The Viscoelastic Properties of the Cervical Mucus Plug

Sara Kjær Bastholm[1], Naja Becher[2], Peter Reimer Stubbe[3],
Ioannis S. Chronakis[3] and Niels Uldbjerg[1]

[1] Department of Obstetrics and Gynecology, Aarhus University Hospital, Denmark
[2] Department of Clinical Genetics, Aarhus University Hospital, Denmark
[3] Technical University of Denmark, DTU Food, Lyngby, Denmark

ABSTRACT

The objective of this study was to characterize the viscoelastic properties of cervical mucus plugs (CMPs) shed during labor at term. Spontaneously shed cervical mucus plugs from healthy women in active labor, were tested. The viscoelastic properties of cervical mucus plugs were investigated with using frequency and stress sweep experiments within the linear viscoelastic region. Random-effects regression was used for statistical analysis. The CMPs are solid-like viscoelastic structures and the elastic modulus dominated the viscous modulus at all frequencies. These rheological characteristics are probably essential for the CMP's ability to form and sustain a plug in the cervical canal during pregnancy, thereby reducing the risk of ascending infections.

INTRODUCTION

During pregnancy, the cervical mucus plug (CMP) occludes the cervical canal and forms a barrier between the sterile uterine cavity and the microbe-rich vagina[1]. CMPs are shed spontaneously during labor and are rather heterogeneous structures. They vary from clear transparent to cloudy yellow in visual appearance and in the amount of visible blood (Fig. 1). They also vary in size (3g - 10g) and in consistency – some seem thinner and more fluid-like whereas others are more rubbery and solid.

The immunological barrier functions of the CMP is well described and comprise high concentrations of antimicrobial peptides, in addition to an abundance of immune cells[2]. However, to date the properties that constitute the mechanical barrier of the CMP have never been described.

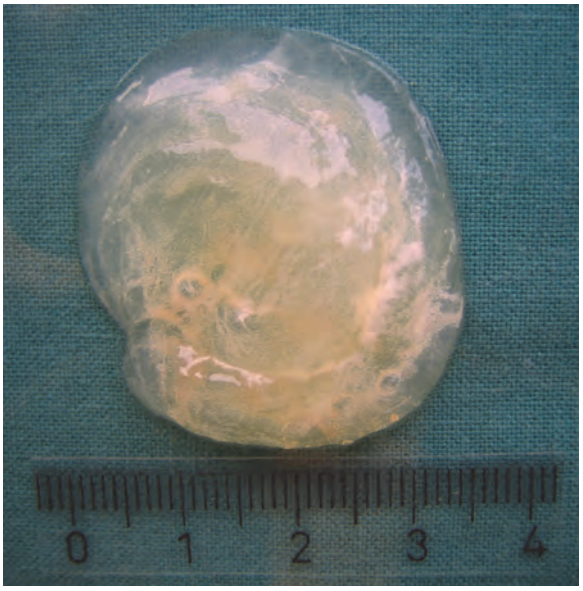

Figure 1. Cevical mucus plug shed during normal active labor.

The CMP resembles the lutheal-phase cervical mucus rather than the ovulatory cervical mucus[3], and we hypothesize that viscoelastic properties enable the CMP to

occlude the cervix during pregnancy. Hereby, the CMP constitutes a mechanical barrier which is important for the prevention of ascending intrauterine infection during pregnancy. With this study we aim to characterize for first time the rheological properties of CMPs shed during labor at term[4].

MATERIALS AND METHODS

Eleven CMPs were collected, stored at 4°C and analysed within 24 hours. Prior to analysis they were cut into 2-7 specimens (median weight 1.37 g; IQR = 1.30 – 1.38) depending on the original CMP size, resulting in a total of 41 specimens. When preparing the specimens, care was taken to avoid mechanical agitation. Rheological properties were assessed in the linear viscoelastic region and at 37.0°C in a dynamic oscillatory rheometer (Reologica Instruments AB, Lund, Sweden) equipped with a serrated parallel-plate with a diameter of 30 mm and a gap of 1.0 mm. To avoid evaporation during the experiment, the edges of the CMPs were covered with silicone oil (Synperonic PE/L 6l). All specimens were temperature calibrated for 120 seconds prior to analysis.

Statistical analyses were conducted in Stata IC 11. Descriptive statistics are given by mean values and standard deviation (SD) if nothing else is stated. Fixed effects (within) regression analysis was used to estimate the difference in rheological variables between specimens evaluated fresh or after freezing. Random-effects GLS regression analysis was used to estimate the influence of blood contamination and specimen weight on the viscoelastic variables (elastic and loss modulus). Random-effects ML regression was used to estimate CMP inter and intra variability. All statistical methods take account for the clustering of the data where specimens are grouped and matched. Details are provided in the relevant results sections. *p*-values below 0.05 were considered significant.

RESULTS

Though no statistical significant correlations between hemoglobin concentration and the viscoelastic modulus (G′ and G″) was found when performing a Random effects GLS regression analysis (G′: $P = 0.28$, G″: $P = 0.32$), specimens with blood contamination > 10% were excluded to ensure comparability.

Frequency Sweeps. All CMP specimens showed solid-like viscoelastic behaviour as seen in the representative example shown in Fig. 1. This behaviour was substantiated by the fact that the elastic modulus (G′) was 3-4 times greater than the viscous modulus at all frequencies. It was also reflected by the mean tan delta which was below 1 at all frequencies (0.38 at 0.01 Hz, 0.27 at 1Hz). The small variation with frequency for both the elastic (G′) and the viscous modulus (G″) (Table 1a) and the frequency dependent behaviour of the complex viscosity with a mean (95%CI) slope of -0.88 (-0.87– -0.90) also emphasize this solid-like viscoelastic behaviour.

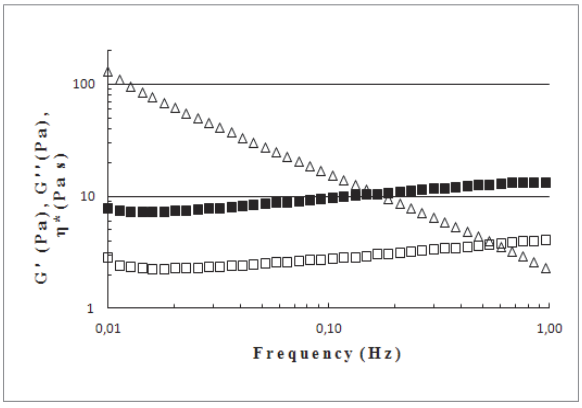

Figure 1. Representative frequency sweep recorded from one CMP specimen using 5 Pa stress. Elastic modulus (G′, ■), viscous modulus (G″, □), and complex viscosity (η*, Δ).

The elastic modulus followed the power law relation $G'(\omega) = K\,\omega^{A}$; where A was equal to 0.13 (0.09 – 0.15) (median (IQR)) and K to 24.1 (17.8 - 33.1) and R^2 to 0.98 (0.93 – 0.99). The G″ did not follow this power law relation.

Stress Sweeps. Two representative examples of CMP stress sweep behaviour are shown in Fig. 2. Despite variation in their rheological behaviour with shear stress, all CMP specimens showed a linear viscoelastic region from 1 – 15 Pa. Within this range the elastic modulus was approximately 4 times greater than the viscous modulus (Table 1b). At higher stresses the elastic and the viscous modulus decreased, culminating in a crossover between the two.

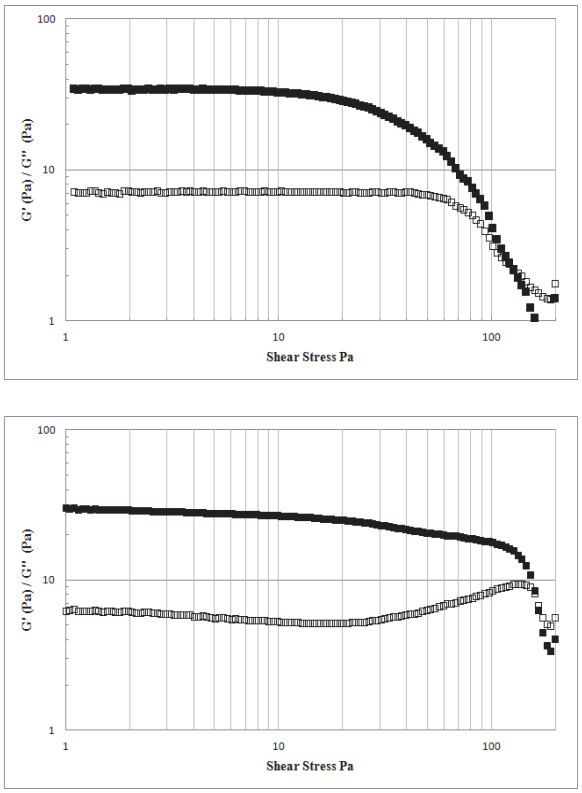

Figure 2. Representative stress sweeps showing the viscoelastic behaviour recorded from two CMP specimens. Elastic modulus (G′, ■) and viscous modulus (G″, □), using frequency of 1Hz.

Table 1. Analysis of variance of CMP rheological variables obtained at 0.01 Hz and 1 Hz from frequency sweeps (Table 1a) and at 1 Pa and 15 Pa from stress sweeps (Table 1b). Mean ± Standard deviation (SD), Explained variation $(1 - \frac{\text{Residual MS}}{\text{Total MS}}) * 100$ shows the percentage of variation explained by CMP intervariability.

Table 1a.

Rheological variable n = 33		Mean ± SD	P-value	Explained variation
Elastic modulus				
	0.01 Hz	15.6 ± 7.5	0.10	28.4%
	1 Hz	25.0 ± 10.5	0.025	42.1%
Viscous modulus				
	0.01 Hz	5.1 ± 1.8	0.014	47.0%
	1 Hz	5.7 ± 2.0	0.02	44.1%
Tan delta				
	0.01 Hz	0.36 ± 0.1	0.053	32.9%
	1 Hz	0.25 ± 0.1	0.007	51.3%

Table 1b.

Rheological variable n = 31		Mean ± SD	P-value	Explained variation
Cross over point				
	Pa	149 ± 21.0	0.21	22.4.4%
Elastic modulus				
	1 Pa	26.2 ± 9.5	0.058	36.4%
	15 Pa	22.2 ± 10.6	0.054	37.1%
Viscous modulus				
	1 Pa	5.9 ± 2.0	0.07	35.1%
	15 Pa	5.9 ± 2.0	0.05	38.7%

Variation within and between CMPs. The regression analysis evaluated to what extent the variation between the specimens reflected differences between CMPs or by CMP heterogeneity and methodological imprecision. Despite the relatively large variation within CMPs, significant differences between CMPs were found. The statistical significant differences between CMPs, however, were only present when comparing rheological variables obtained from frequency sweeps. Among the rheological variables obtained from frequency sweeps the most pronounced differences were found between the CMPs elastic modulus (Table 1a, b). Concerning the cross over point obtained from the stress sweeps, none of the variation could be explained by differences between women.

DISCUSSIONS

The rheological analyses conducted in this study demonstrate that CMPs shed spontaneously during vaginal delivery at term are solid-like viscoelastic materials with predominating elastic properties. Furthermore, they are heterogeneous structures which were reflected by a relatively large variation within the CMPs. Yet when comparing CMPs from different women, the rheological variables G′, G″ and tan delta obtained from frequency sweeps differed significantly.

The large variation in the rheological properties between specimens from the same CMP is in good agreement with their visual appearance showing different colours and density in different parts. A similar heterogeneity has been described within cervical mucus from non-pregnant women[5]. The differences between the individual CMPs may arise from different mucin composition and concentration. Trefoil factor peptides 1-3, which are small mucin co-secreted peptides believed to cross link mucins[6], and shown to increase the viscoelastic properties of mucin solutions, might be of importance[7]. It has also been shown that mucin, in co-operation with trefoil factor peptides, constitute a non-specific barrier against a broad range of insults[8]. In addition, pH seems to influence mucin aggregation as well as the microstructure and rheological properties of cervical mucus from non-pregnant women[9].

One can only speculate on the physiological importance of the CMP. It is, however, well established that the CMP contains antimicrobial constituent that protects against ascending infections from the vagina to the uterus, thus preventing preterm birth[10]. This antimicrobial barrier function is supported by the CMP's solid-like viscoelastic behaviour which enables the CMP to form a plug that occludes the cervical canal without flowing into the vagina during normal daily activities[11].

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REFERENCES

1. Becher N, Waldorf KA, Hein M, and Uldbjerg N. (2009), "The cervical mucus plug: structured review of the literature", *Acta Obstet Gynecol Scand.*, **88**, 502-13.

2. Lee DC, Hassan SS, Romero R, Tarca AL, Bhatti G, Gervasi MT, *et al.* (2011), "Protein profiling underscores immuno-logical functions of uterine cervical mucus

plug in human pregnancy", *J Proteomics* **16**, 817-28.

3. Chretien FC, Cohen J, Borg V, and Psychoyos A. (1975), "Human cervical mucus during the menstrual cycle and pregnancy in normal and pathological conditions", *J Reprod Med* May, **14**, 192-6.

4. Bastholm S.K., Hansen L. K., Samson M. H., Stubbe P.R., Becher N., Nexø E., Chronakis I.S. and Uldbjerg N., (2013) "The viscoelastic properties of the cervical mucus plug", *Acta Obstet Gynecol Scand*., in press.

5. Wolf DP, Blasco L, Khan MA, and Litt M. (1977), "Human cervical mucus. I. Rheologic characteristics", *Fertil Steril* **28**, 41-6.

6. Tomasetto C, Masson R, Linares JL, Wendling C, Lefebvre O, Chenard MP, et al. (2000), "pS2/TFF1 interacts directly with the VWFC cysteine-rich domains of mucins", *Gastroenterology* **118**, 70-80.

7. Thim L, Madsen F, and Poulsen SS. (2002) "Effect of trefoil factors on the viscoelastic properties of mucus gels", *Eur J Clin Invest* **32,** 519-27.

8. Kindon H, Pothoulakis C, Thim L, Lynch-Devaney K, and Podolsky DK, (1995), "Trefoil peptide protection of intestinal epithelial barrier function: co-operative interaction with mucin glyco-protein", *Gastroenterology*, **109**, 516-23.

9. Brunelli R, Papi M, Arcovito G, Bompiani A, Castagnola M, Parasassi T, *et al.* (2007), "Globular structure of human ovulatory cervical mucus", *FASEB J.* **21**, 3872-6.

10. Hein M, Valore EV, Helmig RB, Uldbjerg N, and Ganz T. (2002), "Antimicrobial factors in the cervical mucus plug", *Am J Obstet Gynecol.,* **187**, 137-44.

11. Odeblad E. (1968), "The functional structure of human cervical mucus", *Acta Obstet Gynecol Scand*., **47**, 57-79.