead, a rough sample topology was detected by optical profilometry (Fig. 3). While uncoated hydrogels were characterized by surfaces with typically nanosized pattern (Fig. 3A), pronounced wrinkling was observed for PBCA coating with hexane (Fig. 3F, compare scale of y-axis for insets). Based on the higher contact angle (Table 1) and the trend to a more homogeneous topology for heptane (Fig. 3E) compared to hexane (Fig. 3F) as cyanoacrylate solvent, heptane was used for the subsequent experiments. The microroughness created at the hydrogel surface during PACA synthesis may serve as a proof that water from the hydrogel rather than other nucleophiles such as impurities from the coating medium initiated the cyanoacrylate polymerization. This again confirms the *in situ* polymerization that was initiated at and adapted to the hydrogel surface.

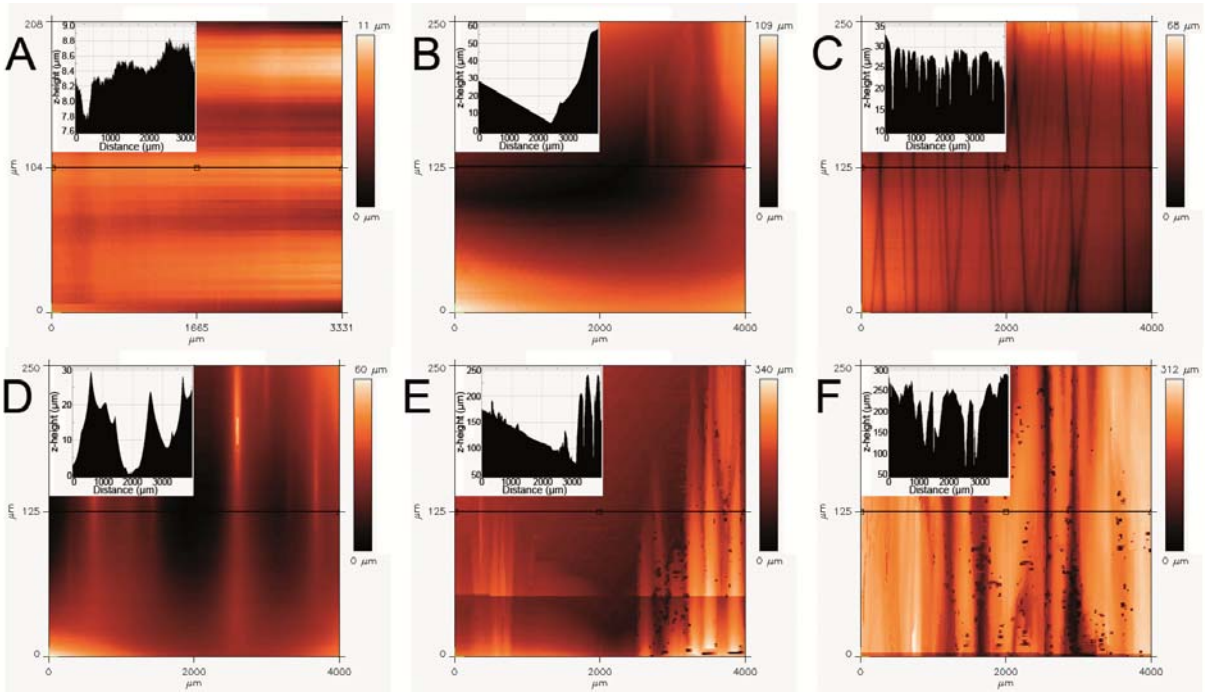

Fig. 3: Surface profiles of unmodified hydrogels and PACA-hydrogel multimaterial systems as determined by optical profilometry for (A) unmodified hydrogels, for heptane-based hydrogel coating with (B) PMCA (C) PECA, (D) PMECA, and (E) PBCA as well as for (F) PBCA coating in hexane. Insets represent virtual optical sections along the black line as indicated in the respective graphs.

Micro- and nanotopology of PACA layers depending on the PACA side chains

In addition to the integrity of the PACA layer, the polymer's physicochemical properties such as its hydrophobicity are of high relevance regarding their efficiency to act as diffusion barrier to water and hydrophilic bioactive molecules. The hydrophobicity of PACA can be tailored by the alkyl substituents, which were selected to be of increasing chain length (PMCA < PECA < PBCA). Additionally, PMECA was explored, which has a substituent with a length similar to PBCA but is of higher flexibility and reduced hydrophobicity due to its ether bond. As illustrated in Table 2, no increase in contact angles was observed for PACA modified hydrogels in case of PACA with short

side chains. Since much higher contact angles were reported before for films of PMCA (55.6°) and PECA (64.7°) [33], there may be doubts on whether a cyanoacrylate polymerization took place at all for these materials. However, FTIR analysis proved the formation of both PECA and PMCA (data not shown; similar pattern as in Fig. 2). Additionally, optical profilometry illustrated a modification of surface topology (Fig. 3B-C). Therefore, it may be concluded that the absence of contact angle alterations was due to a lack of homogeneous coating for PMCA and PECA. In contrast, a distinct increase in contact angle was observed for PMECA, but values remained below that of more hydrophobic PBCA (Table 2). Additionally, the surface topology was modified towards a wrinkled structure as expected (Fig. 3D).

Tab. 2: Effect of PACA side chains on the contact angles of PACA-hydrogel multimaterial systemss using heptane as solvent[1].

Contact angle of films	Uncoated[2]	Solvent treated[2]	PMCA	PECA	PMECA	PBCA[2]
Advancing [°]	35.7 ± 2.4	33.4 ± 3.9	31.3 ± 2.2	30.9 ± 0.8	55.1 ± 8.3	70.3 ± 8.8
Receding [°]	35.4 ± 2.4	30.9 ± 2.5	31.2 ± 6.5	31.1 ± 1.3	55.1 ± 8.2	39.5 ± 1.8
Hysteresis [°]	0.3	2.5	0.1	0.1	0	30.7

[1] $n \geq 9$, mean, ± standard deviation. [2] Data reprinted from Table 1.

While optical profilometry was used to provide an overview on sample topology on the micrometer to millimeter scale, the homogeneity of the PACA layer on the nanoscale as required to inhibit small molecule diffusion cannot be safely assessed. Additionally, it should be noted that optical profilometry demands the sample surface to be outside an aqueous environment, so that artifacts due to water loss cannot be absolutely excluded. Therefore, characterization by AFM in a water chamber was applied to visualize the native structure of the unmodified and PACA-modified hydrogels for later correlation with eventually observed sustained drug release profiles. Besides data on the samples' nanotopology (height signal), AFM provided information on the potential presence of microphases with different mechanical properties (phase contrast).

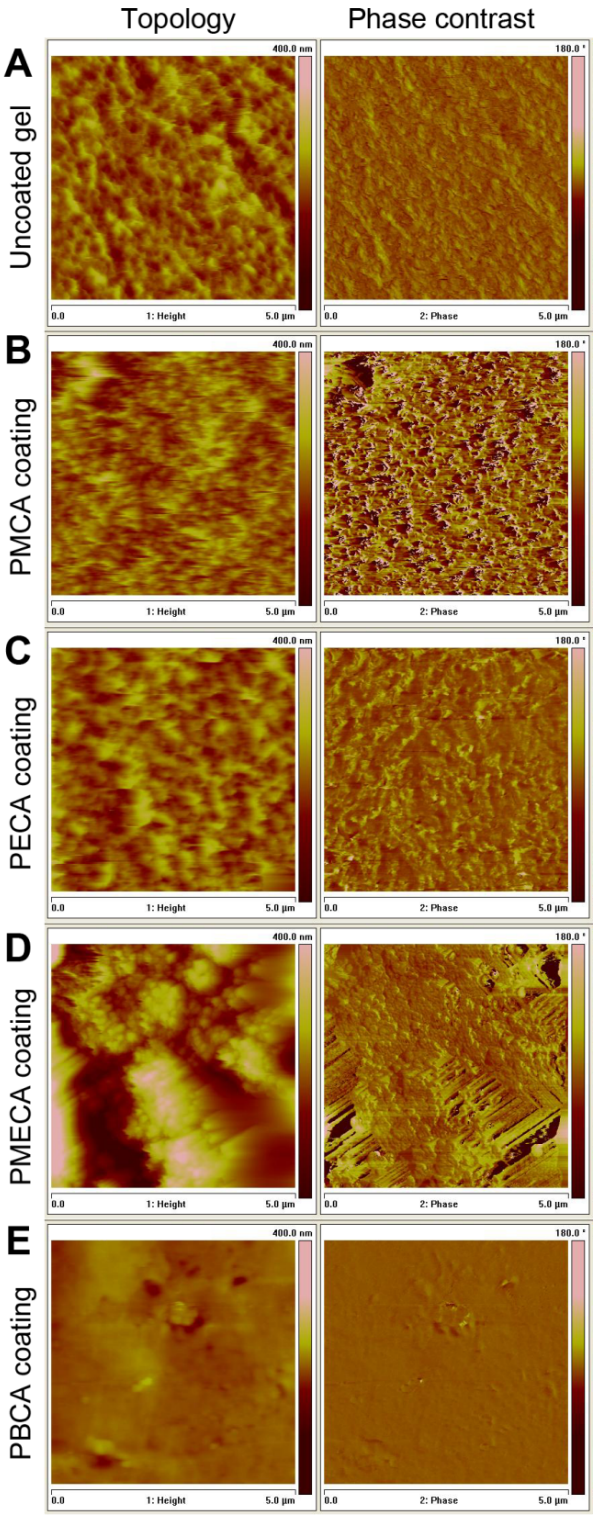

Fig. 4: Evaluation of the surface topology and phase contrast for unmodified gels and PACA-hydrogel multi-material systems by AFM.

Uncoated gels showed random pattern with maximum differences in height of 100-200 nm and, as expected for the pure hydrogel, a very minor phase contrast (Fig. 4A). Although phase images generally contain artifacts from the sample topology as altered upon PMCA coating towards more small-sized structures (Fig. 4B), the strong increase in phase shifts indicated the synthesis of hard PMCA as islets on the hydrogel surface. Alterations in phase images were also evident for PECA (Fig. 4C), while for PMECA a phase morphology with less distinct phase shifts was overlayed by scanning artifacts caused by sample movement during the challenging experiment (Fig. 4D). Remarkably, PBCA coated surfaces were extremely smooth, which suggests the formation of a dense PBCA layer on top of the hydrogel (Fig. 4E). Additionally, the absence of literally any phase contrast strongly supported the absence of a phase separation in the PBCA or of islets of unmodified hydrogel (Fig. 4E). A quantitative description of surface roughness was provided by R_q according to Eq. 1, which was

R_q = 25.2 nm for the unmodified gel. Increased values were obtained for PMCA (R_q = 32.9 nm) and PECA (R_q = 34.7 nm), while roughness was strongly decreased for PBCA (R_q = 9.3 nm).

Due to the selection of alginate gels with physical crosslinks, the hydrogels could be dissolved by extraction of Ca^{2+} ions as physical netpoints in excess of aqueous medium. For PBCA, films were isolated and the thickness was analyzed by SEM. Since no major swelling of PBCA may be expected in water due to its hydrophobicity, the dry state thickness of the PBCA films in the range of ~4 µm (Fig. 5) may be predictive also for wet-state samples. Interestingly, while the surface of the film facing the heptane coating medium was smooth as also observed by AFM, the side facing the hydrogel exhibited roughness with particle like structures (Fig. 5). This finding perfectly corresponded to previous observations for PBCA polymerization at water-solvent interfaces [26] and may be explained by interfacial dynamics resulting in spontaneous emulsification as supported by low solvent viscosity. With ongoing polymerization, the hydrogel phase was covered by a dense layer of PBCA.

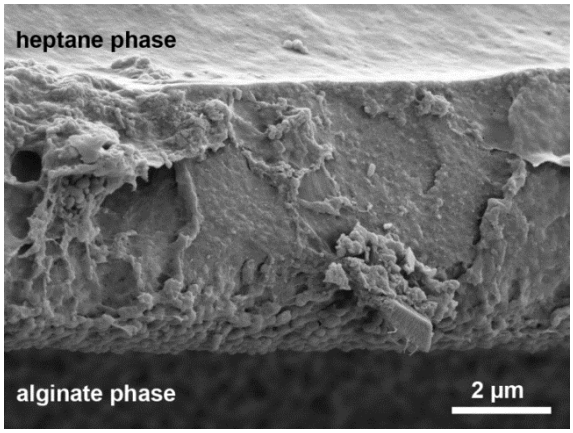

Fig. 5: Morphology and thickness of the PBCA layer as isolated after dissolution of the alginate hydrogel from a PBCA-hydogel multi-material system prepared with heptane as monomer solvent.

Sustained drug release from PACA-modified hydrogels

Due to a high diffusivity and small size, a control of diffusion-driven release from hydrogels may be particularly challenging for small drugs that are well water soluble. Diclofenac sodium is an

example of a water soluble substance and has been selected as a model drug also based on its frequent use with alginate hydrogels [30] and the assumed absence of ionic interaction due to similarly charged, anionic groups of both alginate and diclofenac. A calcium free phosphate buffered saline pH 7.4 was selected as release medium because i) the calcium concentrations in the body differ depending on the application site, age, and disease status and any selected concentration may not be of universal relevance , ii) accepted calcium-substituted PBS media (0.9 mM $CaCl_2$) are not suitable to reflect the physiological calcium concentration e.g. of the serum (~2.5 mM), and iii) the suitability of the coated hydrogels to delay the drug release should be explored under the most challenging conditions without the presence of calcium ions as a hydrogel stabilizer.

As expected, the majority of molecularly dispersed diclofenac was released from uncoated hydrogels in a short time frame, e.g. ~77 wt.% after 6 hours (Fig. 6). It should be noted that the dissolution of drug powder without hydrogel was much faster with similar relative values being reached in less than 5 min. Since the hydrodynamic diameter of the diclofenac ion (~0.5 nm [35]) is much below the typical average mesh size of calcium crosslinked alginate gels (~5-10 nm [36] [37]), an increased diffusion length of the drug in the physically crosslinked gel may not be the only explanation for the sustained diclofenac release mediated by the hydrogel. Additional contributors may include the interactions of diclofenac carboxyl groups with calcium ions resulting in a salt with decreased solubility and/or the presence of physical interactions such as hydrogen bonding between drug and alginate. Still, the effect of PACA layers to further sustain the release of the hydrophilic model drug was substantial. The release pattern could be altered by the selected PACA (Fig. 6). With increasing length of the alkyl substituent, the release kinetics could be systematically controlled allowing a continuous release with cumulative values of, e.g., ~77% being reached after 2 d for PMCA, 4 d for PECA, and 10 d for PBCA. Interestingly, the PMECA coating illustrated a similar capability to sustain the drug release as PBCA despite its more hydrophilic structure and apparently less perfect layer formation as indicated by the AFM data. It should be noted that the

substantially higher mobility of alkoxyalkyl compared to alkyl side chains is generally associated with a substantially decrease in the PACA glass transition temperature T_g [38], e.g., from 118 °C for PBCA to 74 °C for PMECA [26]. Based on its structure and reduced T_g, it was expected that PMECA would allow faster diffusion of diclofenac sodium than PBCA. However, another aspect is the extreme brittleness of PACA, which might result in microcracks of the PACA layer that would contribute to the release profiles. Based on the substantially lower T_g and reduced brittleness, contributions of such cracks as potential explanation for the observed release pattern may be less likely for PMECA compared to PBCA.

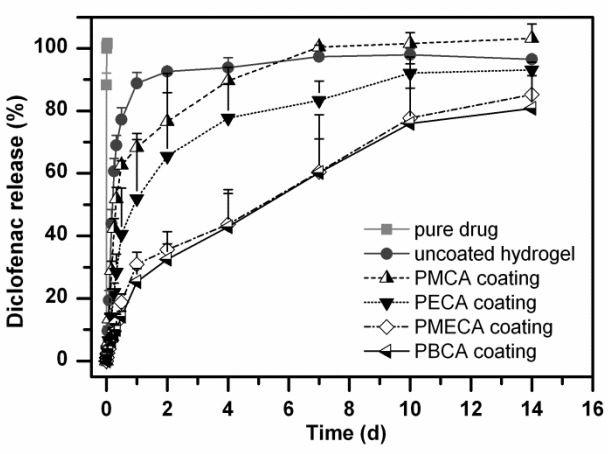

Fig. 6: Control of diclofenac release from PACA-hydrogel multimaterial system depending on the presence and nature of the PACA layer. For comparison, the unmodified gels and the the dissolution of pure drug are shown (n = 3, mean, S.D.).

Overall, this study provided a proof-of concept for the preparation of multimaterial systems by hydrogel-modification with an *in situ* polymerized PACA layer on the hydrogel surfaces and explored the effects of cyanoacrylate solvents and side chains on the properties and structure of the formed PACA layers on the micro- and nanoscale. Based on this, PEMCA and PBCA can be suggested as candidate materials to serve as physical barriers to control the release of hydrophilic substances from hydrogels. While soft planar hydrogels as reported in this fundamental study may face challenges in practical applications e.g. due to a sensitivity of brittle PACA layers to bending, hydrogel matrices of smaller sizes and modified shapes such as with convex surfaces or alternative cyanoacrylates with altered side chains to reduce brittleness may facilitate translation of the

reported concept into a clinical use. Generally, when applying this technology to specific therapeutic drugs in the future, the modification of the bioactive compounds by cyanoacrylates may need to be excluded. Based on the large quantity of available water molecules compared to other nucleophilic groups such as from drug molecules, it may be postulated that an undesired covalent PACA binding to the drug may statistically not be preferred. This was also supported by a recovery of typically 100±2 wt.% of diclofenac from release experiments as determined by HPLC.

Conclusions

The combination of advantageous hydrophilic environments on the one hand and a suitable control of release rates for hydrophilic drugs on the other hand is an inherent challenge of hydrogel based carriers. By using the general presence of water in hydrogels as trigger to polymerize cyanoacrylates *in situ*, micro-thin hydrophobic layers could be established on hydrogel surfaces that enabled tailoring drug release rates and their correlation with the PACA structure. As this approach to establish multimaterial systems may neither be limited to a specific hydrogel type or shape nor to a specific drug, it may be widely applicable also for more complex shaped hydrogel carriers.

Disclosure

Parts of this work have been shown as a poster and an oral presentation at the Second Symposium on Innovative Polymers for Controlled Delivery (SIPCD 2012) held from September 11-14, 2012 in Suzhou, China, with an abstract being published online in the Journal of Controlled Release along with the conference.

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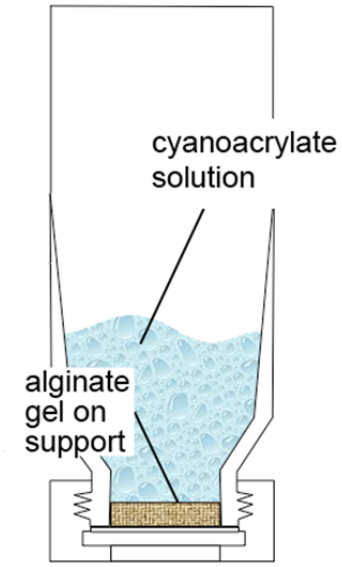
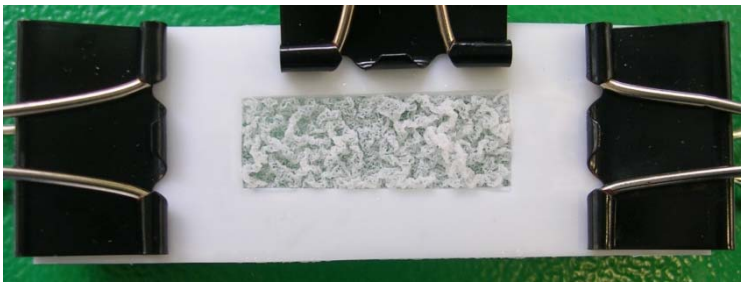
Supplementary Material



Suppl. Fig. 1: Setup for modifying hydrogel samples in a closed vessel with a poly(alkyl-2-cyanoacrylate) based diffusion barrier. The orifice diameters of the vessel corresponded to the diameter of the gel samples, while the paper support was clamped in the lids of the vials and hold the gel film in place. Organic solution of the cyanoacrylate monomers were brought in contact with the samples by inverting the glass vial.



Suppl. Fig. 2: Alginate hydrogel film upon exposure to high cyanoacrylate concentrations (25% (v/v) *n*-butyl-2-cyanoacrylate in hexane) with apparent alteration of surface morphology by the massive parallel initiation of cyanoacrylate polymerization.