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Short communication

Work of adhesion between mucin macromolecule and calcium-alginate gels on molecular level

Riste Popeski-Dimovski*

Institute of Physics, Faculty for Natural Sciences and Mathematics, Skopje, Macedonia

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ABSTRACT

The bioadhesion of biopolymers to mucus layers is of great interest for the development of drug delivery systems. Herein we use AFM force measurements to evaluate the interaction on molecular level between a mucin macromolecule attached to an AFM tip and a calcium-alginate gel layer. The total work of adhesion is measured from the AFM force curves depending on different parameters: time of contact, G/M ratio of the alginate, and crosslink ratio of the gel. The total work of adhesion is found to be in the range of 1×10^{-19} to 6×10^{-18} J. The results show that the work of adhesion increases with the time of contact but it is independent from the molecular mass of the alginate, the G/M ratio of the alginate and crosslink ratio of the gel.

1. Introduction

In the past several decades alginate gels have been extensively studied because of their controlled release and bioadhesive properties (Gremiao, Carvalho, Bruchi, & Evangelista, 2010). In a biocompatible system for the delivery of an active pharmaceutical ingredient, the bioadhesive substrate is usually some type of mucus, thus the mucoadhesive force between the mucin and the alginate macromolecules becomes of interest (Henriksen, Green, Smart, Smistrad, & Karslen, 1996; Woodley, 2001). There are different theories and experiments that describe bioadhesion but they each give different results that are hard to compare, so the understanding of the dynamics of the mucoadhesive process is of interest (Andrews, Laverty, & Jones, 2009). Here we use atomic force microscopy (AFM) as a tool to measure the interaction force between a mucin macromolecule and a sodium alginate gel layer to estimate the work of adhesion that comes from the molecular interaction of the adhesives in order to understand the dynamics of mucoadhesion (the time dependence of mucoadhesion) (Lee, Park, & Robinson, 2000). This method is good because it simulates in vivo conditions, gives precise measurement of the forces of interaction and can be used to measure the adhesion arising from weak electrostatic interactions and interpenetration of single macromolecule chains. In this experiment we established a bottom-up approach in which the mucin macromolecule is attached to an AFM tip, instead of the mucin being as a mucous substrate, and the alginate is in the form of a thin gel layer.

2. Materials and methods

2.1. Chemicals

The sodium alginate is from FMC Biopolymer Norway. We use three types of sodium alginate with different guluronic to mannuronic (G/M) acid residue ratio, viscosity and molar mass as supplied, Protanal LF120M, LF200M and LF240D. Data for the alginates is given in Table 2.1 (Shen, Rendevski, Kavakli, Sepenhrianazar, 2010, data reproduced with authority from the authors).

Mucin is Porcine stomach type II Mucin from Sigma Aldrich. $CaCO_3$, glucono-δ-lactone (GDL), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDAC), N-hydroxysuccinimide (NHS), (3-aminopropyl)triethoxysilane (APTES), HEPES are from Sigma–Aldrich.

2.2. Preparation of sodium alginate gel layer

Sodium alginate is dissolved in water with a 1% (w/v) concentration at room temperature. For gel preparation we use in situ gelling

* Tel.: +389 70339103.
E-mail address: ristepd@gmail.com

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Table 2.1
Molar weight, G/M ratio and viscosity data of the alginates used in the research.

Type	$\overline{M_w} \times 10^3$ (g/mol)	$\overline{M_n} \times 10^3$ (g/mol)	G/M ratio	Viscosity at 1% (w/v)
LF120M	670	340	70/30	92.0 mPas
LF200M	690	310	50/50	94.0 mPas
LF240D	500	275	45/55	108.0 mPas

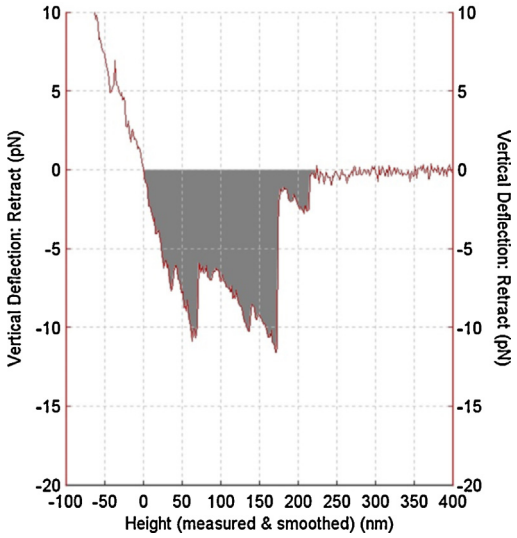

Fig. 2.1. Representative retraction curve of the interaction between mucin macromolecule and sodium alginate gel layer; and calculation of the work of adhesion as an integral under the retraction curve (alginate LF200M at 1% alginate concentration, 1X crosslink ratio and 15 s contact time).

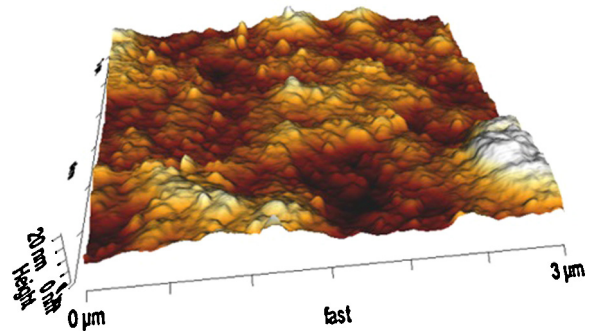

Fig. 2.2. AFM 3D image of the surface of the thin sodium alginate gel layer for LF200M at 1% (w/v) alginate concentration and 1X crosslink ratio.

method with calcium ion as a cross linker and GDL as activator, as previously described in literature (Kuo & Ma, 2001). In short, $CaCO_3$ is added to the alginate solution and vortexed for 1 min, then GDL is added and vortexed for 45 s. After this, the mixture is homogenous and is poured as a thin layer over a glass slide and it is allowed to crosslink for 24 h. We prepare four crosslink ratios noted as 0.5X, 1X, 1.5X and 2X where 1X is the base value. In all preparations GDL to $CaCO_3$ ratio is held at 0.5 to maintain neutral pH of the solution. Before measurement, the layer is hydrated in the liquid cell of the AFM for 20 min in HEPES buffer solution at pH 7.4.

2.3. AFM tip activation with mucin macromolecules

For the preparation of AFM tips we use a covalent linking procedure as explained at www.piercent.com, Carbodiimide Crosslinker Chemistry and literature (Gunning et al., 2013). In short, first we prepare fresh 1 mg/ml mucin solution in HEPES buffer at pH 7.4.

Table 3.1
Time dependent average work of adhesion for all alginates.

Time of contact (s)	LF120M (10^{-19} J)	LF200M (10^{-19} J)	LF240D (10^{-19} J)
5	3.532	3.791	3.633
10	6.552	6.482	6.655
15	13.27	12.45	14.89
30	23.25	20.84	24.96
60	29.79	27.35	30.91
120	36.09	35.93	38.76

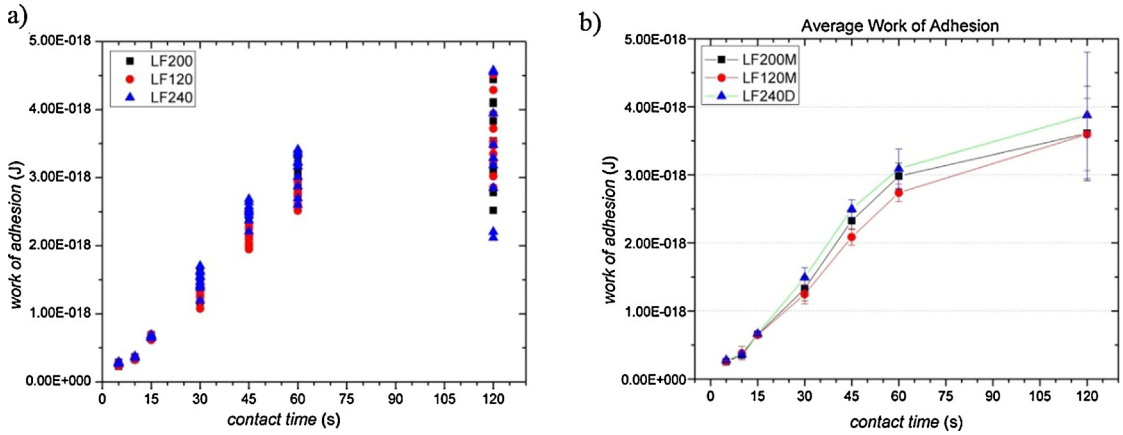

Fig. 3.1. (a) All measurement values and (b) average work of adhesion with standard deviation for mucin macromolecule on calcium-alginate gel dependent from time of contact for all alginate types.