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pH-responsive materials for optical monitoring of wound status

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ABSTRACT

The monitoring of infection status of wounds is an emerging field and the pH of wound exudate is considered one potential indicator of infection. pH indicators intended for use in medical devices, such as swabs or dressings, need to be fixed in place, however, visual pH indicators are usually soluble molecules so are not inherently suitable for use in devices. To address this, we developed a rapid and simple immobilisation method for coupling pH-responsive dyes onto solid phases. The use of a silane based coupling agent for immobilisation of bromocresol purple led to a shift in the pH dependent spectral properties of the resulting material. The pH responsive material changes from yellow to green to blue with rising pH providing an ideal contrast to the reddish colour of most wound exudates. This is a key advantage over currently available alternatives when considering the suitability of this material for incorporation into various medical devices. In addition, we analysed clinical study samples to verify the association between wound infection and elevated pH-values. A device with an embedded indicator that changes to a contrast colour could represent a simple and easy-to-use system for detecting wounds at risk of infection.

1. Introduction

Real-time monitoring of wound status is gaining importance in modern health care systems. Early detection of incipient infections, especially in chronic wounds, is an important step towards better wound management and reduction of antibiotic therapy. Timely treatment of infected wounds is crucial for wound healing and can actively reduce patient suffering. This has led to significant effort being put into the development of novel diagnostic systems to gain more information about wound status [1–11].

A key parameter that can provide information about wound status is the pH value. While low pH values around 4.0–6.0 are commonly typical for healing wounds [12–16], elevated pH values of 7.0 and above are mainly associated with non-healing wounds [12,14,15] or infected burns [17]. One aspect that links slow healing to high pH is the fact that many proteases are more active at higher pH meaning that elevated pH would favour tissue degradation by endogenous proteases [15,18]. These data suggest that elevated pH values could be used as one within a set of potential markers for wound infection, enabling an appropriate treatment to be administered in a timely manner. Consequently, a number of different approaches for monitoring the pH value of wounds has recently been published [7,8,19–23]. However, various limitations have been identified for many smart bandages/materials previously developed to monitor wound pH. The readability of fluorescent indicators [24] requires excitation (often UV) lamps or even more sophisticated equipment which is a disadvantage when compared with a visual contrast colour system under ambient light. Other recent approaches, for example, the combination of indicator and inert dyes, led to a “traffic light” system, where the colour changes from green to red with increasing pH [4]. The red colour for elevated pH-values is a familiar warning signal, but could be a problem, especially for bloody wounds where the indicator colour is similar to the colour of wound

Abbreviations: BCP, bromocresol purple; FBCP, functionalised bromocresol purple; GPTMS, 3-glycidyloxypropyltrimethoxysilane; AA, acetic acid; NPV, negative predictive value; PPV, positive predictive value; MS, mass spectrum

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exudates. Other strategies involve the release of indicators into the wound and thus safety and biocompatibility aspects need to be carefully assessed [2]. Given the challenges described, there is a need for improved technologies to enable monitoring of the pH of wounds in an easy and practical manner.

The primary goal of our work was the development of a pH responsive material that gives a clearly visible colour change from yellow to blue in order to achieve the best possible contrast to colours of wound exudates. The secondary objective was the development of an immobilisation method for the covalent binding of pH responsive dyes onto relevant materials that are frequently used in wound care products such as cotton or cellulose.

A wide range of different alkoxysilane coupling agents can be used for targeted modifications of OH-bearing surfaces (e.g. cotton, cellulose) and therefore present many options when considering the development of pH responsive materials. Even in more complex systems like macroscopic switches reactive silane species can be used for the building of sensing surfaces [25]. Recently published approaches involved 3-glycidyloxypropyltrimethoxysilane (GPTMS) as a coupling agent and methyl red or resorufin as a pH indicator [26,27]. Moreover, Plutino et al., previously demonstrated that the coupling of methyl red with GPTMS led to a higher photo-stability of the reactive dye [28] which adds additional value to the use of GPTMS as coupling agent.

To achieve the primary goal of a pH-sensing material with a colour change from yellow to blue, bromocresol purple (BCP) was selected as indicator dye in this work. Following the functionalisation of BCP with GPTMS, a spectral shift occurs that leads to a blue instead of a purple colour at pH values above 7.0. Therefore, the combination of GPTMS as coupling agent and BCP as pH-indicator matches the described needs. Fig. 1 shows the predicted coupling mechanism of BCP onto OH-bearing surfaces, based on previous published findings [26,28–31].

In this article, we describe a new mechanism for covalently coupling indicators onto surfaces and potential applications, mainly in the field of medical devices. Finally, we conducted an ex-vivo clinical study with 156 patients to validate the clinical significance of wound pH by determining the sensitivity and specificity of the immobilised indicator functionalised BCP (FBCP), versus wound infection status.

2. Materials and methods

2.1. Materials

Bromocresol purple (BCP), acetic acid (AA), (3-glycidyloxypropyl) trimethoxysilane (GPTMS), phosphoric acid, citric acid, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium carbonate, sodium hydroxide, sodium chloride and hydrochloric acid were all of analytical grade, obtained from Sigma-Aldrich (St. Louis, USA) and used as received. The cellulose matrices (Whatman filter paper grade 1 and Sigmacell cellulose Type 20, 20 µm) were also obtained from Sigma-Aldrich. The cotton swabs (EH11.1) were obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). The dressing (AQUACEL® Foam, Non-Adhesive, 10 cm × 10 cm) was obtained from ConvaTec Ltd (Deeside, UK). The sterile wound swabs (FLOQSwabs, Regular, Sterile Single Wrapped, Molded bp 100 mm) were obtained from Copan (Brescia, Italy).

2.2. Methods

2.2.1. Immobilisation reactions

2.2.1.1. Functionalisation of filter paper and cotton swabs with BCP. 89.0 ml 57 µM AA was stirred with a magnetic stirrer at 400 rpm and 11.0 ml of GPTMS was added dropwise. The reaction solution was stirred for additional 15 min until no turbidity was observable. The Whatman filter paper and the cotton swabs were fully soaked in the reaction solution and dried with a heat-gun (generating a surface temperature of 75 °C on the paper) for a homogenous distribution on the surface. The dry GPTMS-modified material was then soaked in a 1 mg/ml BCP solution (in 57 µM AA) and pre-dried with a heat-gun. The condensation reaction was carried out in a compartment drier at 120 °C for 20 min. Excess dye was removed from the FBCP by several washing steps with phosphate buffer (pH 3.0, 10 mM, total volume 2 L). Dye removed in the eluate was monitored by spectrophotometer and washing continued until no dye could be detected after incubation in buffer for 60 min.

2.2.1.2. Determination of staining degree. The determination of the immobilisation efficiency was carried out by comparing GPTMS – pre-treated filter paper vs pure filter paper. Therefore, the above

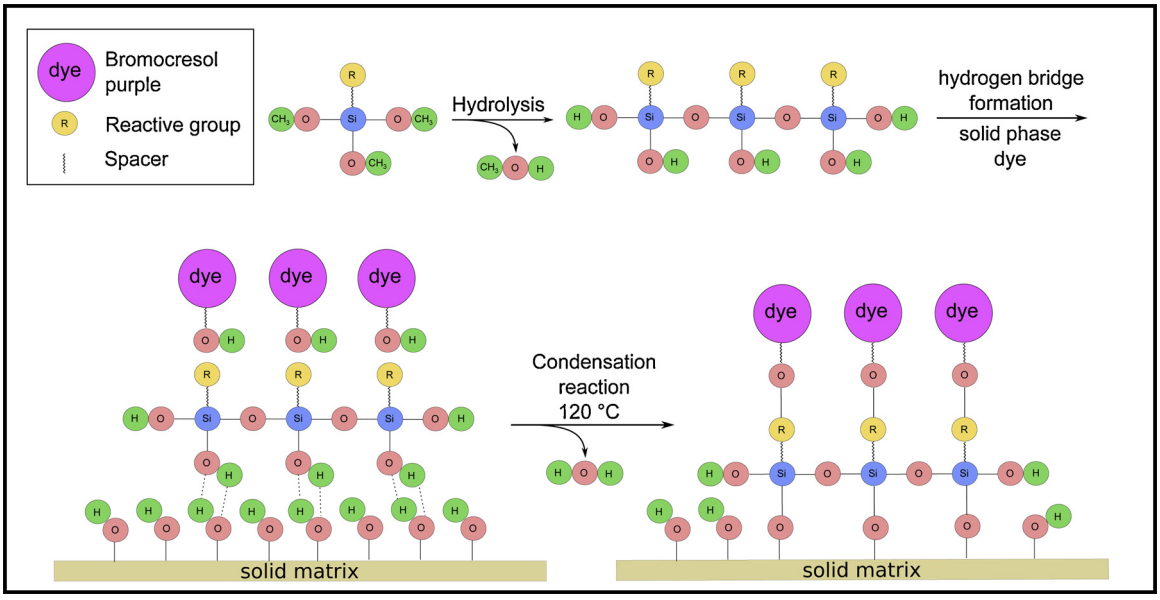

Fig. 1. Reaction overview: Schematic representation of the immobilisation reaction of the pH indicator bromocresol purple with (3-glycidyloxypropyl)trimethoxysilane onto materials containing hydroxyl groups on the surfaces. (Reactive group: e.g. epoxy group; spacer: e.g. propyl; solid matrix: e.g. cellulose). (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

described method was used, but in one case the first step of functionalisation with GPTMS was omitted and the dye solution was directly applied on the filter paper. The concentration of dye in the washing solution was quantified using a microplate reader (Infinite M200 pro, Tecan) and a calibration curve of bromocresol purple. All analysed samples were measured at pH 8.0.

2.2.1.3. Identification of liquid phase reaction products. 1.58 g of Bromocresol purple and 11.4 g of GPTMS were combined in a closed vessel, heated with occasional shaking to 54 °C until a homogenous solution was achieved and then incubated at the same temperature for 2 days. The products of reaction were observed using negative ion mass spectrometric analysis (LC-Duo ion trap instrument Thermo Fisher), via direction injection in a 50% methanol, 0.1% formic acid solution. Spectra were analysed using Excaliber software. Upon further reaction the material gelified consistent with formation of silane bonds. The unreacted BCP was eluted from the gel by washing with water. The resulting gel was light orange in colour. This gel was pH responsive becoming blue with increasing pH. On prolonged incubation with basic solutions, unaltered BCP was released in the blue supernatant as detected by a single peak at *m/z* 539.

2.2.1.4. Functionalisation of cellulose particles with BCP. 18.6 mg of the cellulose particles (20 µm) were suspended in 100 µl of a 10% (v/v) GPTMS solution in 57 µM AA and incubated in a compartment drier at 120 °C for 20 min. The incubation step was carried out in an open glass vial. The dried functionalised particles were transferred into a new glass vial containing 100 µl of a BCP solution (1 mg/ml in 57 µM AA). The second reaction step was also carried out at 120 °C for 20 min in a compartment drier. The stained particles were re-suspended and washed in phosphate buffer (pH 3.0, 10 mM) until no dye could be detected in the supernatant.

2.2.2. Integration into a wound dressing

A single layer of FBCP filter paper was inserted between the polyurethane layer and the top opaque adhesive layer of a non-adhesive AQUACEL® Foam dressing. 0.5 ml of pH 5.0 (0.1 M, sodium acetate) and pH 8.0 (0.1 M, sodium phosphate) buffer was pipetted onto the bottom hydrofiber layer of the dressing, respectively. After 5 min, the buffer reached the FBCP layer and colour change documented by photography.

2.2.3. Characterisation of the pH-responsive material

2.2.3.1. UV–vis, FTIR and colour measuring device. The pH value of all buffered solutions was measured with an InLab Expert Pro pH electrode (Mettler Toledo). 0.1 M acetate buffers were used for the pH range of 4.0–5.5. 0.1 M sodium phosphate buffers were used for the pH range of 6.2–8.0. 0.1 M Tris buffers were used for the pH values 8.5 and 9.0. 0.1 M carbonate buffers were used for the pH values 9.5 and 10.0.

Absorbance spectra were recorded with a micro plate reader (Infinite M200 pro, Tecan). 5 mm diameter FBCP-filter paper disks were placed in a 96-well plate (Greiner Bio One). 100 µl of different buffers with pH values ranging from 4.0 to 10.0 were applied before measuring absorbance scans. The absorbance spectra of the FBCP filter paper was compared with the unbound BCP in solution (25 µg/ml, 100 µl). The average absorbance of the cellulose baseline was 1.8 and subtracted in the diagrams for a better comparability. While the paper itself blocks significant light transmission, there is sufficient transmission in wet paper to measure differential absorbance due to the dye.

The initial cellulose layer and the FBCP filter paper were characterised by FTIR using a Perkin Elmar Spectrum 100. The resolution of the recorded spectra was 1 cm^{-1} for 8 scans. The spectra were normalised to the peak around 2880 cm^{-1}.

Colours of the FBCP filter paper at different pH values were measured using a ColorLite sph850 (ColorLite GmbH). Therefore, the material was soaked with different buffers as indicated above and colours were determined according to the CIELab concept.

2.2.4. Clinical testing

Wound fluid samples were obtained from 156 patients with open chronic wounds suitable for sampling via wound swab. The study was conducted at the departments of vascular surgery of Medisch Spectrum Twente hospital (Enschede), Ziekenhuisgroep Twente (Almelo), Streekziekenhuis Koningin Beatrix (Winterswijk), St. Jansdal hospital (Harderwijk), and at Livio homecare (region of Twente) [32] and with informed consent of the patients and following approval from the Medical Ethical Committee Twente. Non-eligible patients (Under-age persons or the mentally incompetent) and patients without informed consent were excluded. Wounds were evaluated by clinicians based on the clinical appearance of the wounds and were classified into two groups: “infected” and “non-infected”. Regular sterile swabs were used for the sampling of 156 wounds and after the sampling, all swabs were suspended in 1 ml of unbuffered physiological saline solution. All wound fluids were analysed by applying 5 µl of sample onto the FBCP filter paper. The occurrence of a blue colour corresponding to pH values above 7.0 was used to classify the samples as positive. The pH results were compared with the expert clinical judgment of the infection status of the wound for the calculation of sensitivity and specificity of pH values above 7.0.

3. Results and discussion

3.1. Immobilisation reactions

A procedure for covalent coupling of BCP onto solid matrices using alkoxysilane coupling agents was developed (Fig. 1).

The slightly acidic conditions (57 µM AA) during the hydrolysis of the GPTMS appeared to improve immobilisation, possibly because it accelerates the hydrolysis reaction and ensures a more stable intermediate product [31]. The BCP dye was dissolved in 57 µM AA because this was found by empirical means to increase apparent immobilisation. A high reaction temperature for the immobilisation step was required for a successful condensation reaction. Lower temperatures led to an increased leaching of unbound dye during washing and hence, led to reduced immobilisation.

Analysis of paper following application of BCP in the optimised protocol suggested immobilisation of 68 ± 1.3% of the applied dye with the GPTMS-pre-treated filter paper, whereas no dye was immobilised without the prior functionalisation of the paper. This corresponds to an amount of 11.5 ± 0.2 µg/cm^2 BCP on the filter paper.

3.2. Characterisation of the pH responsive material

3.2.1. UV–vis & MS analysis

Absorbance scans of the acidic, neutral and the alkaline form of the pure BCP dye were compared with the immobilised dye, FBCP (Fig. 3). The background of the cellulose layer was subtracted from the FBCP scans to enable comparison. Both free and fixed dye showed a clear pH-dependent colour change. Nevertheless, upon immobilisation a shift of the absorbance maximum could be observed in both, the acidic and the alkaline form. Whereas a shift from 432 nm to 406 nm was observed for the acidic form, a shift from 588 nm to 606 nm was recorded for the alkaline form. Similar shifts were recently reported for a GPTMS functionalised methyl red derivative [28].

In attempting to characterise the solid-phase reaction product we observed that the fixed BCP (or any derivatives thereof) was not eluted in acid or neutral water, or by sodium fluoride solution, but was released by treatment with basic solutions (ammonia, KOH, ethanolamine). This suggested that the covalent linkage was base labile consistent with an ester.

To evaluate where the ester could form, we conducted model reactions in liquid phase. Mass spectra suggest that there is a direct

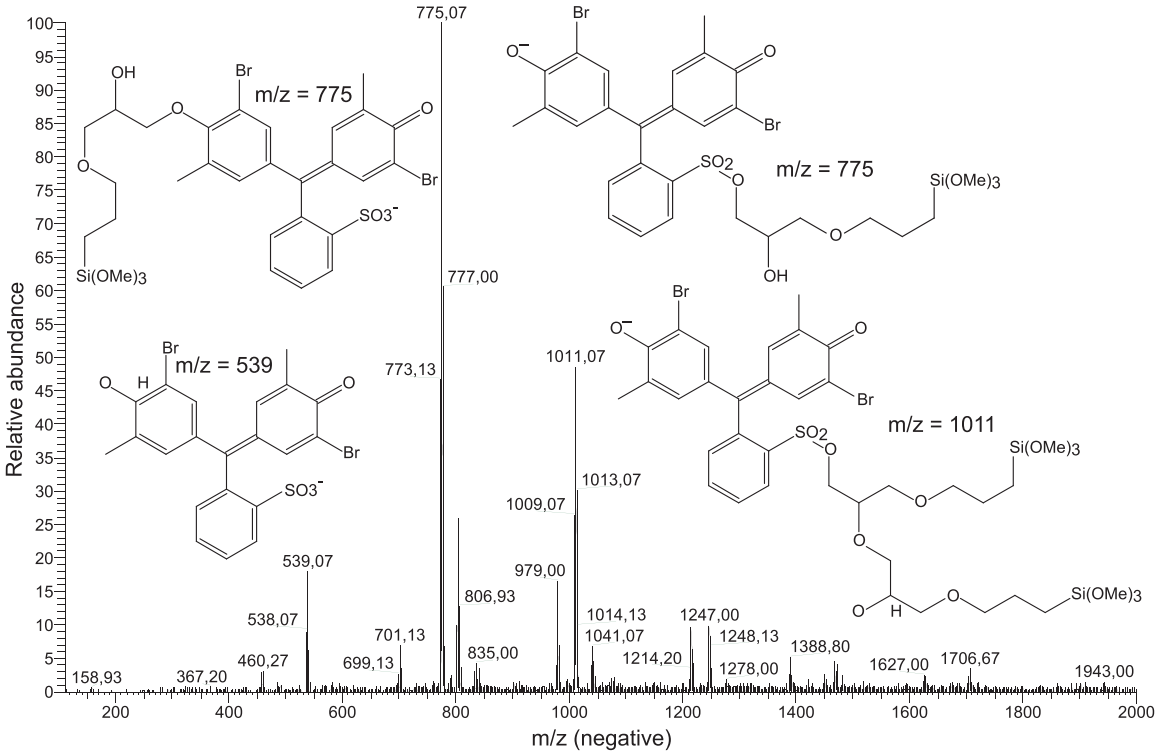

Fig. 2. Mass spectrum of reaction products: Mass spectra from the unpurified reaction mixture of BCP and GPTMS is overlaid with possible structures consistent with signals at *m/z* H- of 775, 1011 and 1247 corresponding to mono-, double-, and triple alkylation products of bromocresol purple with (3-glycidyloxypropyl) trimethoxysilane.

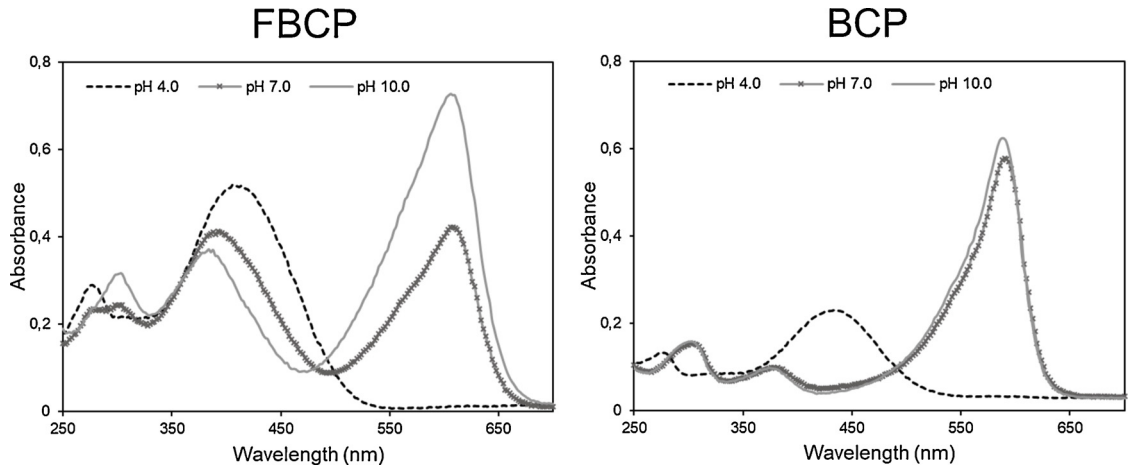

Fig. 3. Absorbance measurements: Absorbance spectra of the pH responsive dye bromocresol purple (BCP) and of the immobilised dye (FBCP) under acidic (pH 4.0), neutral (pH 7.0) and alkaline (pH 10.0) conditions. The background absorbance of the cellulose itself was subtracted (only for the FBCP) for a better comparability of the graphs.

reaction between GPTMS and BCP either on the phenolic OH groups, or the sulfonic acid. The mass spectrum gives clear signals for mono-, double- and triple-alkylation products of BCP with multiple GPTMS molecules. Possible structures for the resulting mono- and double alkylation products are given in Fig. 2. The analysis of the products is also further complicated by the potential for silane groups to bond. Similarly, the beta hydroxy group that is formed from the opening of the epoxide is potentially able to open another epoxide adding an additional GPTMS, which in turn can provide a new beta hydroxy group as a reaction site for a further GPTMS. Thus, if allowed to proceed these reagents can provide a large polymer via multiple reactive entities which are not easily structurally characterised.

Nonetheless, if analysed early in the reaction period, the possible reaction products of initial reactions (and thereby, those likely to form on paper during the short heating phase) could be interpreted. These data indicate potential opening of the epoxide by the phenolic OH and sulfonic acid groups (Fig. 2).

Reaction on the phenolic hydroxy groups would not be easily reversible but could explain the absorbance shifts of the coupled dye. An alteration of the dye molecule is very likely to change the absorbance properties of an indicator depending on the site of reaction. Forming a phenolic ether would, most likely, prevent formation of a quinoid system and thus impair pH mediated colour change. The additional peak of absorption at 384 nm could be the result of a multiple-coupled dye derivative which may no longer be pH responsive. The sulfonic ester should not impair quinoid formation and may remain pH

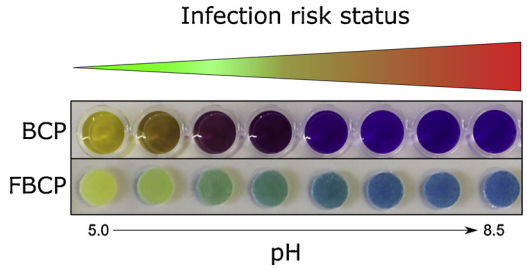

Fig. 4. Infection detection based on FBCP: Colour change of immobilised (FBCP) and free dye (BCP) over a pH range of 5.0–8.5 and a schematic comparison to infection risk status. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

responsive. Similarly, this is a reversible linkage and one that is known to be base labile. This derivative could release unmodified BCP.

A key question is whether the pH indicating activity is provided by a bound species, or BCP following de-esterification. Given release by alkaline solutions, the reaction at pH 9.0 or above could be ascribed to free BCP that has de-esterified. However, the shift observed in the alkaline pH absorption maxima suggests that it is the spectrum of another species and not free BCP.

The apparent green colour of the new material at pH-values between 6.0 and 6.5 (see Fig. 4) is not associated with chemical hydrolysis and is a different colour reaction to that of free BCP. The apparent green colour may be the result of a blue reaction combined with a non-changing yellow background (likely phenolic ether reaction products). Similarly, the trend for alkaline pH to provide blue in these immobilised forms suggests that the immobilised species provides the colour reaction and not the free BCP.

The responsive range of pH indication and the spectrum of colours obtained is highly appropriate for wound assessment. Comparable effects were achieved in previous approaches using a second dye to achieve more favoured colour combinations [4], however, here we have obtained suitable indicating properties with a single indicator species.

3.2.2. Colour measuring device

One of the most important aspects of the development of pH-responsive materials for detection of wound infection risk was simple readability of the colour response by the naked eye. The visual inspection and evaluation must be as simple as possible and should give the best possible contrast to colours likely present in wound exudate. Hence, a yellow to blue shift from regular to elevated pH values as infection risk increases is the preferred option. The colour change of pH-responsive solid materials was quantified using the CIELab colour space concept which is based on the measurement of 3 values: L*, a* and b*. The L* value in this concept represents the brightness, the a* value the colour change from green to red. The most relevant value for our pH-responsive material is the b* value which represents the colour shift from yellow to blue. The most clinically relevant pH range for the detection of infection in wounds is between the pH-values of 5.0 and 8.0. Hence, the b* value was used to quantify the colour change of the functionalised material in this range. Fig. 5 shows the relationship between apparent colour and the corresponding pH indicating that pH values between 5.0 and 8.0 can be distinguished with this material and give measurable colours from yellow to blue.

3.2.3. FTIR characterisation

FTIR spectra recordings from the blank cellulose matrix and the FBCP are shown in Fig. 6. The main differences were detected in the area between 1200–700 cm^{-1}. The peaks arising around 1184 cm^{-1}, 1085 cm^{-1} and 814 cm^{-1} can be related to Si—O—Si stretching and bending [33–35]. The peak around 1184 cm^{-1} has also previously been associated with Si—O—cellulose binding [36]. In summary, the FTIR

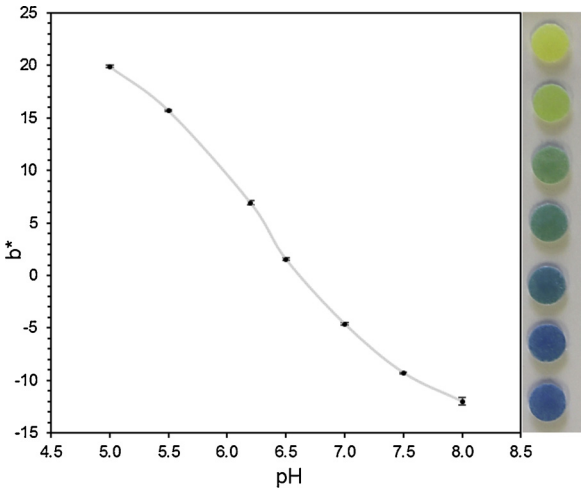

Fig. 5. Quantified colour change: pH-responsive colour changes of the pH responsive material (FBCP) shown as b* values including standard deviation (pH range from 5.0 to 8.0). (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

analysis confirms the hypothesis of the coupling mechanism. The peaks at 2870 cm^{-1} could be due to remaining methyl groups of the GPTMS. In general, the area around 900–700 cm^{-1} is strongly influenced by different substituted aromatic ring systems and can therefore be related to a combination of BCP as well as diverse Si—O—X combinations. Another spectral characteristic of the BCP would be in the range of 3100–3000 cm^{-1} where the =C—H stretch in aromatics occurs [35], but would likely not be detected due the relatively low concentration of BCP compared to the other chemical components.

3.3. Clinical judgement

Infection is one of the most common causes of delayed wound healing with corresponding effects on morbidity and loss of quality of life. In search of infection markers and indicators, we performed a clinical study with our sensing material to evaluate solid state indicators of wound pH as a potential diagnostic aid to assess changing wound state.

Wound samples were gathered as part of routine wound care which includes the submission of swabs for microbiological assessment as well as the visual assessment by a wound care practitioner for signs of non-healing or infection (redness, swelling, pus, secretion, odor or pain). Of 156 wound fluid samples assessed by blinded operators using our pH-responsive material 114 wounds had a pH < 7.0 (indicated by green colour, considered as low likelihood of infection) and 42 wounds had a pH ≥ 7.0 (blue colour, considered likely to be infected). In parallel, the expert visual assessment at the time of sampling and the results of microbiology from the same sample were gathered. Using these data, wounds were assigned the status of “infected” or “non-infected”.

The sensitivity and specificity of the pH measurements to associate with wound infection status, were calculated by comparing with wound status determined by expert clinical observation. The sensitivity was plotted against (1 – specificity) resulting in a receiver operator characteristic curve (Fig. 7) to visualise the diagnostic properties of the sensing material.

More than 50% of infected-classified wounds (Sensitivity 53.8%) showed an elevated pH and were detectable with our material. This leads to a positive predictive value of 33.3% (Table 1). The specificity was 78.5% with a false positive rate of only 21.5% and a negative predictive value (NPV) of 89.5%. The usual high positive predictive value (PPV) of clinician’s judgment combined with the high NPV of the pH-value could help avoid unnecessary and costly treatments. A device with a high NPV would give clinicians more confidence to select

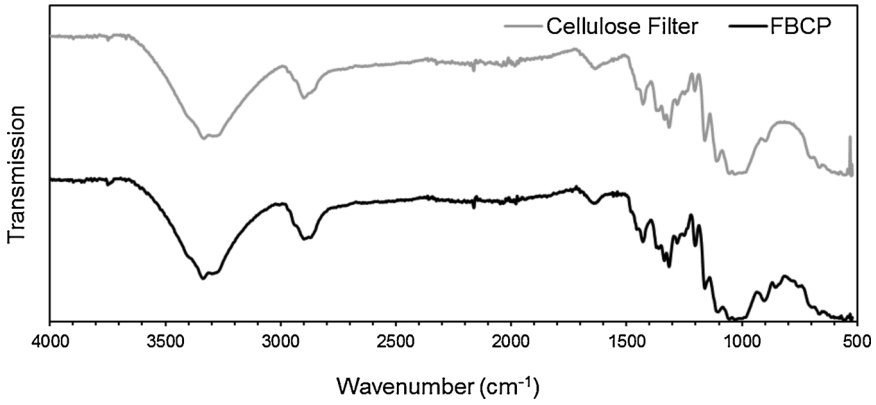

Fig. 6. FTIR: Normalised FTIR spectra of the pH responsive material FBCP and cellulose filter.

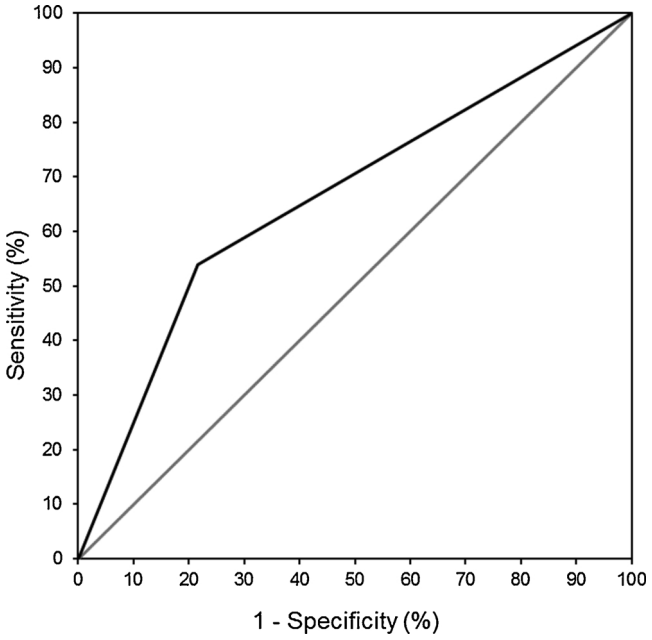

Fig. 7. Diagnostic properties: Receiver operator characteristics curve for the pH responsive FBCP in an ex-vivo setup (156 patients) for the detection of infection. (grey line reference line; black line pH).

Table 1
Diagnostic properties of wound pH compared to clinical judgment: Sensitivity 53.8% and Specificity 78.5%. (TP – true positive; FP – false positive; FN – false negative; TN – true negative; PPV – positive predictive value; NPV – negative predictive value; AUC – area under the curve).

		Clinical judgment				
	156	Infected	Not infected	PPV	NPV	AUC
pH	Elevated (pH ≥ 7.0)	TP 14	FP 28	0.33	0.89	0.66
	Not elevated (pH < 7.0)	FN 12	TN 102			

appropriate treatments for patients with a negative microbiology test result [37]. These results support the idea of use as an aid in detecting wound infections and reveals a potential association between elevated wound pH and infection. Another clinical study by Metcalf et al. used an alternative method for the determination of wound pH and showed a similar association between elevated wound pH and wound infection [38].

While examining pH in wound fluids from clinically infected wounds provides some confirmation of the association, it is not, by definition clinically helpful because these wounds were considered infected by current clinical assessment (e.g. redness, pus, discolouration, smell, swelling). The more useful attribute would be to identify ambiguous or cryptically infected wounds that are currently under-treated due to the absence of overt clinical signs, which is often the case in non-healing chronic wounds. This makes high pH "non-infected" wounds worthy of more detailed studies.

To investigate this further, one approach may be to undertake a longitudinal study examining the predictive potential of rising wound fluid pH with development of clinical infection. In this context, increasing pH, even if not yet above pH 7.0, may be a leading indicator of a trend to non-healing in chronic wounds. Thus, so called "false positives" in our study may merit observation for long-term outcomes to determine whether, A) the observation of high pH was repeatable in time, and B) if it was associated with later onset of overt infection. In such studies, it would also be possible to assess the effect of wound cleansing agents and dressing buffering on the results obtained.

An alternative approach is to regard pH as one of a set of biomarkers associated with incipient or overt clinical infection. Recently published studies also showed that the evaluation of a single parameter might not be sufficient for a definitive classification of wounds into "infected" or "non-infected" [39]. Rather, data suggest that the combination of multiple parameters can give information about the status of a wound that has higher positive predictive power. In current studies, we are examining whether the combination of the pH-sensing material FBCP with other markers, such as elevated levels of enzymes associated with wound infections [40–44], will lead to improved diagnostic properties including greater sensitivity to sub-clinical or incipient infections.

That a degree of association between infection status and pH exists is supported by a range of observations on wound biology. The production of various microbial metabolites such as amines with basic pH reaction from devitalised wound tissue is one example (ammonia derived from urease activity). In addition, the simplicity and cost effectiveness of these sensors make them suitable for implementation in devices and in standard wound care practices. The simple read out by an unaided eye, the colour change from yellow to blue as well as the easy incorporation into existing medical devices make the approach inexpensive and convenient.

3.4. Applications in medical devices

A large field of applications is conceivable for this new pH-sensing material. Here we demonstrate only a view feasible options for use in medical devices. Fig. 8a–b shows small cellulose particles for pH-sensing. The small particle size of only 20 µm makes them compatible with printing or liquid systems, where they could be centrifuged to give readable spots (Fig. 8a) or simply be used in suspension (Fig. 8b) for colouring the whole suspension. Fig. 8c shows sensing swabs that could directly be used in wounds. Swabs are commonly used in wound

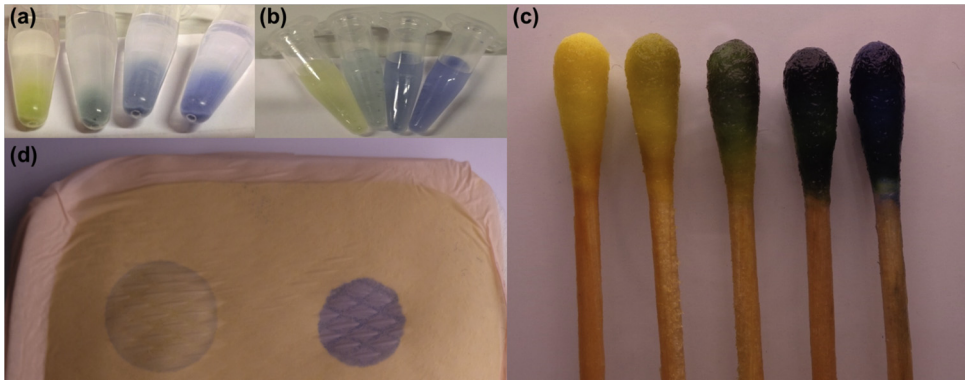

Fig. 8. Different possible applications of the new pH-responsive material: (a) settled particles (pH 5.0, 6.0, 7.0, 8.0) different pH values; (b) particles in suspension (pH 5.0, 6.0, 7.0, 8.0); (c) modified cotton swabs (initial colour, pH 5.0, 6.0, 7.0, 8.0); (d) integration of FBCP into a dressing (pH 5.0, 8.0). (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

management and whilst mainly used for microbiology testing, they have many other uses such as wound cleansing [45,46]. As swabs are ubiquitous in wound care, they could simultaneously be used for wound cleansing and pH sensing, and therefore provide important information about the wound status. Details of other pH-responsive swabs have recently been published indicating a high interest in such multi-functional swabs [4,7]. The last medical device in Fig. 8d shows a wound dressing with an integrated pH-responsive layer. Such a dressing could be used to monitor the wound status without the need to change the dressing. One can easily see the difference between an elevated pH (blue spot, right side) compared with a low pH (yellow spot, left side). Noteworthy effort is being made today in the establishment of smart wound dressings [1,2,5,6,8–11,20,27,47]. The crucial benefit of sensing dressings is the monitoring and timely identification of possible risks. An early stage detection of emerging wound infections will gain even more importance in the future when considering patient outcomes, health care costs association with treatment of wound infections, and antibiotic stewardship [48].

4. Conclusion

A pH-responsive material was developed which shows a reversible colour change from yellow to blue when the pH value changes from acidic to alkaline. Both the pH range and the type of colour change was specifically chosen to allow detection of increased infection risk in sampled wound fluids. The results of the clinical study showed a correlation between elevated pH values and wound status. Nevertheless, measurement of pH alone might give only limited information on the infection status of wounds, and thus may need to be used in combination with additional parameters to increase sensitivity and positive predictive power. For the integration into a medical device, more studies on the stability and sensitivity of FBCP to sterilisation techniques must be performed. More generally, these materials appear to have potential for integration into medical devices. This could have clinical utility in that it could increase the relevance and use of pH as an infection risk parameter.

Declaration of Competing Interest

A. Heinzle, E. Sigl, M. Burnet and G. Guebitz are co-founders of Qualizyme Diagnostics GmbH & Co KG. C. Gamerith and D. Luschnig are employees of this company, but this has not influenced the content of this manuscript.

M. Burnet is the founder of Synovo GmbH, J.-H. Guse and N. Pietrzik are employees of this company, but this has not influenced the content of this manuscript.

M. Haalboom, J. van der Palen and A. Ortner have no conflicts to declare.

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