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Silane surface modification for improved bioadhesion of esophageal stents

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ABSTRACT

Stent migration occurs in 10–40% of patients who undergo placement of esophageal stents, with higher migration rates seen in those treated for benign esophageal disorders. This remains a major drawback of esophageal stent therapy. In this paper, we propose a new surface modification method to increase the adhesion between self-expandable metallic stents (SEMS) and tissue while preserving their removability. Taking advantage of the well-known affinity between epoxide and amine terminated silane coupling agents with amine and carboxyl groups that are abundant in proteins and related molecules in the human body; we modified the surfaces of silicone coated esophageal SEMS with these adhesive self-assembled monolayers (SAMs). We utilized vapor phase silanization to modify the surfaces of different substrates including PDMS strips and SEMS, and measured the force required to slide these substrates on a tissue piece. Our results suggest that surface modification of esophageal SEMS *via* covalent attachment of protein-binding coupling agents improves adhesion to tissue and could offer a solution to reduce SEMS migration while preserving their removability.

1. Introduction

Cancer of the esophagus is one of the most lethal cancers, which has a cure rate less than 50% and a five year survival rate of only 5–10% [1]. Chemoradiation, laser ablation therapy, brachytheraphy and argon beam coagulation therapy with self-expanding stents have been widely used for palliative treatment of esophageal cancer [2,3]. Due to the advances in minimally invasive medicine and the developments in interventional endoscopy, the use of stents for either treatment or alleviation of strictures in the gastrointestinal (GI) tract including the esophagus has become more prevalent [4]. There are a variety of commercially available self-expandable metal stents (SEMS) designed for different portions of the GI tract. These stents have different physical characteristics such as dimensions, material composition, expansion properties and are deployed using varied delivery methods depending on their manufacturer and the intended GI organ.

While endoluminal stenting is a common procedure, stent migration occurs in upto 40% of the patients, especially in those who are treated for benign disorders, such as benign strictures, leaks and fistulae; migration is a major drawback in esophageal stent therapy [5–10]. One of the main reasons that the migration rate is higher in benign disorders is that therapies for these conditions utilize removable SEMS. In contrast, when SEMS composed of pure nitinol, which is a flexible shape memory metal alloy of nickel and titanium, are utilized, these stents expand, eventually embed into tissue and migrate less often. However, these uncoated SEMS have a higher risk of tumor ingrowth and they become nonremovable which makes them unsuitable for the treatment of benign disorders [11]. In an attempt to keep SEMS removable, they are partially or fully coated with silicone, which results in decreased ingrowth, but increases the possibility of stent migration [12]. It is possible to address this problem by introducing surface roughness which partially resolves both migration and ingrowth problems, but it also creates challenges in the retrieval of the stent [13–15].

In this paper, we investigated the possibility of increasing the adhesion strength of the SEMS to the surrounding tissue while preserving their removability by modifying the stent surface with

Abbreviations: APTES, (3-aminopropyl)triethoxysilane; GI, gastrointestinal; GPTMS, (3-glycidyloxypropyl)trimethoxysilane; SAMs, self-assembled monolayers; SEMS, self-expandable metallic stents; PDMS, polydimethylsiloxane; XPS, X-ray photoelectron spectroscopy.

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self-assembled monolayers (SAMs). SAMs offer unique opportunities for surface modification of biomedical implants and are particularly attractive due to their commercial availability, ease of modification and tunability of head- and tail-groups, consequently altering the surface properties. SAMs of alkylalkoxysilanes spontaneously form on the surface of hydroxyl terminated silicone surfaces by chemisorption [16]. The activation of these silane coupling agents through hydrolysis is the driving force behind the silane-SAM formation. Specifically, reactive siloxane groups undergo condensation with water and with surface silanol groups (—SiOH) and neighboring siloxanes to form a cross-linked polysiloxane $(—Si—O)_n$ network. The cross-linking of such organic molecules through head-groups onto a substrate from either liquid or vapor phase is followed by a slow organization of the tail-groups [17,18]. This phenomenon, the slow packing of the monolayer, is attributed to polysiloxane cross-linking and van der Waal interactions between hydrocarbon chains.

Our hypothesis is that modifying the surface of the silicone covered esophageal SEMS with silane coupling agents would improve adhesion of the SEMS to the surrounding tissue and thereby reduce its migration. Silane coupling is a well-known phenomenon that is based on a reversible hydrolytic bond mechanism between silane and a substrate with a stress relaxation capacity [19,20]. Under stress, the bonds between the silane and substrate break, allowing the system to slip across the substrate plane to reform bonds at new sites in the presence of water. The reversible hydrolytic bond mechanism, *i.e.* spontaneously reforming the broken bonds, prevents any loss of adhesion in silane coupling [21,22].

For the initial experiments, we used polydimethylsiloxane (PDMS) strips to mimic the silicone coated stent surface and reduce the use of expensive commercial stents. We focused on silane coupling agents with epoxide and amine tail-groups, (3-glycidyloxypropyl)trimethoxysilane (GPTMS) and (3-aminopropyl)triethoxysilane (APTES), respectively, due to their affinity for amine- and carboxyl groups which are abundant in body [23–26]. Fig. 1 shows the chemical structures of these silanes and the SAM formation on a silicone surface.

We developed a silanization technique which is applicable to silicone coated esophageal SEMS. After confirming the functionalization success using X-ray photoelectron spectroscopy (XPS), we tested the effect of the silane-SAM formation on the strength of the adhesion in *ex vivo* experiments. Importantly, we also measured the strength of adhesion with and without mucin gel to simulate adhesion in the presence of the mucus lining in the GI tract.

2. Materials and methods

2.1. Preparation of PDMS substrates

PDMS elastomer kit (Sylgard© 184; Dow Corning) was used to prepare PDMS substrates by vigorous mixing of 10:1 (w/w) ratio of the base polymer to the curing agent for 5 min. This mixture was then degassed under 700 mmHg vacuum for 35 min to remove all embedded bubbles. Once the bubbles were completely removed, the prepolymer mixture was pipetted into Fischer Scientific 47 mm petri dishes, which served as the mold, and the prepolymer was subsequently cured at 65 °C for 4 h in a gravity convection oven (Thermolyne®; Product no. Z111007; Sigma-Aldrich).

2.2. Preparation of mucus gel

Mucin from porcine stomach (Type II) was purchased from Sigma-Aldrich (Product no. M2378; Sigma-Aldrich). A 2% (w/w) mucus gel was prepared in PBS (Product no. 10010023; Life Technologies) to simulate the mucin content in the GI tract. The solution was placed on a stir plate at 550 RPM at room temperature for 45 min to ensure good, homogenous mixing. No heat was added to the system during gel preparation and a fresh batch of gel was made for each experiment to avoid protein aggregation and changes in protein conformation.

2.3. Surface modification protocols

PDMS substrates and the SEMS (Evolution, Cook Medical, Winston Salem, NC and Wallflex, Boston Scientific, Natick, MA) were exposed to an 18 W (at High setting) air plasma (Product no. PDC-32G; Harrick Plasma) for 30 s after pumping down for 4 min in order to reach a background pressure of 200 mTorr. This low power plasma oxidation converts surface methyl groups to hydroxyl groups [27]. Post-activation, substrates were jetted with nitrogen gas and placed in a desiccator for vapor-phase silane deposition.

Control samples of both PDMS and SEMS were removed and the rest of the samples were silanized as follows. Silanes, APTES (99%) and GPTMS (≥98%) (Product no. 440140 and no. 440167; Sigma-Aldrich), were purchased and used without further purification. The substrates were positioned in the desiccator and a tiny droplet (0.1 mL) of the silane of choice was injected onto a Petri dish *via* a 1 mL syringe. Silicone grease was applied to all the connection points to ensure good sealing and the chamber was degassed for 3.5 min under 700 mmHg vacuum. At the end of the 3.5 min, the vacuum valve was shut and the substrates were exposed to silane-vapor for 1 h. The chamber was then pumped down once again for another 3.5 min, followed by an additional hour of exposure. Since silane mono layers do not possess long term stability [28,29], once the functionalization was complete, samples were jetted with nitrogen gas and sealed with Parafilm under an inert atmosphere to preserve the quality of the SAM.

2.4. XPS measurements

XPS analysis was performed using an X-ray photoelectron spectrometer (Model 5400; PHI XPS System). Each measurement was made over 10 sweeps with a non-monochromatized Mg K_α radiation source (1253.6 eV, 15 kV, 300 W). The binding energies were referenced to O (1s) at 532.0 eV [30]. The emission angle of electrons was set at 45° with respect to the sample normal.

2.5. Force measurements

The measurement of bioadhesive force between the stents and tissue represents a challenge due to the relative roughness of the interfaces, presence of mucus, stent pattern and the heterogeneity of tissue. We developed an approach to measure the bioadhesion using a force meter to measure the lateral pull-off force which represents the important criterion for testing lateral migration of the stent. We used the approach with flat samples and stents while measuring the influence of the presence of mucus using a simulated mucin gel.

2.5.1. Adhesion of surface modified PDMS samples on PDMS substrates

Functionalized PDMS surfaces were removed from the petri dishes by cutting out a disk with a scalpel. The disks were then cut into half-disks and 4 μL of 2% (w/w) mucus gel was dropped on the bottom half-disk for the samples; control samples without the mucus gel were also studied. While pairing the half-disks, 0.5 cm was left skewed from each other to allow the attachment of force meters. Once the disks were paired and placed in a petri dish, a uniform weight of 32.2 ± 0.2 g was placed on each pair to ensure good contact between the surfaces. Samples were then left out at

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