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Self-healing Fuel Cells by Biological Actuators

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Abstract

A completely novel approach aims to extend the lifetime of polymer electrolyte membrane fuel cells (PEM-FCs) many times over through a self-healing mechanism, thus contributing to the transition to a sustainable economy and mobility. Many start-up cycles in truck or car applications lead to pinholes in the proton-conducting polymer membranes (Nafion™). Since there is no way to repair this type of defect when the fuel cell is already assembled, the performance of the fuel cell stack is continuously reduced towards the end of its lifetime. The BioHealing project, an interdisciplinary consortium at the Karlsruhe Institute of Technology, investigates an innovative biohybrid technology that can repair occurring pinholes selectively in fuel cell membranes by a self-healing mechanism without disassembling the fuel cell stack. This is enabled by a biohybrid system, i.e., integrating biological and technical components. The filler-forming enzymes are functionally immobilised on the polymer membrane surface via a special peptide linker to enable site-directed filler production during membrane electrode assembly. For self-healing, a substrate is injected into the gas inlet of the fuel cell stack, the enzymatic reaction is catalysed, and the substrate is converted into the filler, a biological polymerised product that seals the existing pinholes. In this publication, the concept is presented with initial research results. The concept includes characterisation of surface properties and functional groups of the Nafion™ membrane, immobilisation of the specially designed fluorophilic peptide linker, selection of suitable enzymes to be screened for activity and stability, and investigation of the filler to be successfully esterified with the membrane.

Keywords: Biologicalization; Biohybrid Technology; Self-healing; Repair; Circular Economy; PEM-FC; Pinholes; Enzymes; Immobilisation

1. Introduction

Inspired by nature's self-healing mechanism, this paper aims to integrate biological and technical components into a fuel cell to make electric car driving greener. The objective deals with creating a biohybrid system that uses the mechanism of self-healing to plug pinholes in the polymeric membrane of fuel cells selectively. The described application of self-healing in fuel cells is a flagship approach but subsequently transferable to several kinds of polymeric membrane or foil showing pinholes or fine cracks that should be sealed.

The new approach for optimising fuel cells' effectivity contributes to the transition from an economy based predominantly on fossil raw materials to a sustainable

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economy. The innovative biohybrid technology focuses on developing a hybrid product and the associated hybrid process created through the integration of biological components in the technical system and benefits from this integration. As biological actuators, enzymes are selected. Enzymes have the characteristics of being selective and specific in terms of reaction and substrate binding. The enzyme takes up the role of a small factory within the fuel cell. It serves as a material producer and fulfils sealing pores in the membrane by manufacturing a polymeric product.

The fuel cell is generating great interest as a future energy converter, which can be operated emission-free with high efficiency and independent of fossil fuels. The central element of a fuel cell is the electrolyte, which enables the chemical reaction of hydrogen and oxygen and consequently allows electricity generation. The polymer electrolyte membrane fuel cell (PEM-FC) is used in small devices and vehicle applications and is run between 60-80°C [1]. The choice of enzymes is preliminarily limited for the described approach in terms of operating temperature as it has to be compatible with the operating conditions of PEM-FC.

The technical problem to be solved deals with defects, so-called pinholes, in polymer membranes. Proton-conducting polymer membranes are particularly susceptible to pinholes. These can occur during the production process, e.g. because of handling errors or the protrusion of gas diffusion electrode fibres, and in PEM-FC operation. In particular, frequent start-up cycles lead to pinholes during operation due to the membrane's chemical, mechanical, and thermal stress. As a result, pinholes are only the beginning of a longer ageing process of polymer membranes. The defects increase over time, the pinholes grow and lead to the loss of function of the respective application, which becomes noticeable in a drop in performance and, finally, the premature end of life of the application.

Nafion™ developed by DuPont is the most commonly used membrane material in PEM-FCs. It offers a good compromise between stability and proton conductivity, making it one of the best-selling membrane materials on the market. Several approaches were undertaken to optimize it to that affect. Research focusses on composite membranes with inorganic fillers, or metal organic fillers, or ionic liquid fillers. Most composite materials result in higher stability, better proton conductivity, and can operate in lower humidity conditions than pristine Nafion™. [2]

But even better performing material can not completely avoid wear and pinholes in the long term. Since there is no possibility to repair this sign of wear when the fuel cell is already assembled (besides the disassembly of the fuel cell, which is highly inefficient economically), the lifetime and performance of the fuel cell stack are continuously reduced. [3, 4]. A reduced functionality directly raises fuel consumption and maintenance costs. Early self-healing of the defects thus would decrease the total cost of ownership of a vehicle through increased functionality and performance. This contributes to a long-lasting, sustainable technology and motivates the examination of the mechanism to repair pinholes in proton-conducting polymer membranes of PEM-FC in this research.

2. State of the Art

Polymer self-healing mechanisms exist for polymer hydrogels, in which embedded enzymes heal cracks by crosslinking the polymer environment [5, 6]. In addition, the self-healing of an elastomeric film by chemical crosslinking has been described [7]. The production of polymer films by enzymes through enzymatic polymerisation has also been well studied [8]. Regarding self-healing mechanisms in PEM fuel cells, several patents are specified, where the self-healing process is induced physically, either by melting an additional material in the modified membrane [9] or by encapsulating a filler that is released by cracking [10]. Other enzymes and polymer membranes application include biosensors [11] and biofuel cells [12]. The transferability of the new self-healing mechanism to these applications would be conceivable.

Table 1. Overview of state-of-the-art solutions similar to the approach.

	Enzyme-regulated healable polymeric hydrogels, 2020	A highly stretchable autonomous self-healing	Enzymatically synthesised polyaniline film, 2012	WO2004082813A2	US 2017/ 0209837A1	Biosensors based on immobilisation of enzymes in polymer-coated with Nafion, 2006	Perfluorosulfonic Acid-type membrane for an enzymatic fuel cell, 2016
Self-healing of polymers	X	X	-	X	X	-	-
Contains polymeric membrane	-	X	-	X	X	X	X
Contains enzymes	X	-	X	-	-	X	X
Enzymatic polymerisation	-	-	X	-	-	-	-
Enzymatic fillers are polymers	X	-	X	-	-	-	-
Fuel cell application	-	-	-	X	X	-	X

The described approaches to self-healing in fuel cells have the deficit that the membrane must be fundamentally modified. In addition, multiple self-healing at one point of the membrane is not possible because the filler is finite.

The new approach, described in the following, shall have the advantage that the filler is infinite, and thus multiple self-healing cycles are possible. In addition, the modification of the membrane is small since only the surface is modified.

3. The Self-Healing Concept

This project aims to create a biohybrid system that uses a self-healing mechanism to repair pinholes in the membrane of fuel cells selectively. This is made possible by the use of filler-forming enzymes. A proof-of-concept of the mechanism will first be demonstrated on a fuel cell membrane.

For realisation, the enzymes will be immobilised on the membrane surface via a special peptide linker to enable site-directed filler production. Enzymes have the advantage of carrying out chemical reactions relatively quickly and selectively and are robust to changing process conditions, such as changing temperature or acidity. The enzymes shall be integrated into the membrane during membrane electrode assembly. Immobilisation grants them increased stability to withstand frequent fuel cell operating cycles. Then, the self-healing mechanism takes place 'offline' when the vehicles or trucks are in parking mode. For this purpose, a substrate from a special subtrate-tank (similar to an AdBlue® tank) is fed into the gas inlet of the fuel cell stack, where the enzymatic reaction is catalysed, and the substrate is converted into the filler. The filler is a polymerised biological product, similar to the polymer membrane, and seals the existing pinholes, see Fig. 1.

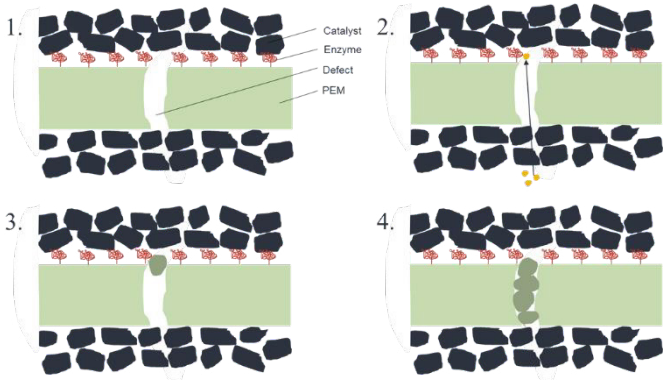

Fig. 1. The self-healing mechanism in four steps. 1. Cross section of PEM-FC including an exemplary defect. Enzymes are immobilised at the PEM surface. 2. Substrate is fed to enzymes through pinholes and triggers reaction. 3. The substrate is converted into a filler, a polymer. 4. Pinholes are closed by filler.

For the realisation of the self-healing mechanism, three steps are needed to be investigated: (1) the immobilization of enzymes or to be more precisely of lipases on the membrane surface, (2) the enzyme reaction, catalyzing the substrate to a polymer, and (3) the adhesion of the polymer in the membrane defects by interactions. The self-healing process can be applied to various polymer membranes. The application to Nafion™ 212, a sulfonated PTFE, is shown here.

4. Methodologies & Experimental

To achieve the proof of principle of the self-healing fuel cell membrane, the first results and methods of investigation are presented. The use-case specific characterization of the membrane was discussed in section 4.1. Then the initial results for the immobilization strategies were investigated in section 4.2. In sections 4.3 and 4.4, the selection of enzyme and the investigation of filler were shown, respectively.

4.1. Membrane characterisation

The characterisation of the membrane is divided into the measurement of the size of the artificially introduced pinholes to examine the effectiveness of the healing process after the enzymatic treatment and the interactions of the solvents of the enzyme solution with the membrane during wet application of the functional film.

The examination of the pinholes before and after the enzymatic healing process is intended to confirm the effectiveness of the process in terms of a reduction in the hole size or complete filling of the pinhole and thus serves as a quality assurance measure. Pinholes, which normally occur during fuel cell operation due to chemical and mechanical degradation, were artificially introduced by us into the Nafion™ membrane using needles. A digital microscope (VHX-7000, Keyence with lens VH-Z100R, Keyence) and a surface profilometer (Dektakt XT, Brucker) are used to identify and characterise the hole size. The pinholes can be detected, and their sizes can be determined by different characterising methods, as shown in Fig. 2.

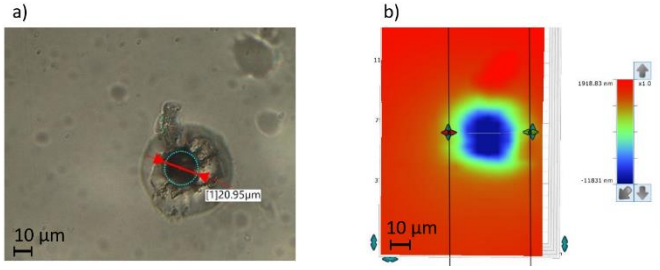

Fig. 2. a) photomicrograph, b) height analysis using the surface profilometer for an exemplary artificially made pinhole.

Due to the needle-based perforation, the area around the actual pinhole (Fig. 2 b) green area) is also visible. The investigations carried out in this work focus explicitly on the size measurement of the actual pinhole. The artificially inserted pinholes are reproducible in size based on analysis using the digital microscope D = 21.07 µm ± 1.35 and the surface profilometer D = 21.10 µm ± 0.92. The standard deviations of approx. 1 µm can be attributed to the not exactly round shape of the holes. The artificially inserted and investigated pinhole sizes in a range from 10 µm to 450 µm in diameter fit into the real diameter range of occurring pinholes in fuel cells [3, 13, 14]. The possibility to accurately insert and determine artificial pinholes form the initial basis for later measurements of the healing effectiveness.

Moreover, the interactions of the Nafion™ membrane with different solvents of the enzymatic solution and the catalyst ink were analysed. In order to apply the enzymes at their site of action, they are to be coated directly onto the membrane. Therefore interactions of the membrane and solvents affect the effectiveness of the coating process. Nafion™ interacts differently with solvents due to its molar structure consisting of hydrophobic (main chain) and hydrophilic areas (side chains). These different Nafion – solvent interaction are closely related to the swelling behaviour of the membrane. Different potential solvents for the enzyme solution such as, alcohols, water and Dichlormethane (DCM) were investigated in this study. As already shown, alcohol interacts strongly with Nafion™ [15, 16]. Water showed a lower absorption behaviour, also confirmed in [17]. With DCM no interactions between the solvent and the membrane were optically visible. For this reason, alcohols should be avoided in the formulation of the enzymatic coating solution. Further interactions of solvents and the Nafion membrane need to be considered regarding the influence on the stability of the connection between membrane,

linker and enzymes. In addition, the biocompatibility of the solvents used with the enzyme must be investigated.

4.2. Immobilisation

Strategies to immobilise enzymes or larger particles onto polymeric substrates typically rely on utilising linker molecules, which covalently bind to both species. To form covalent bonds, both enzyme and polymer surfaces must carry functional groups like carboxyl-, hydroxyl-, or amino groups. While this is guaranteed in the enzyme, many polymers like polypropylene do not carry such moieties. The necessary anchor points can then be introduced, e.g., by surface oxidation prior to linker immobilisation. However, this functionalisation protocol does not apply to perfluorinated polymers like Teflon™ or Nafion™ due to the high stability of the C-F bond. Even though Nafion™ copolymers theoretically carry anchor points in the form of sulfonic acid groups in their side chains, conversion (esterification) of these functions to immobilise a linker may be ill-advised since this could restrict the PEM in its primary function in the fuel cell due to the crucial role of the sulfonic acid moieties in the proton transport mechanism.

The initial inaccessibility of such perfluorinated polymer surfaces for conventional covalent linking strategies can be circumvented by utilising basic principles of fluorous chemistry. The term fluorous chemistry involves compounds in which the majority of hydrogen atoms are replaced by fluorine atoms providing these molecules with special physicochemical properties [18]. Their most characteristic features are a hydrophobic nature and a strong affinity to fluorous phases.

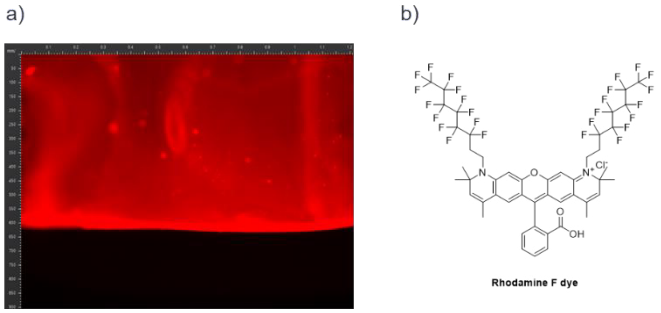

Fig. 3. a) Model experiment result showing F-tagged rhodamine dyes (b)) attachment to Nafion™ membrane in a fluorescence microscope. 10 µM dye was dissolve in 50 wt% methanol. Then, membrane was dipped into solution for 5s, washed with methanol solution, and dried.

Applying this principle of fluorous chemistry to our linker system, we sought to design a linker building block carrying such an F-tag to introduce a fluoric domain that enables fluorophilic interaction with the perfluorinated Nafion™ surface. For proof of principle, readily available F-tagged Rhodamine dyes from previous work [19] were immobilised on Nafion™ membranes by treating the membrane according to methanolic dye solutions. Adsorption/adhesion was verified via optical detection methods (fluorescence microscopy), see Fig. 3. To our delight, the non-covalent fluorophilic attraction between dye molecules and membrane turned out very strong, resisting a re-solubilisation of the dye by various solvents, even perfluorinated ones.

We designed an F-tagged lysin building block with these promising results in hand, which can be integrated into the peptide spacer via common peptide chemistry, see Fig 4. A further advantage of this modular approach is certain flexibility. If one F-tag does not enable a sufficient attraction between membrane and enzyme-linker-construct, a second or third one can be easily implemented by incorporating the several F-lysin building blocks in the peptide chain.

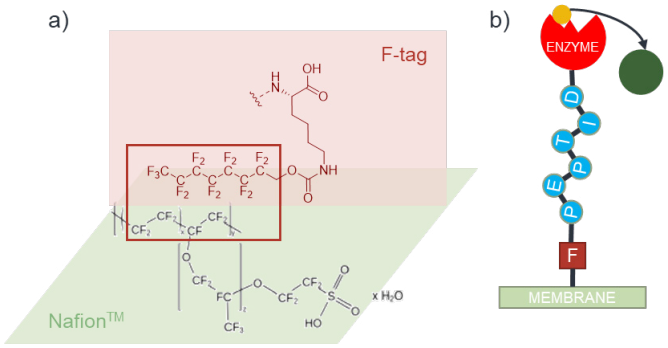

Fig. 4. a) F-tagged lysin building block attached via fluorophilic attraction to Nafion™ membrane, b) Immobilisate's structure comprising F-tag, peptide and enzyme.

While the F-tag performs the function of stable attachment to the membrane, the peptide serves as a spacer, optimising the enzyme's activity and stability, for instance, by adjusting its distance to the membrane surface.

We focused on the attachment of the enzyme to the peptide. A high-density peptide array was fabricated using a photolithographic method [20]. The size of the peptide spot was set to 34 µm, and the distance between the spots to 34 µm as well. These spots were arranged on the standard microscope slide in the form of eighth windows. A special template on top of the peptide slide enabled eight separated volumes of 250 µl in which the binding efficiency and the functionality of the immobilised enzymes could be studied separately under different concentrations of the substrate. In all windows of the chip, only one DAAAA peptide (D = aspartic acid, A = alanine) was synthesised, although the used technology allows synthesising up to 170.000 different peptides on the same surface of a microscope slide. The purpose of this experiment was to demonstrate the fundamental possibility of attaching the enzyme on the surface of the chip, as this would open up opportunities for high-speed screening of the optimal peptide linker.

During the peptide synthesis on the chip, the amino group of the head amino group of acid D (aspartic acid) was blocked, and the carboxyl side group was deprotected by applying trifluoroacetic acid to the chip surface. Then, the peptide array was incubated with the lipase to achieve its coupling to the carboxyl group of the D with carbodiimide as a coupling reagent. The coupling duration was two hours. After coupling the enzyme with the peptide, the enzyme's functionality was tested using an using *para*-nitrophenyl acetate (*p*NP-Ac) assay. We observed linear turnover of the substrate during the first 20 minutes after incubation. It was comparable to the turnover of substrate observed by us in an early experiment in a microwell plate. Thus, we experimentally demonstrated various elements of enzyme immobilisation on the membrane and the high-density peptide array.

4.3. Enzyme selection

Living cells are constantly producing different macromolecules, among them polysaccharides, proteins (which can be seen as polyamides from a chemical point of view) and polyesters, involving enzyme-catalysed chain-growth polymerisation reactions of activated monomers [21]. Enzymes are already successfully applied in many fields of organic synthesis including several approaches of polymer synthesis, offering advantages such as avoidance of organic solvents [22], replacement of potentially toxic (heavy metal) catalysts, and rendering costly protection/deprotection procedures unneccessary [23]. Especially lipases are commercially available in larger quantities and have been shown to be highly stable towards reaction conditions usually considered as harsh for biomolecules (e.g. elevated temperatures or acidic pH values) after immobilisation. So already a decade ago, an immobilised lipase from the yeast *Candida antarctica* (CALB) has been investigated for a higher scale polyester production, more precisely the enzymatic production of glycerol adipate on a 500g scale in a heated, solvent-free system [24]. This enzyme CALB immobilised on acrylic resins (commercially available as Novozym 435) is perhaps the most widely used commercial biocatalyst in both academy and industry due to its interfacial activation and thus unusually elevated activity, whereas immobilization normally leads to less flexible and therefore stable, but less active enzymes [25]. However, for the desired application on PEM-FCs, these highly active CALB immobilisates are not applicable due to the acrylic resin particle size of up to 1mm. So a free lipase had to be immobilised *de novo* on Nafion™ membranes.

As a first model reaction to quantify the immobilised enzyme's activity, a colour assay using *para*-nitrophenyl acetate (*p*NP-Ac) was performed. Hydrolysis to *para*-nitrophenol (*p*NP) leads to the development of bright yellow colour, whereas the condensation reaction of *p*NP + acetic acid results in a colour decrease. Both reaction directions can be quantified in high-throughput by measuring the absorbance at 410 nm (Epoch microplate spectrophotometer, Agilent Technologies) which is depicted in Fig. 5.

First experiments were carried out with the free lipase from the yeast *Candida rugosa* (CRL) as a first model enzyme extensively investigated at the chair [26]. The enzyme appeared highly useful for a proof-of-principle study due to its pH activity profile with no activity, but maximum stability at pH 3. 100% activity was regained after overnight storage at pH 3 when exchanging the buffer to pH 7 again. So, no inactivation of the enzyme is to be expected at the acidic fuel cell operating conditions, and no hydrolysis activity that might lead to degradation of a polymer synthesised by this enzyme. However, "thermal inactivation above 45°C limits CRL's applications", although certain improvement appears feasible by rational design [27]. Moreover, no activity against fluorinated compounds has been described for this enzyme. So, in parallel to the establishment of novel immobilisation methods for CRL (see above), a functional-based screening for other lipases is performed, starting with the lipase basic kit (Sigma-Aldrich, SKU: 62327-1EA).

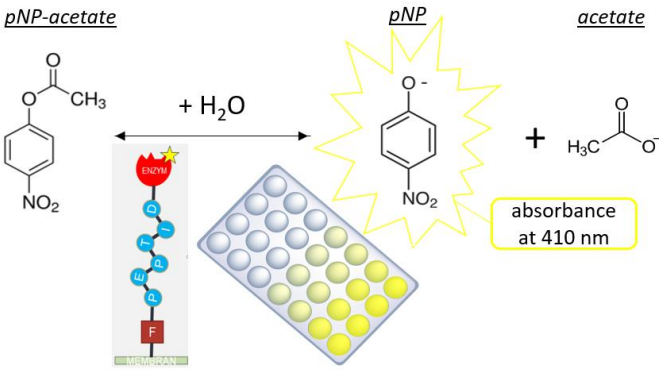

Fig. 5. Scheme of model reaction analysing enzyme's activity using the colour *p*NP-Ac assay.

4.4. Investigation of filler

Initially, the polymeric filler and its precursor monomers must fulfil two criteria: (1) A certain "combability" between filler material and membrane. (2) Enzymatic polymerisation of the precursor monomers must be possible and lead to macromolecules of sufficient molecular weights.

The first criteriuma can be addressed based on the previously investigated linking strategy exploiting the attractive fluorophilic interactions between a perfluorinated membrane and similarly fluorinated molecules. Polymers carrying CF3-groups or short F-tags could likewise show attractive interactions with the membrane material and among themselves, resulting in adhesion at the edges of pinholes and accumulation of material, thereby effectively sealing the pinhole. However, to the best of our knowledge, only one example for the polymerisation of fluorinated monomers by CaLB is mentioned in literature. The reason most probably is that fluorine groups are not widely prevalent in natural products – a fact commonly exploited in drug development to introduce higher metabolic stabilities [28].

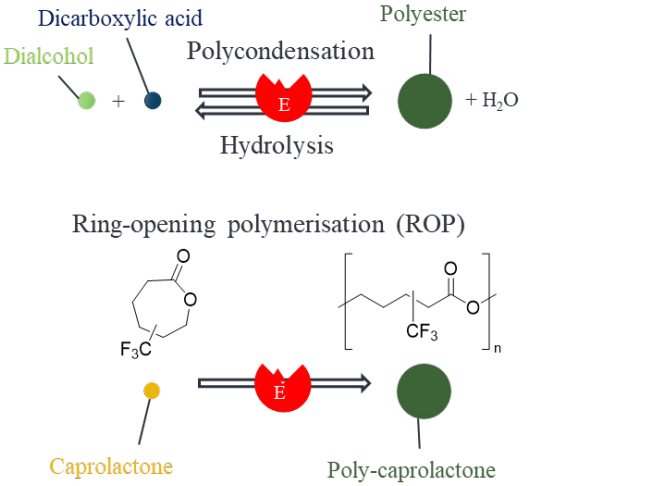

Fig. 6. Enzymatic polymerisation reactions catalysed by lipases, in the upper part the polycondensation, in the lower part the ROP with appropriate substrates and polymeric products.

A further obstacle is a comparably low degree of polymerisation typically reached in polycondensations like the one mentioned above. In step-growth, high degrees of polymerisation can only be achieved at high conversion rates

(>99%). Polycondensation often requires techniques like fractioned distillation to remove by-products such as water, which is not feasible to implement in a fuel cell. Instead, step-growth polymerisation based on ring-opening polymerisation (ROP) of, e.g. lactones or cyclic carbonates, was considered a more reasonable approach to ensure sufficient molecular weights of the polymeric filler material while relying on only one type of monomer. The polymerisation reactions mentioned are shown in Fig. 6.

5. Conclusion & Outlook

A novel approach for a self-healing mechanism of polymeric membranes, using enzymatic immobilisation and polymerisation strategies was conceptualised. At the example of a Nafion™ membrane, used in PEM-FCs, initial investigation results were shown. Artificial pinholes with a diameter of 21.1 µm could be introduced and reproducibly measured, providing the basis for evaluating the healing process. Interactions between the membrane and possible enzyme solvents for the coating step were investigated. In addition, different coating and drying strategies for the functional layers will be developed and tested, considering the combability of the process and the bioactive substances. For enzyme attachment to the membrane, a linker construct consisting of an F-tag and a peptide linker was designed. Successful binding of the enzyme to the peptide array opens the possibility of high-speed screening for linkers to optimise enzyme kinetics in further work. A new strategy for immobilisation onto a fluorine-containing membrane relying on non-covalent fluorophilic interactions was designed and investigated. Successful immobilisation of an enzyme via fluorophilic interactions could provide biochemists and biologists with new opportunities to biofunctionalise fluorous substrates in a straightforward way. In particular, the emerging field of hydrogenase-based biofuel cells [29], which currently rely on nonspecific enzyme immobilisation by drop-casting methods, could benefit significantly from this novel strategy. The presented work will now be further elaborated and the proof-of-concept of this biohybrid system will be realized using the PEM fuel cell as an example. Transferability of the self-healing mechanism to other applications with polymer membranes is conceivable.

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