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Reduced curvilinear velocity of boar sperm on substrates with increased hydrophobicity

Matthew Mears,[1,*] Thomas M. Kennelly,[1] Jonathan R. Howse,[2]
Drew S. Tarmey,[3] Mark Geoghegan,[1] and Allan A. Pacey,[4]

[1]Department of Physics and Astronomy, The University of Sheffield, Sheffield, S3 7RH, United Kingdom

[2]Department of Chemical and Biological Engineering, The University of Sheffield, Sheffield, S1 3JD, United Kingdom

[3]School of Medicine, University of Nottingham, Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3DT, United Kingdom

[4]Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Level 4, The Jessop Wing, Sheffield, S10 2SF, United Kingdom

[*]corresponding author: m.mears@sheffield.ac.uk

Abstract

The curvilinear velocity (VCL) of boar spermatozoa between standard microscopy glassware decreases when the slides are coated with the hydrophobic polymer polystyrene (PS) compared to the less hydrophobic poly(methyl methacrylate) (PMMA) coating. Sperm from three boars were observed and analyzed using particle tracking software. VCL did not differ significantly between coatings of different thickness, indicating no penetration of the sperm into the coating and that only the surface layer of the polymer film interacts with the sperm and buffer medium. The curvilinear velocity of sperm between PS-coated surfaces was significantly reduced compared to PMMA surfaces ($p < .0001$), and this was attributed to a stronger hydrophobic effect between PS and water. The size of this effect varied between different boars, perhaps as a consequence of variations in hydrophobicity of sperm from different boars or different ejaculates. The modification of surface properties in this way may improve our understanding of sperm behavior and may provide improvements to assisted conception techniques as animal or human sperm used in assisted conception are frequently manipulated in laboratory plastics as part of diagnostic procedures (e.g. semen analysis) or before injection into an oocyte or during the co-incubation with the oocyte in IVF. Controlling the velocity of sperm using the interaction properties of inert polymer coatings could lead to new sperm selection procedures for clinical use or the development of model systems to better understand sperm-surface interactions.

1. Introduction

The propulsion of mammalian spermatozoa occurs as a consequence of the forces generated by the beating flagellum as it translates through a viscous fluid; these forces are significantly affected by the presence of nearby fluid-solid interfaces [1]. However, the interactions between sperm and biological or man-made surfaces have been relatively poorly investigated to date. Many observations suggest that sperm preferentially accumulate near the surfaces of microscope slides between the fluid boundary and the surface [2-4] and theoretical models to explain the observation have been proposed [5]. However, such models are limited in scope in that they assume the physical and chemical properties of surfaces that sperm may encounter in biology are both uniform and identical, which is clearly not the case.

Following deposition, motile sperm typically travel through the female reproductive tract from the site of insemination to the site of fertilisation [6]. Depending on the species concerned, this will invariably involve sperm encountering a number of different epithelial cell types with radically different apical topography and surface chemistry of the glycocalyx. Direct observation suggests that interaction with the epithelial surface is important in many aspects of the sperm's journey [7, 8]. However in addition to surface chemistry, sperm interaction with epithelial surfaces may involve interaction between specific receptors, or may be influenced by mucous secretions or local ionic concentrations [6]. Moreover, during the sperm transport process the sperm surface chemistry may also undergo considerable modification associated with sperm capacitation or sperm ageing [9].

In contrast to the sperm's journey *in vivo*, ejaculated or surgically recovered animal or human sperm used in assisted conception procedures are frequently manipulated in laboratory plastics as they are either prepared to be co-incubated with an oocyte in IVF [10] or directly injected into an oocyte [11]. In either case, sperm may spend several hours suspended in tissue culture fluid or accumulating at the interface between the fluid and surface of the laboratory plastic in the container in which they are held. Clearly this environment is significantly different from that encountered *in vivo* and it has been suggested that improvements to infertility procedures might be possible if laboratory processes and equipment better mimicked *in vivo* conditions [6].

In recognition that the surface chemistry of laboratory plastics may not be optimal for sperm, recent studies have focused on how sperm survival in laboratory plastic [12] or sperm movement through microfluidic channels [13] can be significantly altered by relatively subtle changes to the surface chemistry. This study investigates how detailed measurements of sperm motility can be altered by the hydrophobicity of surfaces. Static sessile contact angle measurements are used to determine contact angles from which surface energy is determined and so a quantifiable measure of hydrophobicity is found. A Computer Assisted Sperm Analysis (CASA) system is used to provide objective data on sperm kinematics.

2. Materials and Methods

Percoll was purchased from Fisher Scientific (Loughborough, United Kingdom). Atactic polystyrene (PS) (molecular weight M_W = 220 kDa and polydispersity D = 1.02) and poly(methyl methacrylate) (PMMA) (M_W = 120 kDa and D = 2.0) were purchased from Polymer Source, Inc. (Quebec, Canada) and had no additional functional groups, copolymer units or side chains added and therefore the chains remain inert. All other chemicals were of analytical grade and were purchased from Sigma Aldrich (Dorset, United Kingdom).

2.1. Sperm preparation

2.1.1. Collection and Washing of Spermatozoa

Sperm-rich semen samples were collected from fertile boars kept by JSR Genetics (Driffield, East Yorkshire, United Kingdom). The semen was filtered through gauze to remove gel material and diluted in Beltsville Thawing Solution (BTS: 206 mM glucose, 20.4 mM trisodium citrate, 14.9 mM $NaHCO_3$, 10mM KCl, 3.4 mM Na_2-EDTA, and 50 μg/mL kanamycin sulphate) by JSR and received the day after collection. BTS is a widely used extender for boar sperm that preserves fertility for at least 3 days at ambient temperature [14].

Sperm were separated from the diluted semen by sedimentation through a density-gradient system of iso-osmotic Percoll in a saline-based medium. Once the supernatant layers were removed the sperm pellets were gently resuspended in Tyrode's medium (116 mM NaCl, 3.1 mM KCl, 0.4 mM $MgSO_4$, 0.3 mM NaH_2PO_4, 5 mM glucose, 21.7 mM sodium lactate, 1 mM sodium pyruvate, 1mM ethyleneglycoltetraacetic acid (EGTA), 20 mM HEPES (adjusted to pH 7.6 at 20ºC with NaOH), and 3 mg/mL bovine serum albumin (BSA); at 38˚C the final pH was 7.6 and osmolality was 300 mOsm/kg). The presence of bicarbonate/CO_2 has been shown to affect the motility of boar spermatozoa [15], and so aliquots of 300 mM $NaHCO_3$ saturated with 100% CO_2 were prepared in advance and a volume added to the resuspended sperm to give a final concentration of 15 mM. These aliquots were stored under 5% CO_2 in air to prevent loss of CO_2 during incubation between experiments.

2.1.2. Incubation and Preparation for Analysis

Preparation of samples is based upon the accepted guidelines for clinical assessment [16] as follows. The sperm suspension was incubated at 38˚C for 10 min before motility assessment. An 18 μL sample was removed from the suspension, transferred to a pre-warmed microscope slide, and sealed by a 22 x 22 mm pre-warmed coverslip; this volume of suspension provides a measurement height of 37.2 μm, which prevents sperm from moving in and out of focus during measurements without constraining rotational motion [17].

2.2. Film coating and characterization

2.2.1. Spin coating

Substrates of silicon wafer (Prolog Semicor, Ukraine) were cleaved into approximately 1 cm^2 sections, sonicated in chloroform and then toluene for 20 min in each, and cleaned for 1 h in an oxygen plasma cleaner. The cleaned substrates were then immediately coated with the relevant polymer using the well-established spin coating technique [18]. A range of polymer concentrations (2%, 4%, 6%, 8%, and 10% w/v) dissolved in toluene were used and all spun at 3000 rpm for 30 s. The resulting thin polymer film coatings form a rigid glassy layer in which the polymer chains remain confined and, as PMMA and PS are both insoluble in water, polymer will not dissolve into the overlying media which contains sperm. PS and PMMA were chosen due to their biocompatibility as well as being exceptionally well studied systems in terms of their surface and bulk properties in their glassy state. Both polymers are components of standard laboratory plastics used in fertility laboratories but the structure of the films produced in this work are better controlled down to the nanometer length scale and their chemical composition is devoid of any additional components required for bulk manufacturing.

2.2.2. Measuring film thickness

The thickness of the films was determined using an M-2000 spectroscopic ellipsometer (J. A. Woollam Co., Inc). The film temperatures were controlled using a Linkam heating stage (Linkam Scientific Instruments Ltd, Surrey, UK) with TMS94 heat controller. A sealed chamber (Linkam Scientific Instruments Ltd) specifically designed for use on the ellipsometer with a nitrogen gas flow was used to minimize atmospheric effects from moisture and dust settling on the films. The raw ellipsometry data were fitted with the widely used Cauchy model, which allowed the thickness values of the films to be determined as shown in Figure 1.

[FIGURE 1 HERE]

2.2.3. Contact Angle

All films were mounted onto the measurement stage of a Theta optical tensiometer (Attension, Biolin Scientific, Espoo, Finland) including a fixed Linkam heating platform (Linkam Scientific Instruments Ltd) with TMS94 heat controller. Images were fitted using the native software to determine static contact angles and surface tensions were calculated from these; contact angles present a more direct observation of hydrophobicity, but surface tension provides a parameter that does not depend upon droplet volume, atmospheric conditions, and other experimental variables. All measurements were performed at room temperature using the static sessile method with Milli-Q filtered water as the liquid phase component.

[TABLE 1 HERE]

2.3. Microscopy and tracking analysis

Videos were recorded for 5-10 seconds using an Infinity2 microscope camera (Lumenera, Ontario, Canada)

mounted on an Olympus BH-2 negative high-phase contrast microscope (Olympus, Tokyo, Japan) fitted with 10 times and 20 times objective lenses. Sample temperatures were maintained at 38˚C using a Warm Stage (Linkam Scientific Instruments Ltd).

In order to extract the curvilinear velocities, a custom-built package was developed in-house using LabView 2012 (National Instruments UK, Newbury, UK) based on previous work developed for tracking self-motile particles [19]. The videos were processed to remove debris and dead cells from analysis; the brightness of each pixel was determined over a frame, and if this brightness remained over all frames the object (either immotile cell or debris) was considered unfit for tracking. These pixels were subsequently removed from all frames to produce a flat-fielded video. Following this processing cells were selected manually from the first frame of the video. Contrast in brightness between the selected cell and the background provided the point of reference from which the package tracked the motion of the sperm, recording the position and temporal co-ordinates for further analysis. On-screen pixels were converted to physical distance using an image of a Neubauer haemocytometer taken under the same microscope settings and analyzed using ImageJ (National Institutes of Health, USA). Analysis of 5 videos (before flat-fielding) were conducted to ensure the video processing did not affect the results, and there was found to be no difference between raw and processed videos.

3. Results and Discussions

3.1. Film Thickness

The contact angle of PS was found to be greater than that of PMMA as seen in Table 1. This difference in hydrophobicity is clear from sample images in Figure 2 used to calculate the contact angle consistent with other investigations on the hydrophobicity of these polymer films. The surface tension was also comparable between the two polymer species in line with other work [20, 21]. It is also important to note that whilst there is a notable difference between the measured contact angles on PMMA and PS, the results between different film thickness are consistent between each polymer species. An approximately 90 nm thick PMMA film was made from 2% (w/v) solution but was discarded as the film had dewetted the surface.

[FIGURE 2 HERE]

The distributions of curvilinear velocities between each polymer surface are shown in Figure 3. This setup acts as a control to ensure that film thickness is not a factor in determining motility characteristics, but the physical nature of the films is such that sperm are not expected to penetrate into the rigid glassy film. Given this expectation, sperm velocities were compared over the thickness range of each polymer species to confirm a lack of effect of film thickness on VCL. The datasets obtained for PS and PMMA were both non-normally distributed. Analysis of Variance (with bootstrapping) was performed on log-transformed data confirmed this, indicating that there was no

statistically significant difference in VCL between PS films of different thicknesses (2% n = 32; 4% n = 39; 6% n = 23; 8% n = 61; 10% n = 62). Similar analysis was performed on the PMMA dataset using Bonferroni corrected Mann-Whitney testing (standard transformations did not yield a dataset that satisfied the assumptions of ANOVA) showed no significant difference between PMMA films of different thickness (4% n = 39; 6% n = 111; 8% n = 134; 10% n = 129). A lack of difference in sperm motility between films of different thicknesses is not unexpected given the previous discussion regarding the similarities in contact angle measurements for each polymer species.

[FIGURE 3 HERE]

These results indicate that film thickness does not affect the velocity of sperm for either of the two coated surfaces and that only the surface layer and film composition is important; this finding indicates that long-range forces due to the substrates are not affecting the results. Thus, in the absence of a good solvent or thermal energy to induce a glass transition (both PMMA and PS have glass transition temperatures above 90˚C), the sperm will be restricted to interacting solely with the surface layer of the film. To confirm this, the films were subsequently examined visually using an optical microscope and no sperm were found to have penetrated into the film at any thickness, confirming the previous result that only the surface of the film influences the curvilinear velocity of the sperm.

3.2. Film composition

Having confirmed that film thickness did not affect the motility of sperm, the data from all film thicknesses in the previous section were combined into two groups, PS (n = 217) and PMMA (n = 417). These pooled data from the same boar (hereafter referred to as boar 1) were non-normally distributed and therefore a Mann-Whitney test was performed to assess differences in motility between the two surface types. Curvilinear velocity was found to be significantly greater for PMMA than PS, $U = 111745$, $p < .0001$, $r = .61$. To ensure that this effect was not due to any abnormality or deficiency in the sample from boar 1, sperm from an additional two boars were measured in the same manner. For both of these additional boars (boars 2 and 3) the VCL was also found to differ significantly between the two types of polymer coating for sperm from both boar 2, $U = 20537$, $p < .0001$, $r = .25$ and boar 3, $U = 9368$, $p < .0001$, $r = .38$. Note that the effect size (r) for boar 3 (r = .38) was stronger than that for boar 2 (r = .25). The distributions of curvilinear velocities for all three boars are shown in Figure 4.

[FIGURE 4 HERE]

In all instances the sperm from all boars moved with a greater median velocity between PMMA coated surfaces compared to PS surfaces. Both polymer films are expected to be completely chemically inert and physically constrained such that any differences in median velocities are not attributed to toxic effects of either surfaces. To test this, the percentage of motile sperm (defined as those moving with speeds greater than 5μm/s) for all three boars was determined from the original videos and no significant difference was found in any boar between the

two surfaces. The larger average contact angle for PS (93.2° ± 0.2°) indicates a higher hydrophobicity for this surface compared to PMMA (67.5° ± 0.2°) as shown in Figure 2. The sample must be considered as a three-component system comprising the water-based Tyrode's buffer, rigid polymer surfaces, and the motile sperm cells. Whilst the hydrodynamic interaction between the solvent and the surfaces is well characterized in terms of the hydrophobicity of the polymer films [20, 21], the sperm cells also display surface charge or hydrophobicity. The exact nature of the surface charge of the sperm cell is difficult to quantify as the sperm surface is highly heterogeneous [22, 23] and displays a significant amount of redistribution and re-ordering of the surface molecules in response to environmental conditions [24-26]. However as these experiments were conducted using sperm prepared in an identical manner and suspended in Tyrode's medium, the considerations relating to the surface structure of the sperm present systematic errors that do not detract from the comparison of PS and PMMA as surfaces for sperm motility.

It has already been shown that the hydrodynamic interaction between two boundaries and a self-motile cell leads to aggregation of the cells at the surfaces [5], but in their work the authors did not consider the properties of the surface beyond the condition that they are flat and rigid. The hydrophobic polymer surfaces will exert a force across the aqueous solution [27], which in turn will affect the distribution and motion of sperm. For instance, the repulsive interaction between the PS surface and the water can be reduced if sperm aggregate near the interface and provide a "screen" between the Tyrode's buffer and the surface. Any such increased aggregation at a rigid boundary may reduce the overall curvilinear velocity of the sperm.

Whilst the balance between the interactions of the surface-solvent, sperm-solvent, and surface-sperm provides a mechanism to explain the difference in the curvilinear velocities between PMMA and PS surfaces, the variation in the magnitude of this effect between different boars is most likely due to differences in the distribution and concentration of surface molecules on sperm [28, 29]. However further experiments to quantify the two-component interaction between sperm-surfaces and sperm-solvent are necessary to accurately model the underlying cause of the difference in median VCL presented in Figure 4.

4. Conclusion

The role of the surface in sperm motility was first highlighted in the 1960's, but to date there has been little progress in determining the effect of surface properties on sperm velocity. We have shown that an increase in hydrophobicity of the two flat polymer surfaces decreases the speed of sperm in a solution between the two surfaces. The absence of any surface molecules for binding as well as a lack of surface structure or topography suggests that the cause of the variation in sperm speed is due to the underlying interaction forces between the three components of the system.

At present there has been little work in understanding the fundamental interaction between sperm suspension and solid boundaries, and yet these systems are routinely used in both research and clinical laboratories. Further standardization of laboratory consumables is required to ensure that a difference in materials used to conduct laboratory procedures does not introduce additional variations in motility assessments. It is noteworthy that in the development of a microfluidic chip the authors modified the surfaces to reduce hydrophobicity of their system [13].

The results of this work highlight a future possible clinical application in manipulating sperm motility through suitable selection of polymer films or coatings of laboratory consumables. Current intracytoplasmic sperm injection techniques use mechanical immobilization [30] or a retardation medium [31] to select the sperm, but suitable use of polymer coatings may provide an alternative mechanism to slow the sperm selected for injection. Moreover, the development of a standardized surface on which to observe sperm motility as part of diagnostic procedures such as semen analysis, may help to reduce the known variations in motility assessments between staff and laboratories [32] and may even provide a new training tool or the development of model systems to better understand sperm-surface interactions [7, 8].

The systems presented here are the simplest possible (a flat, uniform polymer surface) and so a logical progression from this work will be to introduce variations in the surface to affect the hydrophobicity through surface topography [33], or by introducing variations in surface properties [34-36] that are already known to stimulate heptotactic motion in a range of cells [37-39].

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References

[1] Gillies EA, Cannon RM, Green RB, Pacey AA. Hydrodynamic propulsion of human sperm. J Fluid Mech 2009; 625:445-74.

[2] Lord Rothschild. Non-random distribution of bull spermatozoa in a drop of sperm suspension. Nature 1963; 198:1221.

[3] Cosson J, Huitorel P, Gagnon C. How spermatozoa come to be confined to surfaces. Cell Motil Cytoskel 2003; 54:56-63.

[4] Woolley DM. Motility of spermatozoa at surfaces. Reproduction 2003; 126:259-70.

[5] Berke AP, Turner L, Berg HC, Lauga E. Hydrodynamic attraction of swimming microorganisms by surfaces. Phys Rev Lett 2008; 101:038102–1-4.

[6] Suarez SS, Pacey AA. Sperm transport in the female reproductive tract. Hum Reprod Update 2006; 12:23–37.

[7] Pacey AA, Hill CJ, Scudamore IW, Warren MA, Barratt CLR, Cooke ID. The interaction in vitro of human spermatozoa with epithelial cells from the human uterine (Fallopian) tube. Hum Reprod 1995; 10:360-6.

[8] Pacey AA, Davies N, Warren MA, Barratt CLR, Cooke ID. Hyperactivation may assist human spermatozoa to detach from intimate association with the endosalpinx. Hum Reprod 1995; 10:2603-9.

[9] Pizzari T, Dean R, Pacey AA, Moore H, Bonsall MB. The evolutionary ecology of pre- and post-meiotic sperm senescence. Trends Ecol Evol 2008; 23:131-40.

[10] Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet 1978; 312:366.

[11] Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992; 340:17-8.

[12] Sommer AP, Zhu S, Gagsteiger F, Fecht H-J. Sperm performance better on diamond than on polystyrene. MRS Proceedings 2013; 1511.

[13] Huang H-Y, Wu T-L, Huang H-R, Li C-J, Fu H-T, Soong Y-K, Lee. M-Y, Yao D-J. Isolation of motile spermatozoa with a microfluidic chip having a surface-modified microchannel. J Lab Autom 2013; in press.

[14] Johnson LA, Aalbers JG, Grooten HJG. Artificial insemination of swine: fecundity of boar semen stored in Beltsville TS (BTS), Modified Modena (MM), or MR-A and inseminated on one, three and four days after collection. Zuchtygiene 1988; 23:49-55.

[15] Holt WV, Harrison RAP. Bicarbonate stimulation of boar sperm motility via a protein kinase A-dependent pathway: Between-cell and between-ejaculate differences are not due to deficiencies in protein kinase A activation. J Androl 2002; 23:557-65.

[16] World Health Organization. WHO laboratory manual for the Examination and processing of Human semen. 5th ed. World Health Organization; 2010.

[17] Le Lannou D, Griveau JF, Le Pichon JP, Quero JC. Effects of chamber depth on the motion pattern of human spermatozoa in semen or in capacitating medium. Hum Reprod 1992; 7:1417-21.

[18] Emslie AG, Bonner FT, Peck LG. Flow of a Viscous Liquid on a Rotating Disk. J Appl Phys 1958; 29:858-62.

[19] Howse JR, Jones RAL, Ryan AJ, Gough T, Vafabakhsh R, Golestanian R. Self-motile colloidal particles: From directed propulsion to random walk. Phys Rev Lett 2007; 99:048102–1-4.

[20] Harris M, Appel G, Ade H. Surface morphology of annealed polytyrene and poly(methyl methacrylate) thin film blends and bilayers. Macromolecules 2003; 36:3307-14.

[21] Kwok DY, Leung A, Lam CNC, Li A, Wu R, Neumann AW. Low-rate dynamic contact angles on

303 poly(methyl methacrylate) and the determination of solid surface tensions. J Colloid Interf Sci 1998; 206:44 – 51.
304 [22] Kumar S, Chaudhury K, Sen P, Guha SK. Atomic force microscopy: a powerful tool for high-resolution
305 imaging of spermatozoa. J Nanobiotechnology 2005; 3:9.
306 [23] McElfresh M, Baesu E, Balhorn R, Belak J, Allen MJ, Rudd RE. Combining constitutive materials modeling
307 with atomic force microscopy to understand the mechanical properties of living cells. Proc Natl Acad Sci USA
308 2002; 99:6493-97.
309 [24] Boerke A, Tsai PS, Garcia-Gil N, Brewis IA, Gadella BM. Capacitation-dependent reorganization of
310 microdomains in the apical sperm head plasma membrane: Functional relationship with zona binding and the zona-
311 induced acrosome reaction. Theriogenology 2008; 70:1188-96.
312 [25] Gadella BM, Tsai PS, Boerke A, Brewis IA. Sperm head membrane reorganization during capacitation. Int J
313 Dev Biol 2008; 52:473-80.
314 [26] Brewis IA, Gadella BM. Sperm surface proteomics: from protein lists to biological functions. Mol Hum
315 Reprod 2010; 16:68-79.
316 [27] Christenson HK, Claesson PM. Direct measurements of the force between hydrophobic surfaces in water.
317 Adv Colloid Interfac 2001; 91:391-436.
318 [28] Pascual ML, Muiño T, Cebrián-Pérex JA, López-Pérez MJ. Sperm cell heterogeneity revealed by centrifugal
319 counter-current distribution in an aqueous two-phase system. J Chromatogr 1993; 617:51-7.
320 [29] Talevi R, Gualtieri R. Molecules involved in sperm-oviduct adhesion and release. Theriogenology 2010;
321 73:796–801.
322 [30] Yanagida K, Katayose H, Hirata S, Yazawa H, Hayashi S, Sato, A. Influence of sperm immobilization and
323 onset of Ca2+ oscillations after ICSI. Hum Reprod 2001; 16:148-152
324 [31] Zollner U, Zollner K-P, Dietl J, Steck T. Semen sample collection in medium enchances the implantation rate
325 following ICSI in patients with severe aliogasthenoteratozoospermia. Hum Reprod 2001; 16:1110-4.
326 [32] Pacey, AA. Is quality assurance in semen analysis still really necessary? A view from the andrology
327 laboratory. Human Reproduction 2006; 21: 1105-9.
328 [33] Wang J, Feng J, Zou X, Long Y, Tian X. Effect of surface microstructure on the hydrophobicity of coatings.
329 Prog Org Coat 2012; 74:777-80.
330 [34] Burgos P, Zhang Z, Golestanian R, Leggett GJ, Geoghegan M. Directed Single Molecule Diffusion Triggered
331 by Surface Energy Gradients. ACS Nano 2009; 3:3235-43.
332 [35] Park J, Kim DH, Kim G, Kim Y, Choi E, Levchenko A. Simple haptotactic gradient generation within a
333 triangular microfluidic channel. Lab Chip 2010; 10:2130-8.
334 [36] Lund K, Manzo AJ, Dabby N, Michelotti N, Johnson-Buck A, Nangreave J, et al. Molecular robots guided by
335 prescriptive landscapes. Nature 2010; 465:206-10.
336 [37] Carter SB. Haptotaxis and the mechanism of cell motility. Nature 1967; 213:256-60.

[38] Cattaruzza S, Perris R. Proteoglycan control of cell movement during wound healing and cancer spreading. Matrix Biology 2005; 24:400-17.

[39] Solon J, Streicher P, Richter R, Brochard-Wyart F, Bassereau P. Vesicles surfing on a lipid bilayer: self-induced haptotactic motion. Proc Natl Acad Sci USA 2006; 103:12382-7.

Figure and Table Legends

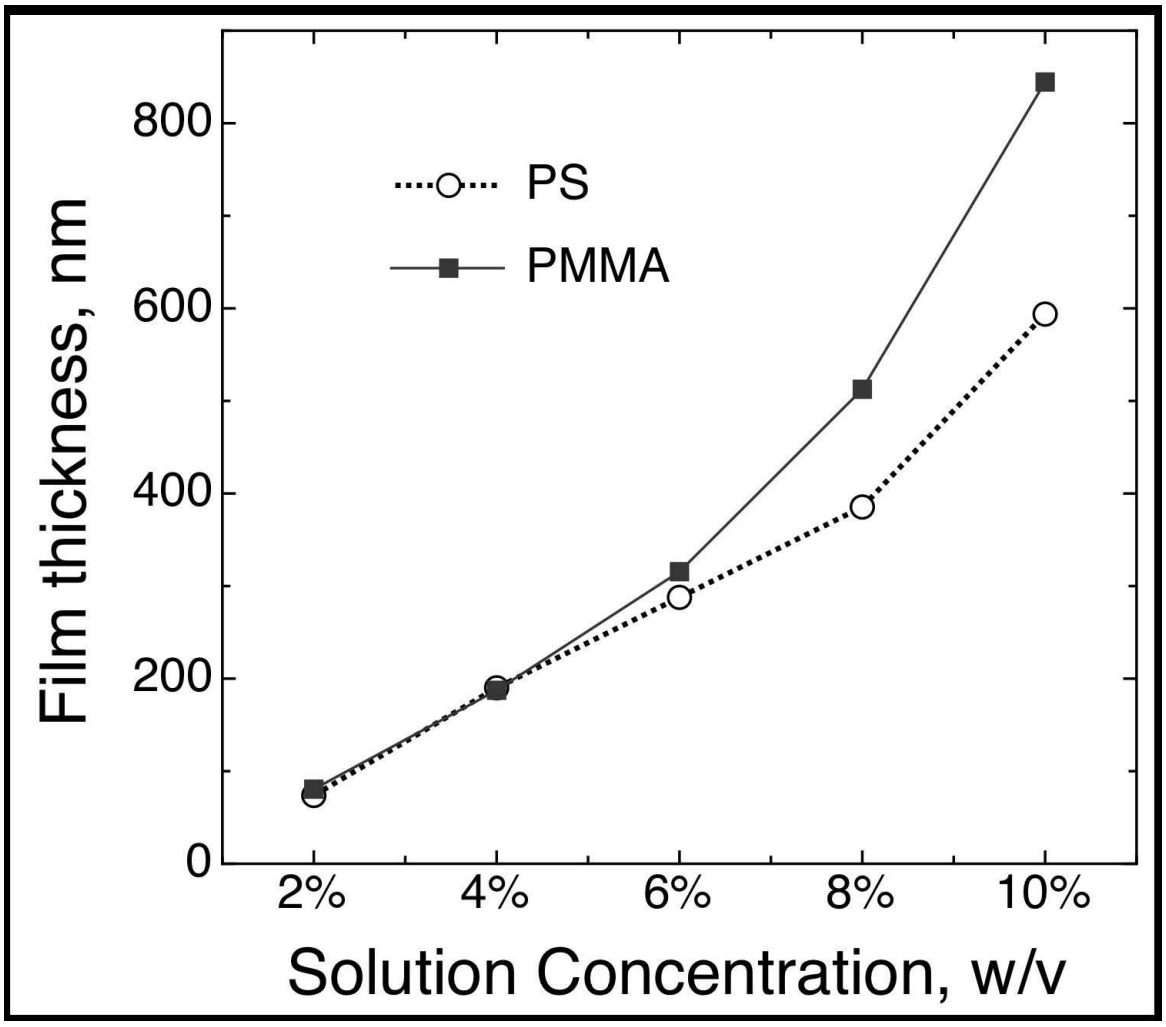
Figure 1: Thickness of polystyrene (PS) and poly(methyl methacrylate) (PMMA) films spun from solutions of different polymer concentrations in toluene. As expected a higher concentration of polymer in the solutions results in a thicker film. All films were spun at 3000 rpm for 30 s, and film thicknesses were measured using ellipsometry.

Table 1: Contact angle and surface tension determined from static contact angle measurements of Milli-Q water on coverslips spin coated with either polystyrene (PS) and poly(methyl methacrylate) (PMMA).

Figure 2: Contact angle images of Milli-Q water droplets on polystyrene (PS) (top) and poly(methyl methacrylate) (PMMA) (bottom) coated coverslips. The curved boundary line shows the fitted model to the droplet, and the straight lines are the tangents at the film-water-air interface. The larger spread of fluid over the PMMA surface results in a smaller contact angle, showing that PMMA is less hydrophobic than PS.

Figure 3: Curvilinear velocities of sperm between poly(methyl methacrylate) (PMMA) (grey) and polystyrene (PS) (white) films of different initial polymer solution concentrations. The resulting film thickness for each polymer and concentration is shown in figure 2. Analysis of variance (with bootstrapping) performed on log-transformed PS data and Bonferroni corrected Mann-Whitney testing of PMMA data showed no significant difference in velocity over the different solution concentrations, implying that the film thickness does not affect sperm motility.

Figure 4: Velocity distributions for three separate boars between poly(methyl methacrylate) (PMMA) (shaded) and polystyrene (PS) (white) coated surfaces. . Mann-Whitney testing showed that the curvilinear velocity between PMMA is significantly greater than PS for boar 1 ($U = 111745$, $p < .0001$, $r = .61$), boar 2 ($U = 20537$, $p < .0001$, $r = .25$) and boar 3 ($U = 9368$, $p < .0001$, $r = .38$).


Film thickness, nm
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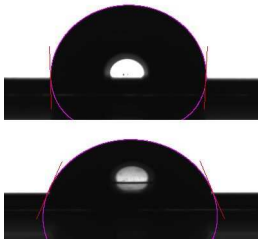
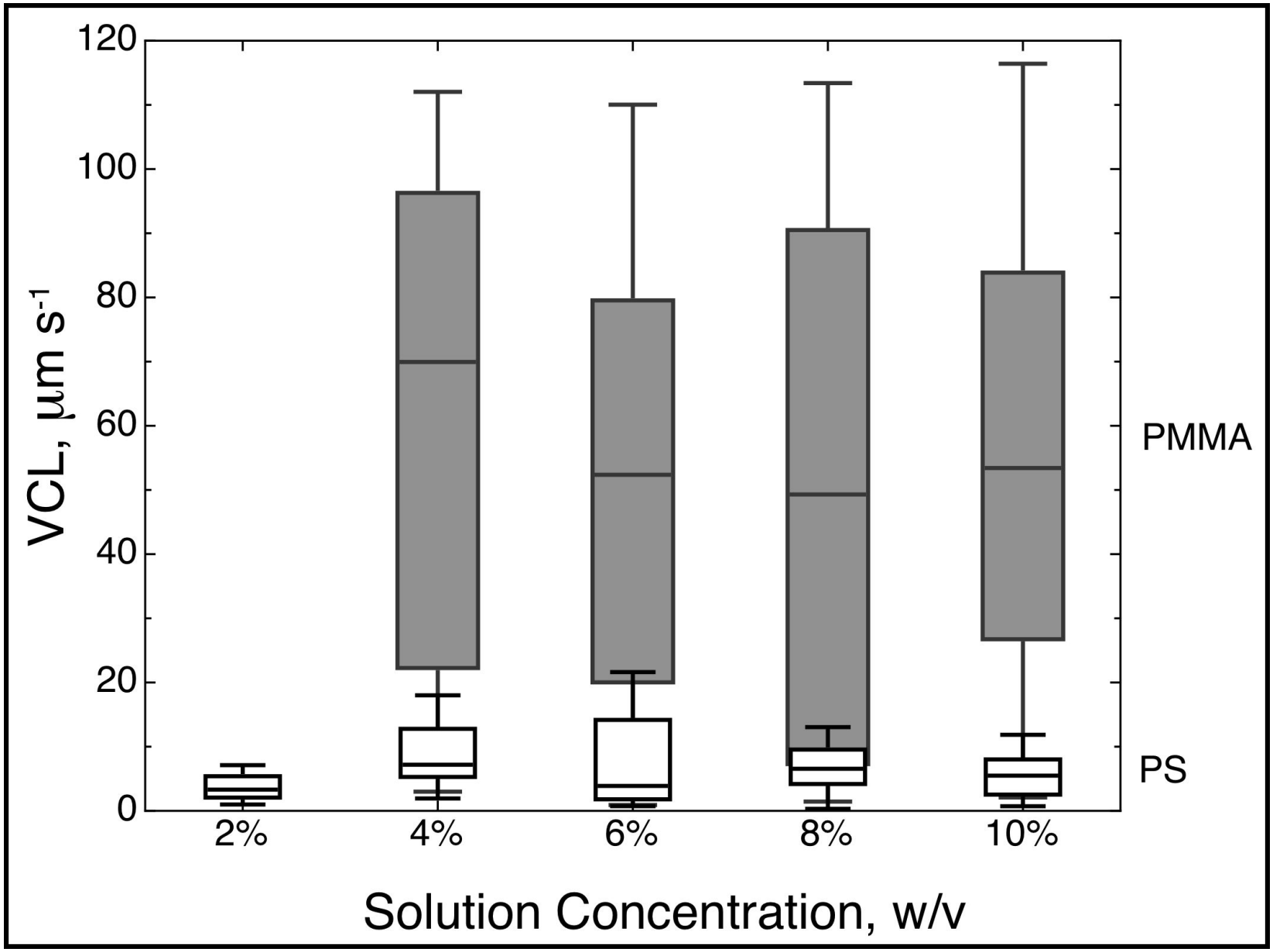
600
400
200
0
PS
PMMA
2%
4%
6%
8%
10%
Solution Concentration, w/v

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VCL, μm s-1
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4%
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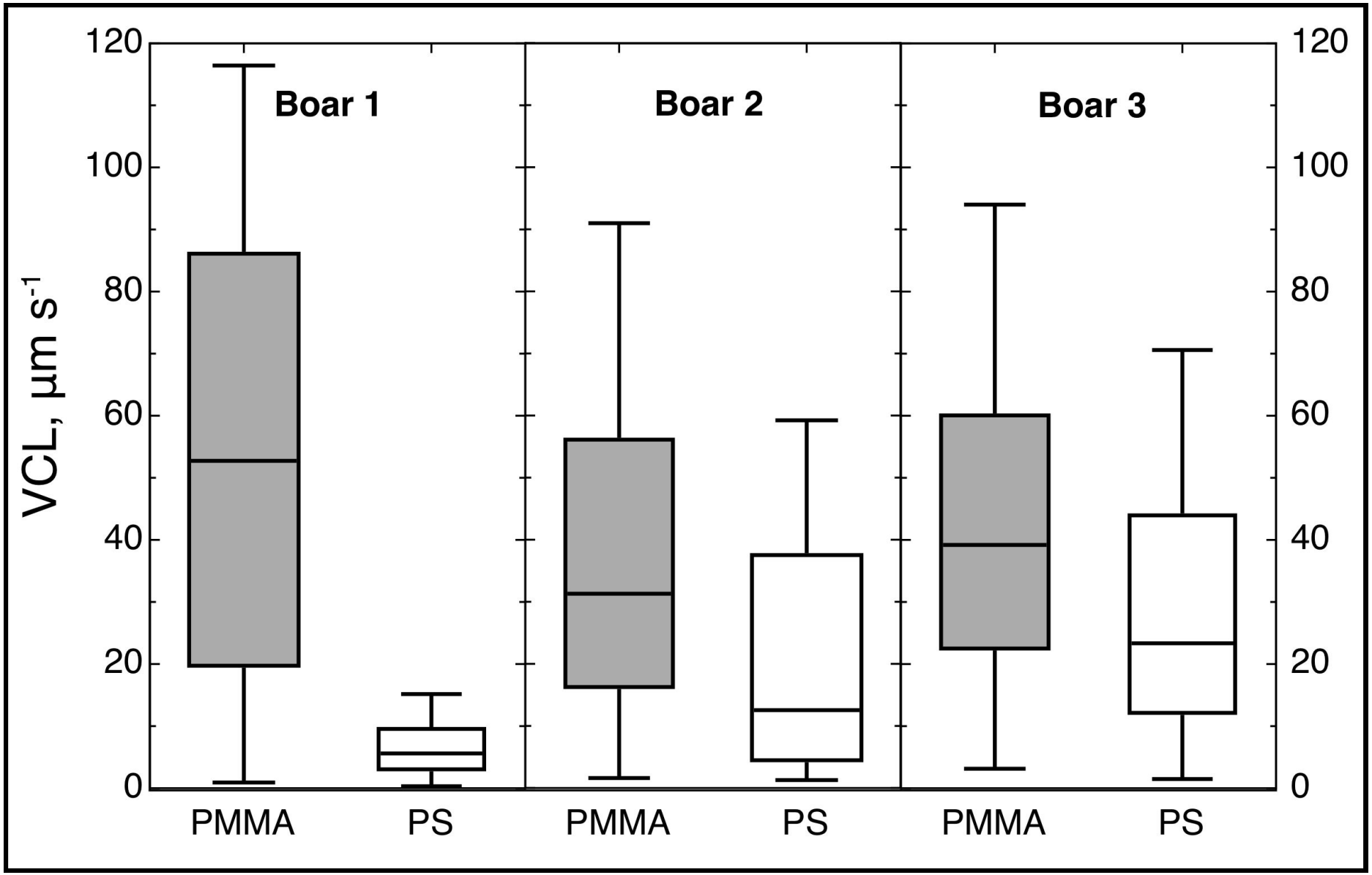
Solution Concentration, w/v
PMMA
PS


Boar 1
Boar 2
Boar 3
VCL, µm s-1
120
100
80
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40
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0
PMMA
PS
PMMA
PS
PMMA
PS

Table 1: Contact angle and surface tension determined from static contact angle measurements of Milli-Q water on coated coverslips.

Solution	Contact Angle, degrees		Surface Tension, mN m^{-1}	
Concentration (w/v)	PS	PMMA	PS	PMMA
2%	94.1 ± 0.1	-	73.8 ± 1.1	-
4%	93.2 ± 0.1	69.5 ± 0.1	72.7 ± 0.9	68.3 ± 1.2
6%	92.5 ± 0.1	65.9 ± 0.1	71.6 ± 0.8	70.4 ± 1.5
8%	92.7 ± 0.1	66.7 ± 0.1	73.8 ± 1.6	71.9 ± 1.4
10%	93.5 ± 0.1	67.9 ± 0.1	73.7 ± 1.0	72.4 ± 1.8