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Investigation of the potential of dipping as a technology for bifunctional assemblies as a coating material

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Abstract

The biologicalisation of production represents an innovative approach to increase sustainability and efficiency, as well as improve the functionality of existing products and processes by incorporating bio-based materials into production. One area of biologicalisation of production involves the use of customized proteins for surface functionalization. The potential functionalities that can be achieved are diverse. For example, customized proteins have the potential to achieve antibacterial and anticorrosive functions. The resulting potentials can be utilized, for instance, in medicine and production technology. However, currently, there is a lack of fundamental knowledge regarding the application of customized proteins as bio-based coating materials for various conventional materials. This leads to new challenges for research and industry. Technologies for the scalable processing and application of bio-based materials such as bifunctional assemblies have not been sufficiently investigated yet. For the application of bifunctional assemblies for coating surfaces, coating technologies have been identified in the past, which are potentially capable of applying customized proteins as coatings to ensure individual functionalities. One of the identified coating technologies with high potential is dipping. The aim of this work is to present the results of initial investigations on the coating of different base materials with the fluorescent bifunctional assembly eGFP-LCI. The fluorescent bifunctional assembly was used because by measuring the fluorescence, an evaluation of the adhesion and based on that, the identification of possible cause-effect relationships in the coating of different materials with tailored bifunctional assemblies is possible. The findings from this series of experiments show that with increasing dipping duration, peptide concentration, and surface roughness of the workpiece cause an increase of the coating quality of PLA. While for other investigated materials, the observations did not apply. These fundamentals are essential for identifying further influencing factors and processability in the dipping process for bifunctional assemblies in production technology for common materials.

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Keywords: Biologicalisation, protein, manufacturing, coating, dipping test

1. Introduction

Global resource extraction has more than doubled in the past years since 1980 [1]. The manufacturing industry is responsible for a significant part of the environmental damage caused by resource extraction [2]. With the ever-increasing product quality requirements due to innovations in technology and material science, as well as society's demand for more sustainable products and thus the decrease of carbon dioxide and other emissions to the environment, manufacturing companies need to make their use of resources much more efficient and sustainable [3]. According Bryne et al. biological transformation takes a central role in the research and development of innovative products and represents an opportunity for resource conservation, as conventional materials can be substituted with alternative newly developed bio-based materials [4, 5]. Previous work has focused on the conceptualization and linking of biology and manufacturing

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and different potentials and fields of application are highlighted [6, 7]. The biological transformation can be divided into bio-inspiration, bio-integration and bio-interaction [5, 6]. Various application fields are influenced by biologicalisation. bio-inspiration [8] and bio-integration approaches are already being researched for technical applications [9]. For example, nanotechnological surfaces that mimic the properties of shark skin are expected to enable more efficient movement in water and increase durability by avoiding fouling [5]. Furthermore, bio-integrated materials like microbial-based cutting fluids be used to reduce the negative impact on environment and human health [10]. Additionally antibacterial functionalities for titanium implants could achieved with bio-integrated materials to reduce the bacterial infections [11]. In the aspect of bio-interaction, bio-intelligent manufacturing is to be achieved through the interaction of the biological, technical and information systems [6].

An example of a complete biological transformation is the concept of a milling machine, where the cooling lubricant is containing vegetable oil in combination with a cooling lubricant system which should work self-sufficient using bacteria and algae. The thermal and heat management is designed according to the biological model of animals to achieve temperature stability during the processing. A further topic is the substitution of harmful materials with ecological biodegradable thermal and acoustic insulating materials, though which all three aspects of biological transformation were considered. Due to the challenges resulting from the biological transformation and high demands of manufacturing bio-based coatings represent a possible solution. Therefore bio-based coatings could be useful for the individual functionalization of surfaces and resource conservation. [6, 12]

The use of bio-based materials, such as functionalized proteins as coating material, can become an essential part of biological transformation [13], which is intended to realize a more sustainable production through the use of bio-based materials, because they are produced of renewable raw materials and are biodegradable [14]. One potential field of research is surface functionalization of components [15]. By functionalizing component surfaces, various properties can be selectively optimized [16]. Functionality describes the ability of a component to reliably perform its intended function under given conditions and external influences over a defined period of time [17]. With the possibility of protein engineering by rational design and directed evolution (Nobel Prize in Chemistry 2018), proteins can be specifically designed to match functional demands in manufactured products. This enables an efficient bio-intelligent manufacturing [18]. To functionalize surfaces with designed proteins, its necessary to combine them with also customized material binding peptides (anchor peptides) which have a high binding strength. A linker is used to connect the two functional modules. The system consisting of the described surface functionalization modules is referred below as a bifunctional assembly and is already under investigation. [15, 19]

The schematic structure of a bifunctional assembly and the various function modules are shown in figure 1 and described in more detail below. The first functional module (anchor peptides) consists of less than 100 amino acids, while the second functional module consists of proteins and enzymes that are larger and have different functions (e. g. fluorescent, antibacterial, superhydrophobic, anticorrosive, etc.) [18, 19]. The anchor peptide (1) enables selective binding with high adhesive strength to various materials and surfaces, such as synthetic polymers, metals, glasses, ceramics, and natural surfaces. A linker (2) is attached to the anchor peptide, which serves as a connector between the second functional module (3) which realizes biological, chemical, or physical functions [20–22]. With the use of bifunctional assemblies as coating material, medical and manufacturing applications can be realized, making a social and economic contribution, for example, to reducing the risk of infection and conserving resources as the population grows. Therefore, knowledge about the processability with coating of bifunctional assemblies is necessary to make the economic and social potentials usable.

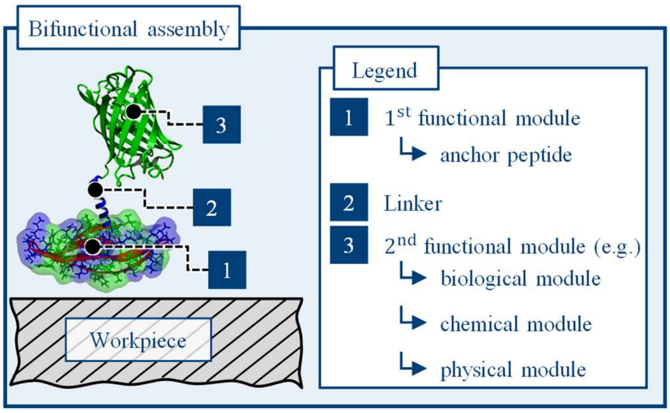

Figure 1 Schematic structure of bifunctional assembly

2. State of the art

In the field of biological transformation, coating with bifunctional assemblies represents a new and innovative approach to the functionalization of component surfaces. The substitution of conventional coatings by innovative bifunctional assemblies as coating material enables sustainable product and process improvement [15]. In the following, coating categories currently used in production technology will be presented to investigate the suitability for bio-based coatings and show their deficits. This is followed by various research approaches dealing with bio-based coatings. In manufacturing technology, conventional coatings can be divided into metallic, inorganic non-metallic, and organic coatings [23]. In the field of organic coatings, mainly liquid high polymer coatings are used [24]. According to the current state of the art, metals and polymers are mainly used for coatings, so various coating technologies have been developed specifically for this purpose [25]. High process temperatures are usually required for the coatings which are far above 100°C. Since proteins denature and lose their functionality at temperatures around 100 °C, the common coating technologies in the manufacturing are not suitable for processing biological systems like bifunctional assemblies [13]. Initial investigations into binding mechanisms and protection of bio-based coatings on surfaces have already been conducted. For example, TAPIA-LOPEZ et al. investigate the attachment of a bio-coating made of laminin-5 proteins to a zirconia surface [26]. SEHR conducted coating experiments on titanium quartz in laboratory scale using lubricin to test coating methods and investigated the influences of the protein on bacterial growth and adhesion [27]. MURUVE et al. studied the binding of a peptide-based corrosion protection coating on stainless steel and QI et al. examined the binding of a multifunctional protein coating for anti-fogging, self-cleaning, and antimicrobial properties [28, 29]. CHEN et al. attempted to integrate bio organisms into a porous latex layer with sufficient permeability to enable mass transfer with the environment [30]. CORTEZ et al. considered various coating methods to coat surfaces with a latex layer in which bio organisms (mixture of cells, latex and porogens) are embedded [31]. The investigated approaches showed the functionality and the effect of the bio-based coating, however none considers which technologies or process parameters are suitable for processing bifunctional assemblies, to apply them on surfaces [6, 18, 32]. In previous work, a bifunctional assembly coating consisting of anchor peptide and an antibacterial functional module was applied to a titanium surface. The bifunctional assemblies adhere to the surface of a particular material with high binding strength due to the functionality of the anchor peptides. Depending on the application, the coated components are subject to wear due to contact and relative movement of foreign bodies. In order to protect the applied bifunctional assembly coating applied to the component surface from mechanical wear, it was investigated how different surface texture could improve the protection of the coatings [15]. GARAY et al. coated poly-caprolactone (PCL, a polymer used in medicine) with a modified bio-based coating consisting of the bifunctional assembly eGFP-LCI and the antibacterial functional module Endlys and demonstrated antifouling and antibacterial effects. The use of the fluorescent functional module eGFP in this hybrid construct serves as a common approach to detect the occupancy density of a protein. The intensity and continuity of the coating can be determined, for example, using an FE-SEM. Due to the fluorescent properties of eGFP, it is possible to determine the relative fluorescence unit (RFU) by measuring fluorescence spectra and determining quantum yields of samples. This provides an possibility to determine the occupancy and quality of the bifunctional assembly as a coating material [33, 34]. This antibacterial functional module of the hybrid construct can minimize bacterial growth in wounds and therefore reducing the spread of infected wounds. This results in enormous social and economic potential [34, 35]. The state of the art has shown that bifunctional assemblies offer significant potential as coating material however, fundamental knowledge of their processability to identify possible process parameter and process parameter windows for industrial applications is lacking. Therefore, various coating technologies need to be investigated first. However, the research focus of previous work was on the coating function itself rather than the coating process. Therefore, in this paper experiments are carried out using the dipping process to gain fundamental knowledge about dipping as a coating process for bifunctional assemblies on different materials to gain knowledge to scale up. The results obtained should provide initial insights into the characterization of processability and potential process parameters. This will enable further investigations and knowledge-based surface functionalization using bifunctional assemblies. The experimental setup and procedure are described below.

3. Dipping experiments with bifunctional assemblies as coating material on various materials

By understanding the cause-and-effect relationships and requirements of the new materials and processes, manufacturing technology will be able to realize processes for the processing of bifunctional assemblies harness their potential. Therefore, the experiments in this paper aimed to identify initial knowledge to achieve a knowledge based processability of bifunctional assemblies to scale up the bifunctional assembly coating process. Dipping was selected as coating technology, because it delivered promising results in previous investigations and it is comparable to the process, which is described in the state of the art (cf. RÜBSAM et al [19]). For the verification of the successful attachment and fulfillment of the functionality of the bifunctional assemblies, a fluorescent functional module (eGFP) is commonly used (cf. RÜBSAM et al [21]).This enables the determination of a quantifiable measurement parameter to show the quality and quantity of the bifunctional assembly binding. The relative fluorescence units (RFU) indicate the occupancy and quality of the bifunctional assemblies as a coating material, which can be used to make specific modifications to improve the functional modules, such as increasing the binding strength or adhesion time of the anchor peptide. As the anchor peptide the peptide LCI is used, which is specifically developed for binding to polymers (cf. RÜBSAM et al [21]). This anchor peptide was chosen because it is already comparatively well available and good results have already been obtained with this peptide [33, 34].

3.1. Experimental setup

Figure 2 shows the experimental setup, the schematic illustration of the bifunctional assembly, the workpiece dimensions, and the investigated process parameter (dipping duration t and peptide concentration cp) of the dipping experiments. The experiments described below for coating various materials (Table 1) with eGFP-LCI were conducted with the FORM WASH from FORMLABS (1). Since the bifunctional assemblies need movement for a better binding, the FORM WASH dipping system, which consists of a plastic container with an integrated propeller that generates fluid movement, was chosen to conduct the dipping experiments. The dipping system was filled with the mixture of solution fluid and bifunctional assembly specification eGFP-LCI (4)

Table 1 Experimental materials

Material	Density (ρ) $\left[\frac{g}{cm^3}\right]$	Roughness (Ra) [μm]
Aluminum [36]	2.71	0.305
Stainless steel [36]	8	0.1
Titanium [36]	4.507	0.075
PLA [37]	1.24	0.17
ABS [37]	1.04	0.06

The mesh grid (2) was attached to a holder that can be moved up and down using a linear motion motor. The workpieces (5) used in the experiments had a diameter (d) of approximately 5 mm and a thickness (b) of 3 mm. A workpiece holder (3) was designed and produced using a 3D printer for fixation of the workpieces. The target peptide concentration c_p of the mixture fluid was achieved by dilution with solution.

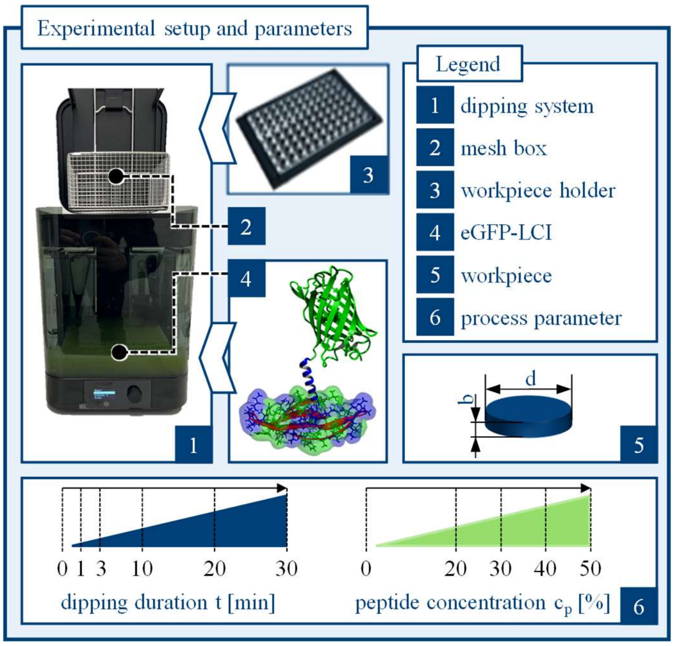

Figure 2 Experimental setup and parameters

3.2. Execution of experiments

In the following, the experimental procedure is described. The investigated test workpieces were fixed in the custom-made workpiece holder and placed in the grid box of the dipping system. The linear motion motor moved the grid box with the sample holder, transporting the samples in and out of the bifunctional assembly solution mixture at each test point. During the dipping process, an integrated propeller rotated with a relative velocity, creating a continuous fluid motion. The roughness of the investigated workpiece surfaces was measured with the HOMMEL ETAMIC-T800 surface roughness gauge. The peptide concentration c_p and the dipping duration t were chosen as the process parameters. The selection of the workpiece materials and process parameters was based on previous investigation experiences as well as commonly used materials in the industry to maximize applicability in manufacturing. The influences of these process parameters on the coating process of the materials listed in Table 1 were investigated by block-wise variation of the peptide concentration c_p and changes in the dipping duration t. The variation of the peptide concentration c_p was achieved by block wise diluting the peptide concentration c_p with a buffer fluid after completion of a test block. To achieve the statistical assurance, the dipping experiments were conducted randomized and twice for statistical assurance.

3.3. Results and analysis of the dipping tests

The experimental results are presented and analyzed below. Figure 3 shows exemplary the determined relative fluorescence units (RFU) of the dipping coating experiments for the materials Aluminum, PLA, and Titanium in a three-dimensional effective area diagram. For the three-dimensional effective area diagram, the dipping duration t is plotted on the x-axis, the peptide concentration c_p on the y-axis and the RFU on the z-axis. The fluorescence measurements ins this work was performed using BMG LABTECH's CLARIOSTAR PLUS to determine the occupancy and quality of the bifunctional assembly as a coating material at the Chair of Biotechnology at RWTH Aachen University. Diagram (a) shows that the bifunctional assembly as a coating material has a much higher RFU than the other two investigated materials in (b) and (c). The determined RFU values of aluminum in diagram (a) are increasing more than the materials in the other two diagrams after a dipping duration $t > 10$ min and increasing peptide concentration c_p. With an RFU of about 75,000 at a dipping duration $t = 30$ min, approximately 10 times as many bifunctional assemblies adhere to the aluminum surface compared to the other investigated materials.

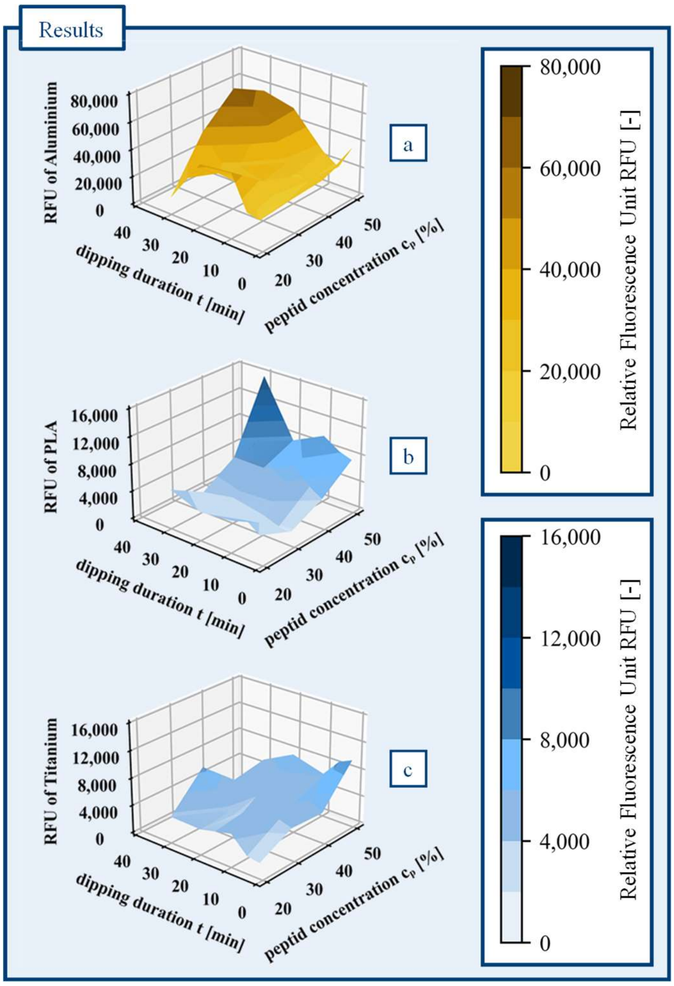

Figure 3 RFU three-dimensional effective area diagram of Aluminium, PLA and Titanium in dependence of the dipping duration t and peptide concentration c_p

A possible reason for this is that the increased dipping duration t and the resulting longer fluid movement in combination with the higher surface roughness Ra can lead to increased binding quantity of the bifunctional assembly as coating material, which has been detected by the fluorescence. Furthermore, at a peptide concentration c_p = 20%, the RFU shows an unusual progression with increasing dipping time t. For PLA (a), the plot of the RFU recorded an increase with increasing dipping duration t and peptide concentration c_p. While the measured RFU of titanium (b) show a decreasing curve with increasing dipping duration t for the peptide concentration c_p = 50% and an increasing RFU for the increasingly peptide concentration c_p for the dipping duration t= 10min. The three diagrams show that processability of bifunctional assemblies is generally possible. The observed correlations for diagrams (a) and (b) indicate that the dipping time has less influence on the coating density and quality than the peptide concentration c_p. Only the combined increase of both parameters leads to a stronger increase of the RFU. Furthermore, contradictory results were observed for the RFU values for titanium, which may be an indication of the selectivity of the adhesion of the bifunctional assembly. As mentioned above, the anchor peptide was developed for polymers. The described observations were also made for the other investigated materials. Finally, these observations support previous work indicating that the selective binding of bifunctional assemblies to certain materials can be achieved, suggesting the possibility of modifying bifunctional assemblies by bioengineering. Here, there is a need for further research on the identification and processing of additional selective anchor peptides for further materials in order to holistically exploit the potential of bifunctional assemblies. To further elaborate the results obtained, Figure 4 shows the RFU as a function of the peptide concentration c_p at a dipping duration t =10 min. Diagram (d) shows the variation of the RFU with increasing peptide concentration c_p for the dipping duration t of 10 min for all investigated materials. The dipping duration t is shown on the x-axis and the RFU is shown on y-axis. Aluminum shows higher RFU values between 30,000 and 45,000, which may be due to the higher surface roughness Ra of the workpieces previously mentioned. Furthermore, diagram (d) indicates that

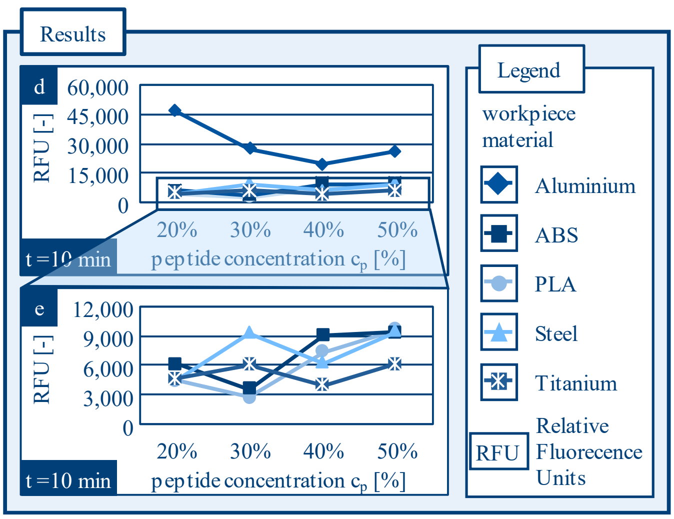

Figure 4 RFU of the investigated materials in dependence of the peptide concentration c_p and the dipping duration t=10 min

the RFU of aluminum is not clearly dependent on the increasing peptide concentration c_p, as already suspected in diagram (a). The reason for this could be the selectivity of the bifunctional assemblies. Further investigations are required to verify this hypothesis. Diagram (e) shows the framed area from diagram (d) and shows that the investigated polymers PLA and ABS have a comparable trend, and it can also be identified that steel and titanium have a similar characteristic without a clear trend. The measured RFUs of these four material specifications were between 3,000 and 9,000. Although an increasing trend with higher peptide concentration c_p was identified for the investigated polymers (PLA and ABS) for c_p >30%, further experiments are needed to confirm this effect or to enable a concrete mathematical description. The results of this study confirm that the coating of several materials with bifunctional assemblies is possible. For PLA a significant influence of the process parameter combination was observed, while the measured RFU for the other materials showed influences which need further investigations to identify concretely influences. Furthermore figure 3 and 4 showed that the peptide concentration c_p had a comparatively higher influence on the RFU and thus the binding density and quality than the dipping duration t. Previously, adhesive holding mechanisms were assumed for the binding of the bifunctional assemblies. These initial investigations show that, further research with specific developed bifunctional assemblies is needed to identify and characterize the cause-effect relationships and the processability of bifunctional assemblies.

4. Conclusion and outlook

This paper presents initial investigations into the coating of various materials with bifunctional assemblies. The state of the art illustrated the enormous economic and societal potential of the use of bio-based materials (e.g., bifunctional assemblies) as well as the knowledge deficits in this field. To provide further basic knowledge to close this knowledge gap, the results of dipping tests for bifunctional assemblies as coating material were presented in this paper. The focus of the experiments was on the occupancy density of the surface of different materials. Dipping was chosen as the coating process to identify possible process parameters for processing bifunctional assemblies. The functional module eGFP fluoresces and thus the RFU value can be used to measure the binding capacity and the occupancy density of the bifunctional assemblies on different materials. Specifically for PLA, a correlation between the dipping duration t, peptide concentration c_p, and RFU was observed. This can be explained by the selective binding of the functional module LCI (anchor peptide). For aluminum, higher values were determined, but the results did not show clear correlations of the process parameters and the coating occupancy and quality. The higher surface roughness of the aluminum samples was deduced as a possible explanation for the higher RFU values. This observation indicates that the coating occupancy density can be influenced by the surface roughness Ra. This means that the surface roughness Ra has an influence on the quality of the coating with bifunctional assemblies, which needs to be further investigated. For the other materials investigated, no clear correlations between the process parameters and the coating quality could be identified. Which may be due to the selective binding of the anchor peptide (LCI) used. Here, there is a need for further research on the identification and processing of additional selective anchor peptides for further materials to holistically exploit the potential of bifunctional assemblies. Thus, further research and collaboration are necessary to transfer the bifunctional assemblies as coating material and identify the process parameters according to the ISO 25178 to an industrial scale in the long term. Due to the arbitrary interchangeability of the functional modules, it could find use in application areas like medicine and manufacturing. For example, the fluorescence functional module eGFP can be replaced by an antibacterial functional module and thus be used in medicine. Identifying additional process parameters and cause-effect relationships for large-scale processing of bifunctional assemblies is a possible approach to closing existing knowledge gaps. Furthermore, durability tests, different bifunctional assemblies with other functional components, the load limits, continuous stress, the fluid temperature T_f, the relative velocity of the fluid $v_{f,rel}$ and stability tests could be considered as possible process parameters.

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