Lectin-Functionalized Polyethylene Glycol for Relief of Mucosal Dryness

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The importance of lubrication between oral surfaces provided by the salivary film is most acutely apparent when it is disrupted, a prevalent consequence of salivary gland hypofunction experienced with aging, a symptom of certain diseases, or a side effect of some medical interventions. Sufferers report difficulty with speech and oral food processing and collectively is detrimental to quality of life. Polyethylene glycol (PEG) is widely employed as a successful biocompatible boundary lubricant in engineering and biomedical applications. It is hypothesized that the immobilization of PEG to biological materials such as oral epithelial cells and tissue can mimic the salivary film and provide durable relief from the symptoms of mucosal dryness. To do so, PEG is functionalized with a sugar binding lectin (wheat germ agglutinin) to enhance epithelial adhesion through lectin-sugar interactions. Retention and lubricity are characterized on an ex vivo oral tissue tribology rig. WGA-PEG coats and retains on mucin films, oral epithelial cells, and porcine tongue tissue, and offers sustained reduction in coefficient of friction (COF). WGA-PEG could be developed into a useful topical treatment for reducing oral friction and the perception of dry mouth.

1. Introduction

The secretion of complex biofluids onto mucosal interfaces deposits conditioning films which are essential to facilitate their physiological functions and confer protection against mechanical and pathogenic stresses. Saliva secreted onto the oral surfaces contributes to the formation of a pellicle layer rich in high molecular weight mucin glycoproteins. Salivary mucins (MUC5B, MUC7) and other protein species associate with epithelial bound mucin (MUC1) anchoring a hydrophilic coating to the mucosa. This retention of water resists dehydration of the underlying mucosa and generates repulsive forces at the interface between the oral surfaces imparting lubrication. The necessity of low friction is acutely apparent during speech, oral processing of food, and swallowing. The biophysical and chemical composition of the pellicle also selectively drives microbial colonization of the oral surfaces and promotes homeostasis of a healthy commensal microbiome. Salivary gland hypo-/dysfunction (dry mouth), reduces the quality and rate of salivary secretion, which impedes pellicle formation. Consequently, the ability of the oral cavity to adequately undertake its functions is impaired, and incidence of infection and oral disease is increased. Dry mouth can be attributed to several diverse etiologies. Sjögren’s Syndrome is a chronic autoimmune disease impairing parenchymal function of exocrine glands such as the salivary and lacrimal glands, resulting in symptoms of dry mouth, and dry eye. While the complete pathology of Sjögren’s remains unclear, it has a population prevalence of 0.5%, disproportionately affecting women 2:1 with the greatest incidence experienced by the post-menopausal population. Iatrogenic causes of dry

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adhm.202101719

DOI: 10.1002/adhm.202101719
mouth are also numerous. Maxillofacial radiotherapy is the primary treatment for head and neck cancers; the sixth most common cancer accounting for 2.8% of malignancies globally.\(^\text{10}\) Radiotherapy for such cancers collaterally hits the salivary glands and results in long lasting salivary gland hypofunction, and dry mouth perception.\(^\text{10,11}\) One study reported 64% of long-term survivors (>3 years post-radiotherapy), experience moderate to severe xerostomia (the perception of dry mouth).\(^\text{12}\) Dry mouth further exacerbates oral infections such as candidiasis in this already immunocompromised cohort.\(^\text{13}\) Pharmaceutical interventions such as widely prescribed, first-line medications for depression, obsessive-compulsive, and anxiety disorders; SSRIs, SNRIs, and atypical antidepressants all report dry mouth as a side effect\(^\text{14,15}\). The consequence of salivary gland hypofunction and xerostomia for these patients includes impaired speech, eating, and pronounced oral infection, ultimately these factors severely impact the individual’s quality of life.\(^\text{11,16,17}\)

A large number of topical, non-pharmaceutical treatments are available for the intended relief from oral dryness, however their efficacy is limited.\(^\text{18}\) A major pitfall for saliva replacements is their inability to maintain a hydration layer on the mucosal surface and therefore relief from friction is short-lived.\(^\text{19}\) The most successful strategies for restoration of lubricity are those which absorb to mucosal surfaces and retain water molecules in proximity. Vinke et al.\(^\text{19}\) recently compared commercially available saliva replacements and identified a clear benefit of component surface adsorption on lubrication. Importantly, the study identified that many saliva substitutes did not adsorb, and consequently offer limited lubrication between oral surfaces. Many oral moisturizers only rely on the viscosity of the formulation to create a layer of hydrated polymer at the epithelial surface. Although the high viscosity of such formulations can help maintain the cohesiveness of the hydrating layer and resist dissolution, they dissolve with saliva flow and eventually efficacy is lost. Previous work has addressed this problem, aiming to promote the immobilization of naturally lubricating biomolecules in the oral cavity, for instance by complexing mucins with mucoadhesive chitosan polymers.\(^\text{20}\)

In this work, we are inspired by the large body of work performed on the hydration of lubrication of synthetic surfaces by hydrated polymers. Polyethylene glycol (PEG) is a highly hydrophilic polymer which hydrates and lubricates very effectively when grafted to surfaces.\(^\text{21,22}\) PEG chains are polymer compounds of repeating ethylene glycol units.\(^\text{23}\) PEG is a widely applied polymer in the food, cosmetic, and pharmaceutical industries, with a long and positive safety track record.\(^\text{24}\) Surface modification by PEG coating is commonly employed in industrial applications for the retention of surface hydration, prevention of fouling, and enhanced lubrication.\(^\text{25,26}\) An interesting example of the application of PEG is in the conservation of Stockholm’s famous Vasa warship which capsized in 1628; upon the ships recovery in 1961 PEG coating was used to maintain hydration, avoiding desiccation and degradation while the ship is on display out of water.\(^\text{27}\) We hypothesize that applying such surface modification strategies to the mucosal epithelium could lead to long-lasting hydration and lubrication of the surfaces and thereby mimic the natural mucinous films. However, successfully grafting PEG onto biological tissues is challenging because of tissue heterogeneity, and cytotoxicity of nearly all grafting chemical strategies used on synthetic surfaces. We have previously shown that the wheat germ agglutinin (WGA) can be used to graft PEG to mucin glycoproteins by binding to sialic acid and N-acetylgalactosamine, restoring hydration without cytotoxicity.\(^\text{28}\) A large number of mucosal glycoproteins carry sialic acids as the terminal moiety of many glycan structures, making them an abundant target.\(^\text{29}\) Functionizing PEG with WGA allows targeted binding of the conjugates to mucosal tissues, in this study we focus on the oral cavity, but treatment could theoretically be applied to other mucosal sites for the treatment of Dry Eye Disease, or Vaginal Dryness, due to the presence of sialated glycans on such surfaces.\(^\text{30,31}\) Here we demonstrated and optimized the anchorage of PEG to oral epithelial cells and explanted tong tissue, assessing coating stability and tribological properties.

2. Results and Discussion

2.1. WGA-PEG Conjugation and Purification

Rhodamine labelled wheat germ agglutinin (WGA) was PEGylated targeting amine groups of lysine residues with linear 5 kDa, and 40 kDa polyethylene glycol functionalized with succinimidyl carboxymethyl ester groups (SCM). The purified products of these reactions will herein be termed WGA-SCM-05, and WGA-SCM-40 respectively. Thiol groups of WGA were targeted with linear 5 kDa PEG, and Y-shaped 40 kDa PEG functionalized with maleimide group (structures can be found in Figure S1, Supporting Information). These will subsequently be termed WGA-MAL-05, and WGA-MAL-Y40 respectively. Size exclusion chromatography (SEC) was used to purify PEGylated WGA. The WGA elution peak could be detected by measuring fluorescence intensity at 565 nm, which corresponds to the fluorescence emission of the rhodamine label on WGA (Figure 1a). Shifts in peak elution volume (e/v) of WGA from 111 mL were observed when conjugated to PEG variants, WGA-SCM-05 (e/v ≈66 mL), WGA-SCM-40 (e/v ≈50 mL), WGA-MAL-05 (e/v ≈59 mL), WGA-MAL-Y40 (e/v ≈53 mL) indicating the increased molecular weight of WGA post conjugation (this compliments UV spectra collected during SEC runs Figure S2, Supporting Information). Fractions from each SEC run which suggested an increased molecular weight of WGA; as determined by UV and 565 nm fluorescence spectra, were pooled. The contents of the pooled samples were then separated by molecular weight with SDS-PAGE and stained with Coomassie blue for protein (Figure 1b), and with barium iodide for PEG (Figure 1c).\(^\text{32}\) Figures 1b,c indicated subpopulations in the purified samples; varying in the number of PEG molecules conjugated to each WGA subunit, which is indicated by the laddering patterns observed.

SDS-PAGE lane profiling was used to identify PEG engraftment numbers (Figure S3, Supporting Information) and is summarized in Table 1. A varying degree of varying PEG chain engraftment is demonstrated. Amine targeted PEGylation with 40 kDa chains (WGA-SCM-40), resulted in 89% of conjugates containing 4 chains per WGA subunit, and 11% containing 5 PEGs. The 5 kDa amine-reactive variant (WGA-SCM-05) led to subpopulations ranging between 1 – 6 PEG chains per WGA subunit, with two chains being the most common at 26%. In con-
Figure 1. PEGylation and purification. a) Fluorescence (545–555 nm) of fractionated eluate from SEC purification. b) Coomassie blue stained and c) barium iodide stained SDS-PAGE gel (1.) molecular weight standard, (2.) WGA control, (3.) WGA-SCM-40, (4.) WGA-SCM-05, (5.) WGA-MAL-Y40, (6.) WGA-MAL-05. Lane profiling is presented in Figure S3 (Supporting Information).

Table 1. The proportion of PEG engraftment number per WGA subunit. The sub-population of WGA-MAL-Y40 could not be resolved by SDS-PAGE and is thus not reported in the table.

<table>
<thead>
<tr>
<th>PEG No</th>
<th>WGA-SCM-40</th>
<th>WGA-SCM-05</th>
<th>WGA-MAL-Y40</th>
<th>WGA-MAL-05</th>
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<tr>
<td>0</td>
<td>100%</td>
<td>–</td>
<td>–</td>
<td>N/A</td>
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<td>1</td>
<td>–</td>
<td>–</td>
<td>18%</td>
<td>N/A</td>
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<tr>
<td>2</td>
<td>–</td>
<td>–</td>
<td>29%</td>
<td>N/A</td>
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<td>3</td>
<td>–</td>
<td>–</td>
<td>27%</td>
<td>N/A</td>
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<td>–</td>
<td>89%</td>
<td>–</td>
<td>N/A</td>
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<tr>
<td>5</td>
<td>–</td>
<td>11%</td>
<td>21%</td>
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<td>6</td>
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<td>–</td>
<td>5%</td>
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In contrast, thiol targeting with 40 kDa Y shaped PEG (WGA-MAL-Y40) was unable to resolve via SDS-PAGE, possibly due to the large Y shaped PEG interfering with phoresies, or the sensitivity of the staining methods used. SEC trace data (UV absorbance/fluorescence at 565 nm) indicate a low yield of WGA-MAL-Y40. Thiol targeting with the 5 kDa variant (WGA-MAL-05) resulted in only partial PEGylation with 37% containing a single chain, and 63% remaining PEG free.

2.2. WGA-PEG Conjugates Bind to Mucin and Salivary Films

Post conjugate synthesis, characterization of WGA binding to salivary films, and lab purified porcine gastric mucin (PGM) was assessed to compare the influence of PEGylation chemistry and PEG molecular weight on binding. We consider these glycoprotein coatings as simplified mimics of the oral epithelial surface. Saliva was collected from 10 self-declared healthy volunteers and was quality controlled before use by flow rate and total protein concentration. All donors had typical resting salivary flow rates ranging 0.29–1.03 mL min$^{-1}$, and total protein concentrations between 0.84–5.48 mg mL$^{-1}$ which are within normal range$^{[34–36]}$ (raw data are presented in Table S1, Supporting Information). Samples were clarified by centrifugation before pooling. Figure 2a/b illustrates the binding of the four conjugates to titrated concentrations of a) saliva and b) PGM with seeding concentrations ranging from 0 to 0.5 mg mL$^{-1}$. All conjugates showed a decreased binding affinity to the salivary and mucin coatings, with the smaller 5 kDa PEG leading to the lowest decrease and the 40 kDa PEG to the greatest. Grafting molecules to proteins can decrease the activity of proteins directly; by sterically hindering access to the ligand binding site, or indirectly; by denaturing the proteins. PEGylation by targeting thiol groups (with maleimide functionality) for example, requires reduction of WGA disulfide bonds, this may cause structural changes upon refolding and thus could be responsible for the decreased in substrate binding.
for these conjugates. Targeting amine groups with SCM functionality does not require pre-reduction of WGA. Figure 2 demonstrates that higher PEG molecular leads to less binding to the salivary and mucin coating substrates, pointing to a steric effect for SCM conjugates. Although the binding was lower than native WGA, the WGA-SCM-05 conjugates bound most successfully to relevant substrates and therefore was selected to be investigated further. The WGA-SCM-40 was also chosen to assess the influence of PEG molecular weight on functionality of the conjugates.

2.3. The Impact of PEGylation of WGA Ligand Binding Specificity

A high through-put glycan array was used to determine the impact of PEGylation on WGA ligand specificity, screening 100 glycan species. (Figure S3, Supporting Information), it should be noted that PEGylation reduced WGA binding to all glycans and a reduction in the number of glycan species which the conjugates bind at a detectable level. Figure 3 presents 10 of the 100 glycans tested which had the greatest binding of a) WGA, b) WGA-SCM-05, and c) WGA-SCM-40 (glycan ID key Table S2, Supporting Information). PEGylation of WGA not only shifted the binding affinity to glycan structures but also shifted its binding preference. For instance, the WGA-SCM-05 bound GalNAc-β-1,4-GlcNAc-β- disaccharide the most but was only the 6th best binder for native WGA. Although the binding preference changed, the oligosaccharides with the best binding were composed of similar sugar units and does not compromise binding to glycosylated mucosal tissues. It is likely that these differences are also due to steric hindrance of the sugar binding site by the PEG chains.

2.4. WGA Conjugation is a Useful Tool for Anchorage of PEG to Human Oral Keratinocytes

Characterization of substrate binding was then advanced with a physiologically relevant oral keratinocyte cell line; this increased the complexity of the substrate surface both topographically and chemically and provided a model for the keratinized human oral epithelium (Human Gingival Epithelial Keratinocytes; HGEK-16 were used) and is illustrated in Figure 4. The HGEK-16 line has been demonstrated to retain morphological and transcriptional similarity to the oral epithelium, and was deemed a physiologically relevant model. In these experiments, the cells were exposed to WGA alone, PEG alone, and WGA-PEG conjugates, and the presence of WGA and PEG was measured separately by fluorescence measurement from WGA-grafted fluorophore (Em 565 nm), or from labeling of PEG by anti-PEG primary antibody, detected by a fluorescent secondary antibody (Em 488 nm).

The fluorescence signal from WGA suggested that there was a strong, and dose dependent binding of unconjugated WGA to HGEK-16 monolayers (Figure 5a), However, similarly to salivary and mucin coatings; the PEGylation of WGA (WGA-SCM-05/ WGA-SCM-40) reduced WGA binding. To confirm the immobilization of PEG at the cell surface (Illustrated in Figure 4c), PEG was detected separately (Figure 5b). PEG with WGA functionalization was retained on the cells after washing, whereas unfunctionalized PEG was completely removed from the surface (Figure 5c). This suggest that the lectin anchor improves retention of PEG at the surface of cell monolayers. There was no large difference in fluorescence signal from the PEG detection between WGA-SCM-05 and WGA-SCM-40, suggesting that the accessibility of the anti-PEG antibody used for the detection was similar in both cases. We hypothesize that for WGA-SCM-05, the large number of PEG per WGA compensated for the shorter chain length.

Imaging of the cell monolayers by fluorescence microscopy (Figure 6) confirmed that WGA and WGA-conjugates bind to HGEK-16 and suggests the binding is largely restricted to the cell membrane. We hypothesize that the lectin binds to glycoprotein components of the cell glycocalyx and secreted extracellular matrix, including transmembrane and secreted mucins. These highly glycosylated molecules exhibit several of the high affinity ligands for WGA, including those containing N-Acetyl glucosamine and sialic acids residues. WGA-SCM-05 and WGA-SCM-40 show colocalization of the WGA and PEG signals and confirm that WGA functionalization of PEG is an effective anchorage mechanism for oral epithelial cells. Gentle washing of the monolayers with PBS was enough to remove signal of unfunctionalized PEG, but not with WGA conjugation (Figure S4, Supporting Information).

2.5. WGA-PEG Offers Sustained Reduction of Friction between Oral Tissues

With the demonstration that the WGA lectin can be used to anchor PEG molecules to biological surfaces, we then investigated whether the immobilized PEG would retain their ability to effectively lubricate such surfaces. For this purpose, coatings, or monolayer cell cultures are not well suited since minimal friction leads to a disruption of the surface, and would not mimic mucosal bio-lubrication properly. Instead, we used the porcine tongue model, which has been demonstrated to have surface morphology and friction coefficients comparable to that of human oral mucosal tissues, making it an easy to procure, applicable model for oral tribology studies. The impact of lectin-PEG conjugates on friction between oral surfaces was assessed by a tribology rig shown in Figure 7, which utilized bovine enamel

Figure 2. Binding of PEGylated wheat germ agglutinin (WGA) to a) salivary films, and b) porcine gastric mucin (PGM). Data points represent mean values of three experimental repeats ± standard deviation.
Figure 3. Ten glycan moieties with the most a) WGA binding out of 100 screened. The ten glycan moieties which bound the most b) WGA-SCM-05, and c) WGA-SCM-40 are presented respectively. Means of four repeats are presented ± standard deviation. All 100 glycans can be found in Figure S4 (Supporting Information), and the glycan ID key is presented in Table S2 (Supporting Information).
sections rubbed against porcine tongue (Figure 7b), in a “to-and-frow” cycle pattern (Figure 7c).

We first validated whether lectin anchoring to tongue tissue would retain PEG to its surface and identified that both WGA and PEGylated WGA bound the dorsum of porcine tongue tissue as measured by the remaining fluorescence signal from WGA-rhodamine detected after gentle washing (Figure 8a). In similarity to the other biological substrates tested, the conjugated WGA bound to a lower degree than native WGA, with the WGA functionalized 40 kDa PEG detectible only at very low levels. The durability of the WGA conjugate binding was then tested by comparing the signals from both WGA (fluorescence at 565 nm), and of PEG (fluorescence at 488 nm) on dorsal tongue tissue after repeated washing of the tissue with PBS (Figure 8b). Significantly more signal from PEG was detected on tongues treated with WGA conjugates, although there appeared to be some level of non-specific binding of PEG 5 kDa with the tissue, suggesting retention on the surface is improved. The retention of the conjugates on the surface is essential for the longevity of relief due to continual oral clearance and shear forces imposed by residual salivary flow, and the consumption of food and drink. All solutions significantly reduced the coefficient of friction when introduced onto the dry tongue. There was no significant difference between samples and controls in the resulting COF (Figure 8c), suggesting that the addition of any fluid can offer relief; it is likely that the application of water hydrates the cellular glyocalyx and any residual protein film on the tongue, which bear lubricating properties when hydrated. This observation is in line with reports that frequent intake of water can help the short-term management of dry mouth. The duration of relief...
Wheat germ agglutinin (WGA) is an effective tool to anchor polyethylene glycol (PEG) to Human Gingival Epithelial Keratinocytes (HGEK) and enhances retention. a) demonstrates the dose dependent binding of WGA and PEGylated forms (WGA-SCM-05/ WGA-SCM-40) to HGEK monolayers by measuring fluorescence from the rhodamine tag on WGA (565 nm). b) Demonstrates fluorescence at 488 nm corresponding to detection of PEG. c) Functionalization of PEG with WGA maintains signal from PEG when HGEK were washed three times with PBS.

is illustrated in Figure 8d, which compares the ten cycle means of COF initially post application (baseline), and at set cycle numbers thereafter. Between the 10th and 100th cycle, control samples of WGA diverged from all other samples and a significant increase in COF was observed. This relates to the fact water can provide relief; however, the relief does not last for more than a few seconds. Lectin-PEG conjugates and PEG controls did not significantly increase COF over the number of cycles tested, demonstrating that PEG was effective at providing long lasting friction relief between oral surfaces whether in free form or adhered to the tissue. Although the coefficient of friction at the higher cycle numbers were on average lower for WGA functionalized PEG compared to free PEG, the differences were not statistically significant due to sample to samples variations. It is likely that in both forms, the PEG, trapped between the enamel and the tongue tissue provide lasting hydration to the tissue, which maintained lubrication. A limitation of this technique is the inability to model more complex oral states such as during drinking or swallowing which may remove free PEG as suggested by Figure 8b. In such cases, and based on tissue retention data, one could predict that WGA functionalized PEG would provide longer relief than free PEG.

3. Conclusion

The data presented in this study indicate that functionalization with wheat germ agglutinin is an effective tool to anchor polyethylene glycol to mucosal surfaces by taking advantage of its sugar binding capacity. Oral physiology presents a number of challenges to a mucosal polymer coating, notably oral clearance and shear forces from food and drink consumption which may reduce retention time, and limit effectiveness and ultimately use. WGA functionalization as an anchorage method aids retention of the polymer on the surface when challenged with shear forces. Further work should be undertaken to optimize the PEGylation chemistry leveraging a more targeted approach such as orthogonal ligation, which could help preserve the full binding affinity of native WGA for mucosal glycans. The perception of oral dryness by a sufferer is largely due to reduced lubrication between the oral surfaces as a consequence of impaired pellicle formation, in this
Figure 6. Binding of wheat germ agglutinin (WGA) and PEGylated WGA to Human Gingival Epithelial Keratinocytes (HGEK). Scale bar represents 50 μm.

Figure 7. a) UMT tribology rig fitted with b) enamel on tongue friction pair, c) cycling in a to-and-fro pattern.
Figure 8. a) Binding of WGA and PEGylated WGA to porcine tongue tissue. b) Retention of WGA (565 nm) and PEG (488 nm) on porcine tongue tissue after multiple washes. c) The difference in coefficient of friction (COF) between dry tongue surface and with the application of test solutions. Data are normalized to dry COF and represent the mean of three independent experiments ± SD. Statistical comparison by Two-Way ANOVA and Šídák’s multiple comparisons test * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \). d) The duration of relief offered by test solutions normalized to initial relief COF. Each datapoint represent the mean of three repeated experiments ± SD. Statistical comparison by Two-Way ANOVA and Tukey’s multiple comparisons test * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \).

study, polyethylene glycol offered long lasting relief from friction when tested between explanted oral tissues. Due to the ubiquity of WGA ligands on different mucosal surfaces, this technology could be used for the relief of ocular or vaginal dryness.

4. Experimental Section

Wheat Germ Agglutinin PEGylation: Amine targeted PEGylation: Rhodamine labelled wheat germ agglutinin (WGA) (Vector laboratories, Inc. Burlingame, CA, USA) was poly-PEGylated by esterification with methoxy polyethylene glycol succinimidyl carboxymethyl ester (Figure 9a) of two molecular weights: 5 kDa; and 40 kDa (JENKEM Technology USA Inc., TX, USA). The reaction took place in PBS, at room temperature, for 4 h, with agitation. For each batch, 500 \( \mu \)g of WGA was incubated at a ratio of 1:100 with one of the four PEG variants. For thiol targeted PEGylation, WGA was first reduced with 10 \( \times \) 10\(^{-3}\) m tris-(2-carboxymethyl)-phosphine (TCEP) (Sigma-Aldrich, St. Louis, MI, USA) in PBS, at room temperature for 30 min. Excess TCEP was removed by size exclusion centrifuge filter tubes. Linear 5 kDa, and Y-shaped 40 kDa polyethylene glycol variants both with terminal maleimide active groups (Figure 9b) (JENKEM Technology USA Inc., TX, U.S.A.), were conjugated to the reduced WGA under the same conditions and ratios as noted above. Conjugates were purified by Size Exclusion Chromatography (SEC) with an ÄktaPurifier System (GE Healthcare, Munich, Germany) with an XK50/100 Sepharose 6B column equilibrated with PBS. Eluate was fractionated by 2.5 mL volumes. UV spectra were used to assess isolation of PEGylated lectin. Each fraction was additionally measured to confirm the presence of the rhodamine-labelled WGA with excitation at 545–555 nm and emission at 570–580 nm using a CLARIOstar plus microplate reader (BMG Labtech, Otterberg, Germany). Fractions associated with a UV absorbance peak, and rhodamine fluorescence peak were pooled and concentrated by size exclusion filter centrifugation. Constituents of the final product were separated by molecular weight using SDS-PAGE on 4–12% bis-tris gels (Thermo Fisher Scientific, Waltham, MA, USA) and stained for protein with Coomassie blue, followed by barium iodide for PEG,[31] gels were imaged on a ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA), and image analysis was undertaken on Image Lab software Version 6.1.0 build 7 (2020) (Bio-Rad).

Resting Whole Mouth Saliva Collection: Resting whole mouth saliva was collected from five female, and five male self-declared generally healthy volunteers (\( n = 10 \)) in concordance with the WMA Declaration of Helsinki, and provided informed written consent. Participants expected into pre-weighted universal tubes for 10 min, tubes were re-weighed post collection. Mass of the sample 1 g of saliva was assume 1 mL, salivary flow rate was then calculated (mL per time). Debris in the saliva was pelleted by centrifugation at 13 000 \( \times g \) before total protein concentration was determined by Bradford Protein Assay, with titrated bovine serum albumin to generate a standard curve. Absorbance (595 nm) was measured using a CLARIOstar plus microplate reader (BMG Labtech, Otterberg, Germany). All samples falling within healthy ranges for flow rate and protein concentration Table S1 (Supporting Information) were pooled, divided into aliquots and stored at −80 °C until use.

Porcine Gastric Mucin Preparation: Porcine gastric mucin (PGM) was purified as published previously.[41] In brief, pig stomachs were processed on the day of slaughter (Lövsta Kött AB, Uppsala, Sweden), mucus was collected by scraping the gastric mucosa. Mucus was diluted in PBS 0.04% w/v sodium azide (pH 7.4) and stirred overnight at 4°C. The diluted mucus then passed through subsequent centrifugation steps 8300 \( \times g \) for 30 min, 15 000 \( \times g \) for 45 min, and 150 000 \( \times g \) for 1 h (all at 4°C). Mucins were then isolated by size exclusion chromatography using an Äkta Purifier System (GE Healthcare, Munich, Germany) with an XK50/100 Sepharose.
Figure 9. Reaction schemes for PEGylation of wheat germ agglutinin (WGA) a) targeting amine groups with methoxy polyethylene glycol succinimidyl carboxymethyl ester, or b) thiol targeting with methoxy polyethylene glycol maleimide.

6FF column, this method has previously been demonstrated to largely isolate MUC5AC and to a lesser extent MUC5B. Fractions were then pooled, and filtered using an Amicon Stirred Cell with an exclusion size of 100 kDa (EMD, Millipore Corporation, Billerica, MA, USA), and washed five times with MilliQ water. The product was concentrated and lyophilized before storage at −20°C.

Saliva and Porcine Gastric Mucin Binding: Lab purified PGM and salivary proteins were serially diluted in triplicate on 96-well polystyrene plates in PBS pH 7.4 to a final well volume of 100 μL. PGM and salivary proteins have previously been shown to adhere the polystyrene surface. Coatings were allowed to develop for 1 h at room temperature, with mild agitation before wells were washed three times with PBS. All wells were then blocked in 100 μL bovine serum albumin, reconstituted to 20 mg mL⁻¹ in PBS for 1 h under the same conditions. Wash steps were repeated before incubating with the PEGylated WGA conjugates at a concentration of 20 μg mL⁻¹ for 1 h. Wells were washed as before, then fluorescence was measured with excitation 545–555 nm and emission at 570–580 nm, using a CLARIOstar plus microplate reader (BMG Labtech, Ortenberg, Germany).

Glycan Binding Specificity: Differences in the binding specificity of WGA with PEGylation was assessed by a high throughput glycan array (RayBiotech, GA, USA). This allowed for direct comparison between glycan binding specificity for the native WGA, WGA-SCM-05 and WGA-SCM-40. Samples were added to one of the four channels on the array, allowing four repeats of conjugate binding to each glycan. The array was washed following the manufacturer’s instructions and loaded for reading with a LuxScan HT24 Microarray Scanner (CapitalBio, Beijing, China).

Coating Oral Epithelial Cells: Human Gingival Epithelial Keratinocytes (HGEK-16) were maintained in DKSFM (Gibco) supplemented with 0.1% v/v defined keratinocyte-SFM growth supplement (Gibco), 1% v/v antibiotic-antimycotic (Gibco), and 100 μg mL⁻¹ G418. Cells were cultured in collagen coated flasks at 37°C, with atmospheric CO₂ of 5%. HGEK-16 were seeded onto collagen coated 24-well plates and allowed to reach confluence. Prior to assaying conjugate binding, cultures were washed 3 times with PBS. WGA, WGA-SCM-05, WGA-SCM-40, and PBS (vehicle control), were added to triplicate wells. The plate was then incubated for 30 min, and subsequently washed three times with PBS prior to reading. Fluorescence was measured by excitation at 545–555 nm and emission at 570–580 nm using a CLARIOstar plus microplate reader (BMG Labtech, Ortenberg, Germany).

Polyethylene glycol was detected by immunofluorescence. Cell cultures were fixed by incubation on ice in 4% v/v paraformaldehyde for 20 min, followed by 3 washes with PBS and 20 min with methanol, the wash steps were then repeated. Bovine serum albumin (50 mg mL⁻¹) was used as a blocking agent. Anti-polyethylene glycol primary (Thermo Fisher Scientific, Waltham, MA, USA) was incubated on the cells at room temperature for 30 min, wash steps were repeated, and a relevant anti-rabbit IgG conjugated to Alexa488 (Thermo Fisher Scientific, Waltham, MA, USA) was incubated at room temperature for 30 min, followed by wash steps. Fluorescence was measured at 545–555 nm and emission at 570–580 nm, using a CLARIOstar plus microplate reader (BMG Labtech, Ortenberg, Germany).

Biotribology: Fresh tongue tissue was collected from six-month-old, 90 kg pigs (Kroon BV, Groningen, Netherlands). Tongues were washed in distilled water and soaked in distilled water on ice for 30 min. Tongues were again washed, and excess water removed before wrapping in cling film. Tongues were then placed with the dorsal surface faced down in a mold and cast in duplicating silicone blocks. Clingfilm was then excised to expose the apical surface of the tongues which were then soaked in adhesion buffer for 30 min. Tongues were washed in distilled water and dab dried before use. Bovine enamel samples were shaped to fit a steel holder by grinding on course sandpaper and slightly rounded by a bench.

pillar drill and sphere-shaped diamond grinder. Blocks were polished with a rubber polishing wheel and finished with 0.05-micron alumina micro pol-
ish. Blocks were rinsed in deionized water and sonicated for 5 min prior to use. Washing and polishing steps were repeated between each sam-
ple measurement. Friction measurements were conducted following the previously published protocol. A universal mechanical tester (UMT-3, Bruker, MA, USA) was used for measurements. Prepared porcine tongue blocks were placed on the lower drive, mounted enamel tongue blocks were in-
serted onto a 0.1–10 N load cell on the upper drive. Statistical Analysis: Statistical analysis was undertaken on Prism 7 Ver-
sion 9.1.0 (216), March 15, 2021(GraphPad Software, San Diego, CA, USA).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors would like to thank Dr. Kai Bao for assistance with this work.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

Keywords

dry mouth, mucosal dryness, polyethylene glycol, tribology

Received: August 18, 2021
Revised: October 21, 2021
Published online: