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Massachusetts Institute of Technology

Characterization of Particle Translocation through Mucin Hydrogels

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ABSTRACT Biological functional entities surround themselves with selective barriers that control the passage of certain classes of macromolecules while rejecting others. A prominent example of such a selective permeability barrier is given by mucus. Mucus is a biopolymer-based hydrogel that lines all wet epithelial surfaces of the human body. It regulates the uptake of nutrients from our gastrointestinal system, adjusts itself with the menstrual cycle to control the passage of sperm, and shields the underlying cells from pathogens such as bacteria and viruses. In the case of drug delivery, the mucus barrier needs to be overcome for successful medical treatment. Despite its importance for both physiology and medical applications, the underlying principles which regulate the permeability of mucus remain enigmatic. Here, we analyze the mobility of microscopic particles in reconstituted mucin hydrogels. We show that electrostatic interactions between diffusing particles and mucin polymers regulate the permeability properties of reconstituted mucin hydrogels. As a consequence, various parameters such as particle surface charge and mucin density, and buffer conditions such as pH and ionic strength, can modulate the microscopic barrier function of the mucin hydrogel. Our findings suggest that the permeability of a biopolymer-based hydrogel such as native mucus can be tuned to a wide range of settings in different compartments of our bodies.

INTRODUCTION

Mucus is a polymer-based hydrogel that covers the inner linings of the body, from the nose to the female genital tract. Mucus fulfills many different functions: it protects underlying cells from contact with noxious agents, assists in the adsorption of food particles, and serves as a lubricant (1–3). One critical aspect of mucus function is to permit selective passage of molecules that are beneficial for the body while rejecting others that are potentially harmful, such as viruses or bacterial pathogens. The filtering properties of mucus are critical for health; changes in mucus properties can cause numerous medical conditions, from bacterial infections to some forms of infertility. Despite the importance of mucus for many body functions, the biophysical principles that govern selectivity in mucus barriers are not well understood.

In a polymer-based hydrogel, the concentration of polymers defines the mesh size of the network. This mesh size sets the threshold beyond which particle diffusion is suppressed: particles with dimensions smaller than this mesh size should be able to pass through the hydrogel, whereas larger particles should not (Fig. 1). Thus, hydrogels with different mesh sizes would show distinct selectivity properties toward particles of different sizes, a concept we will refer to as size filtering. The concentration of mucin polymers found in the human body can vary between 1 and 5% (w/v) (4–6), suggesting that the mucin density might be a key parameter for the regulation of mucus function. Experiments on sputum mucus obtained from cystic fibrosis patients report a decrease in particle mobility with increasing particle size (7)—consistent with a size-filtering mechanism. Yet, the opposite effect was observed in cervical mucus, in which smaller particles were immobilized more efficiently (8) even though certain macromolecules were reported to diffuse almost unhindered (9). In addition, the mobility of microscopic polystyrene particles through native cervical mucus can be enhanced by coating the particles with the inert polymer polyethyleneglycol (PEG) (8–11). These data suggest that the simple concept of size filtering, although appealing, is not sufficient to describe the complex permeability properties of mucus hydrogels. Interactions between traversing particles and the mucus hydrogel might also play an important role for mucus filtering (Fig. 1).

Resolving these seemingly contradictory results requires an in vitro hydrogel system with purified components, which allows control over mucin class and concentration, as well as buffer conditions, such as ionic strength and pH. The gel-like properties of mucus are largely determined by the oligomeric gel-forming mucin MUC5AC, the key constituent of gastric, cervical, and airway mucus (12). Pig stomach mucins are well suited for a systematic reconstitution of mucus model systems because they can be purified in bulk from native porcine gastric mucus (13). Indeed, reconstituted mucin hydrogels have successfully been established (14,15) and have recently been used as a valuable model for *Helicobacter pylori* motility in native gastric mucus environments (16).

Here, we systematically map the permeability of reconstituted mucin hydrogels toward distinct test particles of comparable size (~1 μm) but different surface charges, including negatively charged, positively charged, and neutralized polystyrene beads. We employ single particle tracking to determine apparent diffusion coefficients and quantify the influence of mucin density, pH, and salt concentration on the microscopic mobility of these particles. Our results
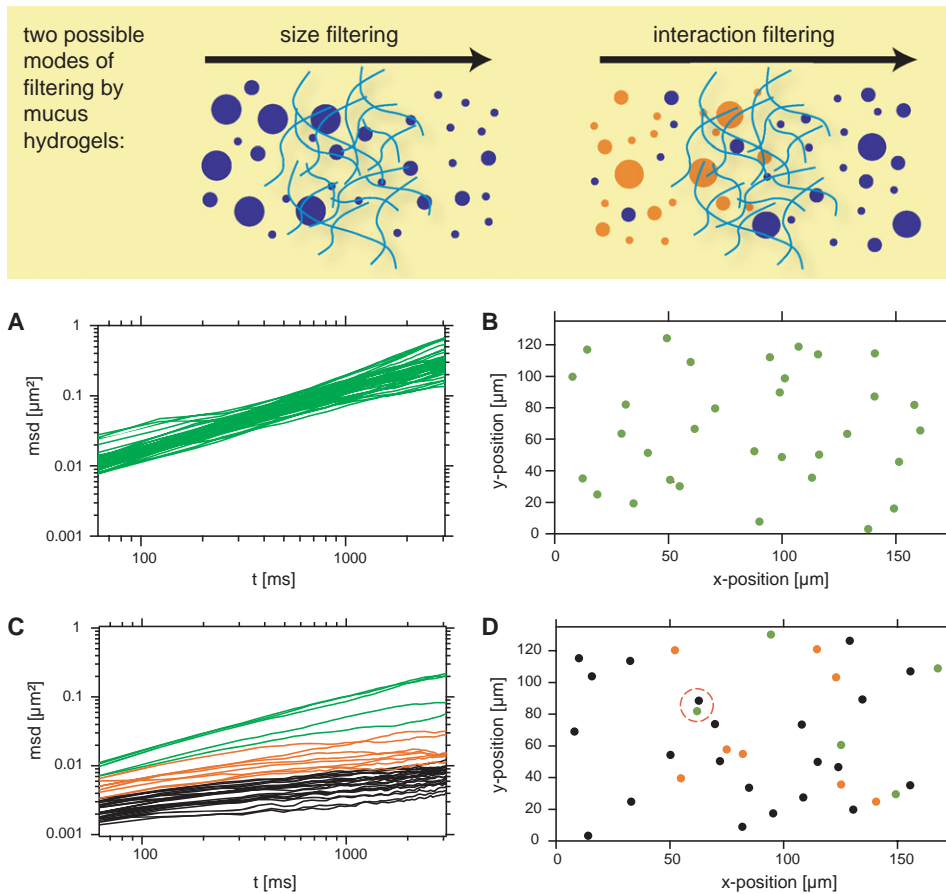
FIGURE 1 (*Top panel*) Two possible filtering strategies could be employed by mucus hydrogels. Size filtering (*left*) allows particles with dimensions smaller than the hydrogel mesh size to pass while larger particles are rejected. Interaction filtering (*right*) allows to distinguish particles according to their interaction strength with the hydrogel polymers as mediated by the particle surface properties: Strongly interacting particles (*orange*) are retained in the hydrogel, whereas weakly interacting particles (*blue*) are allowed to pass. (*Lower panels*) Two examples of the trajectory analysis as conducted for the single particle tracking data (see Materials and Methods) that was obtained for amine-terminated polystyrene particles in a 0.5% (w/v) mucin hydrogel at pH 7 (*A* and *B*) and at pH 3 (*C* and *D*). From the mean-square displacement (*A* and *C*) of the particle trajectories, apparent diffusion coefficients are determined (see Fig. 2 *A* and Table 2 for the ensemble average value). To illustrate the heterogeneity of the obtained diffusion coefficients, particles from a single region of interest are colored according to their mobility. At pH 7, all particles show a highly similar diffusion behavior (*B*). However, at pH 3, mobile and immobile particles can be found in close vicinity, demonstrating a high variance of particle mobility (*D*). The examples depicted in panels *B* and *D* show relative distribution widths of 38% and 178%, respectively.

demonstrate that the interaction between the particles and the mucin polymers is a key parameter that determines the microscopic permeability of the mucin hydrogel. We show that these interactions are electrostatic and thus can be tuned by buffer conditions such as pH and ionic strength.

MATERIALS AND METHODS

Materials

Polystyrene particles

Fluorescent polystyrene particles (carboxyl-terminated or amine-terminated) with 1-μm diameter were obtained from Sigma-Aldrich (St. Louis, MO) and Invitrogen (Carlsbad, CA). Polyethyleneglycol (PEG) coating of amine-modified beads (in the remainder of this article these coated beads are referred to as PEG(a) particles) was conducted using *n*-hydroxysuccinimide-(PEG)$_{12}$-Biotin (Pierce, Rockford, IL) and methyl-(PEG)$_{12}$-*n*-hydroxysuccinimide (Pierce) according to the instructions provided by the manufacturer. The ratio of PEG-Biotin versus PEG was 1:5, and a 5–20-fold molar excess of both reagents was used with respect to the concentration of the amine groups. Biotin was added since the same PEG(a) particles were also used for another study we conducted in parallel. PEG coating of carboxyl-modified beads (PEG(c)) was performed using a carbodiimide-coupling protocol as outlined by Valentine et al. (17). The low molecular weight (<750 Da) of the PEG we use for particle coating reduces the possibility of entanglement

with the hydrogel polymers or other geometrical hindrance effects as induced by long PEG chains (10).

Mucin purification and sample preparation

Porcine gastric mucins were purified from scrapings of fresh pig stomachs essentially as described in Celli et al. (15), with the difference that the cesium chloride density gradient ultracentrifugation was omitted. Mass spectrometry of the purified material confirmed the presence of all major gel-forming mucins: MUC5AC, MUC5B, MUC6, and MUC2. For reconstitution, lyophilized mucins were hydrated overnight at 4°C in distilled water. Polystyrene particles were added to the homogeneous mucin solutions which then were buffered to the desired pH with acetate buffer calibrated to pH 3 or 5, or HEPES buffer calibrated to pH 7. The final buffer concentration in the hydrogel was 20 mM, and NaCl was added to adjust the ionic strength to 20 mM if not indicated otherwise. To minimize errors arising from this preparation procedure, data within a given series of experiments (i.e., those compared horizontally in our graphs) are obtained from identical mucin hydrations. The quality of the reconstituted mucin hydrogels was assessed by rheometry that was conducted at 22°C. Only preparations that showed nearly identical pH-dependent viscoelastic properties as described by Celli et al. (15) were used.

Methods

Particle characterization

Size- and ζ-potential of polystyrene particles were determined with dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments,

TABLE 1 Particle size, polydispersity index (PDI), and particle surface potential (ζ-potential) as determined from dynamic light scattering for different particle types at distinct pH conditions

Particle type	Particle size [nm]	PDI	ζ @pH 7 [mV]	ζ @pH 5 [mV]	ζ @pH 3 [mV]
carboxy	1240	0.16	-37.2 ± 2.2	N/D	-21.5 ± 1.4
PEG(c)	1060	0.35	-6.7 ± 1.3	N/D	2.6 ± 1.7
PEG(a)	1280	0.15	-29.1 ± 1.1	-5.3 ± 1.2	13.3 ± 1.4
amine	1120	0.14	26.7 ± 1.3	N/D	33.3 ± 1.2

Successful PEG-coating results in a partially shielded particle surface charge. PEG-coated particles exhibit intermediate surface potentials at both pH conditions compared to untreated amine- or carboxyl-terminated particles. N/D: not determined.

Herrenberg, Germany). For size and ζ-potential measurements, the particles were resuspended in 20 mM Tris, 20 mM NaCl (pH 7), or in 20 mM NaAc, 20 mM NaCl (pH 5 and pH 3).

Data acquisition

Bright-field or phase contrast images were obtained on an Axioscope microscope (Zeiss, Oberkochen, Germany) with an Achroplan 40× objective (Zeiss). Images were acquired with a digital camera (ORCA-ER C4742-95; Hamamatsu, Hamamatsu City, Japan) using the image acquisition software HCImage (Hamamatsu). Movies were taken at a frame rate of 16.3 frames/s for a duration of 30 s, and three different regions in the hydrogel were analyzed per experimental condition to a total of ~50 particles per experiment. Subpixel precision in particle position was achieved using a tracking routine implemented in the image-processing software OpenBox (18), which employs an algorithm based on a two-dimensional Gaussian fit to the particle intensity profile. From the two-dimensional intensity profile, single particles could be distinguished from smaller aggregates, which were not evaluated for the calculation of apparent diffusion constants.

Data analysis

To quantify the microscopic mobility of the test particles, their apparent diffusion coefficient is calculated from the trajectory of motion of the particles. In brief, the mean-square displacement (msd),

$$msd(\tau) = \frac{1}{N} \sum_{i=0}^{N} [\boldsymbol{r}(i\Delta t + \tau) - \boldsymbol{r}(i\Delta t)]^2, \qquad (1)$$

is related to the diffusion coefficient via $msd(\tau) = 2nD\tau$ where $n = 2$ for the quasi-two-dimensional trajectories $\boldsymbol{r}(t) = (x(t), y(t))$ analyzed here. Note that only the first 10% of the msd is used to determine diffusion coefficients in order to avoid artifacts that arise from statistical limitations. To validate this methodology, we apply it to PEG-coated particles that are freely floating in water. The obtained diffusion coefficient

$$D_{\text{PEG}}^{\text{H}_2\text{O}} = (0.45 \pm 0.09) \ \mu\text{m}^2/\text{S}$$

agrees well with the theoretical value of 0.48 $\mu\text{m}^2/\text{s}$ as predicted by the Stokes-Einstein relation.

This single-particle tracking approach allows us to probe the permeability of mucin hydrogels at various positions (Fig. 1, A–D). For each experiment, we report the average diffusion constant for such an ensemble of particles together with a parameter describing the spatial heterogeneity of the obtained distribution of diffusion constant values. The relative distribution width of a given quantity is well suited to compare its degree of heterogeneity, even if the corresponding mean values vary over orders of magnitude (19). Here, the relative width of the diffusion coefficient distribution,

$$\sigma_{\text{rel}} = \sigma/\overline{D}_{\text{apparent}},$$

is analyzed where σ denotes the standard deviation and $\overline{D}_{\text{apparent}}$ represents the mean value of the apparent diffusion coefficients. For conditions with spatially homogeneous permeability properties, this value is ~20–40% (Fig. 1 B), whereas larger values indicate significant heterogeneities (Fig. 1 D).

RESULTS

Surface charge is a selection criterion for particle translocation in mucin hydrogels

First, we compare the microscopic mobility of different particle species (amine- and carboxyl-terminated polystyrene particles as well as their PEGylated counterparts) (Table 1) in a 0.5% (w/v) mucin hydrogel at pH 3, i.e., at conditions that resemble the environment in the stomach. According to the Stokes-Einstein relation, a 1-μm-sized particle should exhibit a diffusion coefficient on ~0.5 μm^2/s in water. As depicted in Fig. 2 A, the diffusion constants of all four particle types are significantly reduced in the mucin hydrogel (>20–250-fold) compared to free diffusion in water, indicating that the acidic mucin hydrogel constitutes a significant diffusion barrier for these particles. We also observe that the mean diffusion coefficient of PEG-coated polystyrene particles exceeds the corresponding value of untreated carboxyl-terminated (i.e., strongly negatively charged) and amine-terminated (i.e., strongly positively charged) particles up to 10-fold (Fig. 2 A). This implies that the microscopic mobility of the particles within a mucin hydrogel correlates with their surface properties (Fig. 2, A and C): the higher the surface potential, the stronger the particle mobility is suppressed. This finding suggests that charged particles can interact with the mucin polymers via electrostatic forces, and that these interactions may reduce particle mobility.

High salt concentrations increase particle mobility in the mucin hydrogel

Electrostatic interactions are sensitive to the ion content of a solution. In buffer, the surface charge of synthetic particles or polymers is partially shielded by solubilized ions that form shells of counter charges around the surface of the molecule (Fig. 3 A). This Debye screening becomes more pronounced with increasing salt concentrations. As a consequence, the strength of the attractive or repulsive forces between diffusing particles and the hydrogel will depend on the salt content. Thus, if electrostatic interactions between particles and mucin polymers are relevant for particle translocation, then the mobility of charged particles should be sensitive to changes in the ionic strength of the buffer.

Fig. 3 B shows that high concentrations of either NaCl or $CaCl_2$ indeed increase the mobility of charged particles in
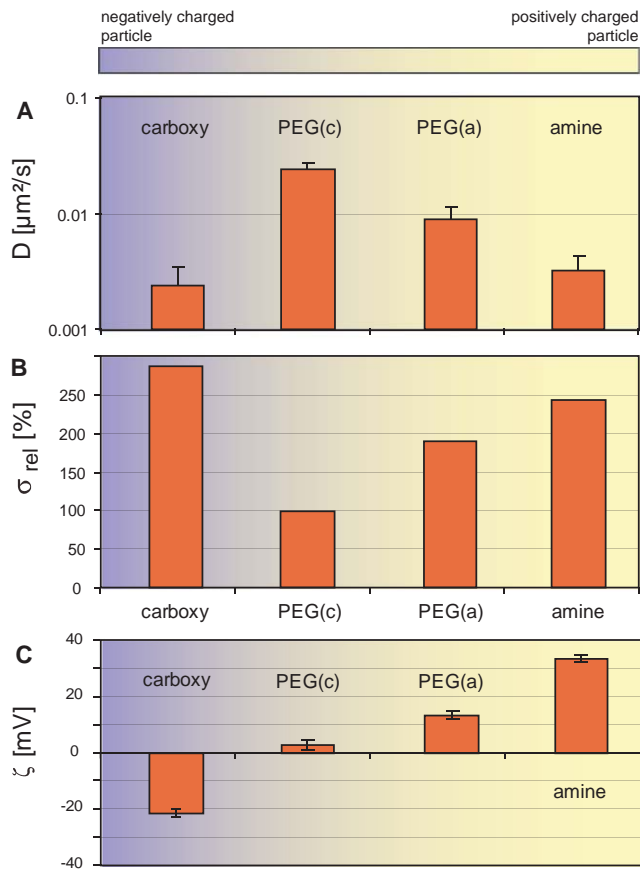
FIGURE 2 (A) Microscopic mobility of particles of ~1-μm size with different surface properties in a 0.5 (w/v) mucin hydrogel at pH 3. The background shading indicates the surface properties of the particles changing from negative to positive charge. PEG(a) and PEG(c) particles were created by PEGylation of amine-terminated and carboxy-terminated particles, respectively. Apparent diffusion coefficients D were determined by single particle tracking for ensembles of ~50 particles per condition (see Materials and Methods). The error bars denote the error of the mean $\sigma/\sqrt{N}$, where σ represents the standard deviation of the obtained distribution and N denotes the number of particles analyzed per condition. (B) Spatial heterogeneity in particle diffusion behavior as represented by the relative distribution width $\sigma_{rel} = \sigma/\overline{D}_{apparent}$ (see Materials and Methods and Fig. 1). Low particle mobilities correlate with higher variances in the diffusion behavior of the particle ensemble. (C) Zeta-potentials for the test particles investigated in panels A and B.

FIGURE 3 (A) Schematic representation of mobile (= neutral or neutralized, *gray*) and immobile (= charged, *yellow*) particles in mucin hydrogels. At high salt concentrations, ions should weaken the electrostatic interaction of the charged particles with the charged mucin biopolymers by Debye screening. (B) The mucin hydrogel permeability depends on the buffer salt concentration. The data shown was obtained at pH 3 for a 0.5% (w/v) mucin hydrogel and a 1% (w/v) mucin hydrogel, respectively. In both cases, the mobility of charged amine-terminated particles is considerably increased at high ionic strengths compared to low salt conditions. This increase in mobility is more pronounced at the higher mucin concentration. In contrast, the mobility of neutral PEG(c) particles in a 1% (w/v) mucin hydrogel at pH 3 is virtually unaffected by changes in the buffer salt conditions.

mucin hydrogels. Importantly, the mobility of neutral PEG particles is largely unaffected by changes in the salt concentration (Fig. 3 B). These data support the previous conclusion that charged particles can interact with the mucin polymers via electrostatic forces, and that these interactions are sensitive to salt. We note that increasing salt concentrations could also destabilize electrostatic interactions that occur between the mucin polymers, and thereby cause rearrangements of the hydrogel architecture that affect its permeability. However, in our experimental conditions this seems to be a minor contribution as only charged, but not neutral particles of the same size gain mobility (Fig. 3 B). Thus, high salt appears to increase the mobility of charged particles predominantly by
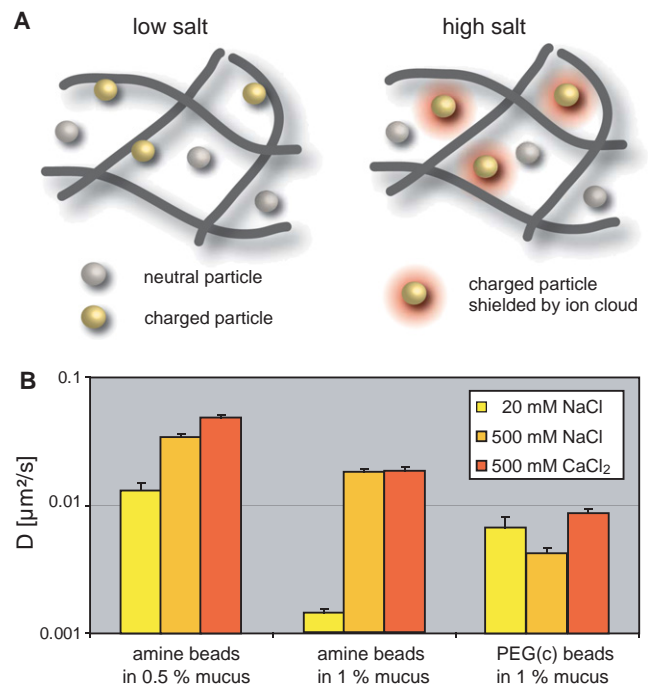
weakening their interactions with the mucin polymers, rather than by reorganizing the mucin network. Our data also show that the average diffusion coefficient of the amine particles is increased ~10-fold in the 1% (w/v) hydrogel compared to a three-to-fourfold increase at 0.5% (w/v) mucin. This shows that the release effect induced by salt is more pronounced at higher mucin polymer density.

Acidic mucin hydrogels form tighter barriers and are more selective than neutral mucin hydrogels

Native mucin hydrogels are exposed to different pH levels throughout the human body (Fig. 4 A): in the oral cavity, nose, or the lung, the mucus layer is neutral or slightly alkaline (20–22), whereas gastric and intestinal mucus is exposed to acidic environments (23). In the female genital tract, pH conditions can change during the ovulatory cycle (24,25). To systematically explore the impact of pH on permeability, we repeat the identical experiment as described before for a 0.5% (w/v) mucin gel reconstituted at neutral pH.

Our results demonstrate that increasing the pH from 3 to 7 results in a general increase in mobility for 1-μm-sized
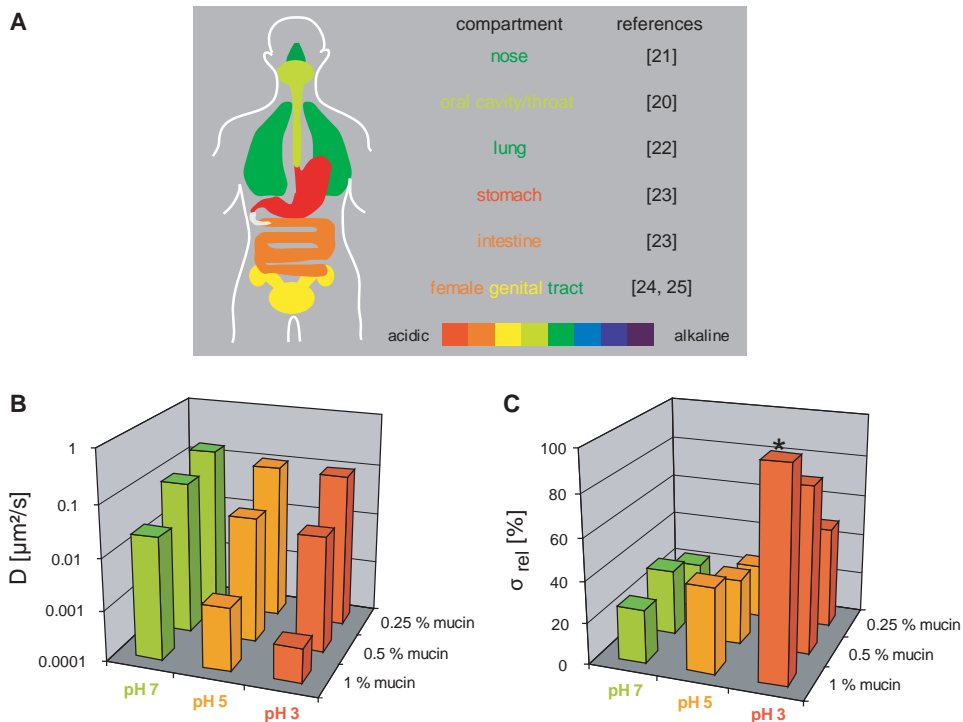
FIGURE 4 (*A*) Mucus hydrogels in the human body exist at different levels of pH as indicated by the color scheme. In the nose or the lung, the mucus layer is slightly alkaline, whereas gastric and intestinal mucus is exposed to rather harsh acidic environments. In the female genital tract, pH conditions can significantly change during the ovulatory cycle, ranging from neutral to acidic conditions. The concentration of mucin polymers in human mucus hydrogels covers a range of 1–5% (see main text). A similar range is explored in our in vitro system albeit at reduced absolute values. (*B*) Mean diffusion coefficient and (*C*) spatial heterogeneity of the particle diffusion behavior, σ_{rel}, for PEG(a) particles in mucin hydrogels at different pH levels and mucin concentrations. Small σ_{rel} values indicate homogeneous distributions of diffusion constants, while large values indicate strong heterogeneities. For 1% (w/v) at pH 3 (*bar* is labeled with an asterisk), $\sigma_{rel} = 100\%$ is chosen for clarity. Note that the calculated value is much larger, $\sigma_{rel} = 600\%$. This is because, at this particular condition, 90% of the particles are completely immobile and show no detectable motion within the resolution of our tracking algorithm. A few particles show weak mobility.

particles. At pH 7, the mean mobility of PEG particles is increased twofold for PEG(c) and fivefold for PEG(a) compared to pH 3. For untreated amine- or carboxyl-particles the effect is even stronger, i.e., ninefold and 13-fold, respectively (Table 2 and Fig. 2 *A*). This experiment also reveals that at pH 7, the diffusion coefficient of PEG particles is only 30–40% larger compared to untreated carboxyl- or amine-particles (Table 2). Thus, in a mucin gel at pH 7, the diffusion of charged particles is not greatly impeded compared to that of neutral (PEGylated) particles. We conclude that a mucin gel at pH 3 forms a more effective barrier toward particle diffusion, and is also more discriminating toward the surface charge of translocating particles than a mucin hydrogel at pH 7.

The interaction of particles with mucin polymers is regulated at small length scales

Earlier studies in gastrointestinal and cervical mucus show that even for rather mobile particles a certain fraction remains immobilized (8,26). This effect might be brought about by geometrical constraints within the mucus gel, and indeed, the organization of mucus at low pH has been described as highly heterogeneous (27,28). However, it might as well be due to a spatially heterogeneous distribution of interaction sites within the mucin network. To test this, we next aim to analyze particle mobility in terms of spatial heterogeneity in more detail, using the relative width of the obtained diffusion

coefficient distributions as a measure (see Fig. 1 and Materials and Methods).

If geometrical constraints are the main reason for spatially heterogeneous mobility, then particles of the same size but with different surface properties should behave alike. Our data show that in a mucin hydrogel at pH 3, this is not the case: for charged particles, i.e., when electrostatic interactions with the mucin hydrogel are strong, the relative distribution width of measured diffusion constant values is relatively broad ($\sigma_{rel} > 240\%$, Fig. 2 *B*). It seems that the diffusion behavior of 1-μm-sized particles can change on distances of 3–4 bead diameters (*red circle* in Fig. 1 *D*), suggesting that the attraction of charged particles by the mucin polymers may be regulated at relatively small length scales. For comparison, neutral PEG(c) particles have more similar diffusion coefficients across larger areas as reflected by narrower distributions ($\sigma_{rel} = 98\%$, Fig. 2 *B*). This indicates that at pH 3, the distribution of interaction sites for charged particles throughout the mucin network is heterogeneous and determines the diffusion behavior of particles. In contrast, at pH 7, the diffusion behavior of all particle types is spatially homogeneous as indicated by small relative distribution widths (~30–40%; see Table 2). Here, the surface properties of translocating particles do not crucially affect their diffusion behavior, and geometrical effects as imposed by the overall architecture of the hydrogel appear to play the dominant role.

TABLE 2 Average diffusion coefficients, $\overline{D}$, and spatial heterogeneity, σ_{rel}, for different particles in a 0.5% mucin hydrogel at pH 7

	Carboxyl beads	PEG(c) beads	PEG(a) beads	Amine beads
$\overline{D}$ [μm^2/s]	0.032 ± 0.002	0.040 ± 0.002	0.041 ± 0.003	0.028 ± 0.002
σ_{rel} [%]	32.6	27.8	40.8	37.9

At this pH, PEG-coating provides a mild mobility advantage of ~30–40% compared to untreated amine- or carboxyl beads, while the relative widths of the obtained diffusion coefficient distributions are very low compared to acidic pH conditions. The corresponding ζ-potential values for all these particles are compiled in Table 1.

Higher mucin concentrations allow for a broader range of permeability control

In the human body, mucin concentrations can vary considerably with both the location of the mucus hydrogel (4–6) and different physiological and pathological conditions (6). We have already shown that the sensitivity of particle mobility in mucin hydrogels toward salt is more pronounced at higher mucin concentrations (Fig. 3 B). To explore the effect of mucin concentration further, we next map the permeability of mucin hydrogels with respect to distinct mucin concentrations.

In Fig. 4 B, the apparent diffusion coefficients of PEG(a) particles are plotted as a function of both the mucin density and pH. At pH 3, low mucin concentrations (0.25% (w/v)) reduce the mobility of the particles approximately threefold compared to free diffusion. This compares well with values obtained for smaller particles in hydrogels with higher mucin density as determined by dynamic light scattering (27). With increasing mucin concentration, the impact of the barrier becomes more pronounced: at 1% mucin (w/v), the microscopic mobility of the particles is almost completely suppressed. In this condition, the diffusion coefficients are in the resolution limit of our particle-tracking algorithm but suggest a 2500-fold reduction compared to free diffusion. This suggests that the mucin concentration is an essential parameter for permeability control.

Our data also show a second aspect of how increasing mucin concentrations alter the filtering properties of the mucin hydrogel: the impact of pH alterations on the hydrogel selectivity becomes more pronounced. In 0.25% (w/v) mucin hydrogels, the apparent diffusion coefficients differ by less than a factor of 2 between pH 7 and pH 3. In comparison, in 1% (w/v) mucin hydrogels, the mobility differs by more than a factor of 100 between pH 7 and pH 3. This demonstrates that the permeability of mucin hydrogels depends on the polymer concentration: the higher the polymer concentration, the more sensitively the mucin hydrogel can recognize the surface properties of translocating particles.

In analogy to Fig. 2 B, where we analyze the spatial heterogeneity of particle mobility, we find that particle mobility becomes increasingly sensitive to the distribution of interaction sites as either pH decreases or the mucin concentration increases: at pH 3 the mobility distributions are relatively broad, being most pronounced in the 1% (w/v) mucin hydrogel (Fig. 4 C). Here, almost 90% of the particles are completely immobilized, whereas very few particles are mobile. In contrast, at low mucus concentrations and at neutral pH, the relative distribution widths are low and the permeability of the mucin hydrogel is very similar at all hydrogel positions tested here.

DISCUSSION

We have shown that the mobility of a microscopic particle within a mucin hydrogel is determined by the mucin polymer concentration, electrostatic interactions of the particle with the mucin polymers, and the detailed distribution of interaction sites within the mucin hydrogel as determined by the microarchitecture of the mucin network. Particle-mucin interactions can be modulated in strength by environmental conditions such as pH and salt content. We propose that mucin polymers generate an absorption filter whose selectivity depends on the density of mucin polymers as well as environmental factors such as pH and ionic strength.

Our detailed analysis of whole ensembles of different particle species in reconstituted mucin hydrogels demonstrates that in addition to the average diffusion constant, the spatial heterogeneity of the particle diffusion behavior is a second important parameter for the characterization of hydrogel filters. We show that high particle surface charges, low pH, and high mucin concentrations tend to generate a strong but spatially heterogeneous barrier toward particle translocation whereas for uncharged particles, at neutral pH, and at low mucin concentrations, the hydrogel permeability is high and homogeneous. Other techniques which rely on more macroscopic data such as translocation chamber assays (26) or scattering techniques reporting only ensemble average values (27) will miss this second piece of information.

Changes in the pH conditions of the hydrogel might not only tune the interaction strength between the translocating particles and the hydrogel polymers but could also affect the microscopic organization of the mucin polymer network. Indeed, the mobility at low pH is reduced compared to neutral pH, not only for charged but also for neutralized PEG-particles (Fig. 2 A and Table 2), suggesting that pH-dependent structural rearrangements of the mucin hydrogel might partly be responsible for the decrease in particle mobility. Both pH and salt have been shown to affect the swelling of mucin gels (29). However, the mobility of PEG-coated particles is affected less by pH than that of more strongly charged carboxyl- and amine-terminated particles of identical size. Thus, mere alterations in the microstructure of the mucin

A

B
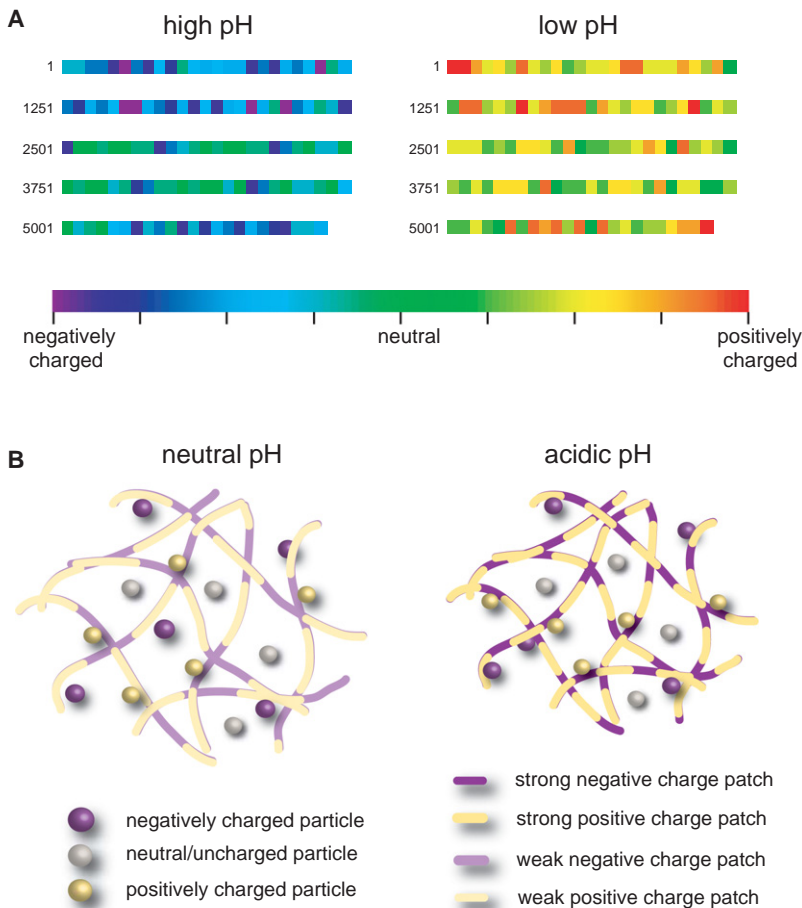
FIGURE 5 (*A*) The human mucin MUC5AC protein contains numerous acidic and basic amino acids. The pK values of Asp and Glu are 4.7, whereas those of His, Lys, and Arg are considerably higher (6.5–12). Thus, the mucin MUC5AC will undergo a major transition in its charge state between high and low values of pH. To visualize the magnitude of this effect, we assume Asp and Glu to contribute one negative elementary charge each at high pH, whereas His, Lys, and Arg each contribute one positive elementary charge at low pH. The entire mucin MUC5AC polypeptide sequence is contracted by pooling subsequent groups of 50 amino acids into virtual domains, which are colored according to their total net charge. The numbers indicate the amino acid position in the mucin sequence. Please note that for this schematic representation the glycosylation of the mucin polymer was neglected, although glycosylated regions might considerably contribute to the barrier function of mucin hydrogels (see Discussion). (*B*) Biophysical model for the filtering function of mucin hydrogels: The mucin hydrogel can be represented as a network consisting of either positively or negatively charged segments. Charged particles (*yellow*, *purple*) are trapped by the opposite charge on the mucin polymer (*purple*, *yellow*), although neutral particles (*gray*) can diffuse nearly unhindered. At neutral pH (*left*), the charge on the mucin polymer is relatively weak, whereas at acidic pH (*right*), its strength is significantly increased. The different sizes of the two hydrogel pieces illustrate gel shrinkage upon pH decrease, as also observed experimentally.

hydrogel do not sufficiently account for the observed suppression of the particle's mobility.

Instead, we propose that one additional critical aspect of mucin polymer function is their ability to trap molecules via electrostatic interactions. The major gel-forming component of mucin hydrogels is the mucin MUC5AC. The human mucin MUC5AC protein contains numerous acidic and basic amino acids. The protonation of these amino acids is sensitive to pH, therefore, the MUC5AC polymer can be expected to undergo a major transition in its charge state upon pH alteration (Fig. 5 *A*). The charge profile of the mucin polymer reveals that the mucin polymer is not uniformly charged, but instead appears to consist of discrete areas of neutral/charged amino acids which are distributed along its backbone. The size and electrostatic attraction potential of these charged areas will depend on both the ionic conditions and the pH of the surrounding medium. In addition, mucin polymers are densely glycosylated (30). Although the function of glycosylation in mucus biology is not known, it is possible that these sugars provide binding sites and contribute to the filtering process in a manner similar to that reported for the proteoglycan heparan sulfate in extracellular matrix hydrogels (31). Thus, both the mucin protein backbone and the

glycosylated regions might provide binding sites for oppositely charged particles (Fig. 5 *B*).

Such a model of a biopolymer-based filter based on electrostatic interactions was proposed to rationalize the filtering properties of extracellular matrix hydrogels (31). Moreover, electrostatic interactions have been suggested to present an important selection criterion for translocation through the nuclear pore complex (L. Colwell, M. Brenner, and K. Ribbeck, unpublished), and thus might turn-out to set the basis for a generic filtering mechanism employed by various biological hydrogels.

Alterations of the pH level or the ionic strength of a hydrogel can be a fast and efficient way to globally adjust its permeability properties. Changes in the biopolymer density or adjustments in the biopolymer charge state by covalent modifications would probably pose a much higher metabolic cost, require more time, and would be more difficult to reverse. Such changes in the pH level can be induced by the human body on purpose, e.g., opening a window of opportunity for sperms in the cervical tract of women during the ovulatory cycle (24). They might, however, as well be created by pathogens in order to penetrate the mucus barrier. For instance, such a strategy is employed by *Helicobacter*

pylori, which is known to locally increase the pH in gastric mucus by enshrouding itself in an ammonium cloud. This enables the otherwise trapped bacterium to swim through mucus (16). The reconstituted mucin hydrogel presented here is an ideal model system for further studies on how bacteria or viruses are effectively shielded from the epithelial layer of the human body—or how this barrier function is compromised by pathogens. It also constitutes a biological template for a smart gel whose mechanical properties as well as permeability can be adjusted by external chemical stimuli. The technical realization of an engineered hydrogel mimicking the outstanding properties of mucus provides an ideal platform for the systematic investigation of other biological hydrogels and the development of new polymer-based materials.

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