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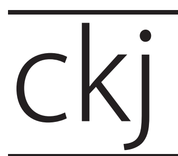

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Original Article

ORIGINAL ARTICLE

Successful kidney transplantation normalizes platelet function

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Abstract

Background: Uraemic platelet dysfunction is not completely understood, in part due to non-physiological platelet function assays. We have developed a physiological flow-based assay that quantifies platelet function in microlitre volumes of blood under arterial shear. The aim of this study was to characterize platelet function before and after kidney transplantation.

Methods: Ten patients scheduled for living donor kidney transplant surgery and nine healthy controls were analysed using the assay. The motional parameters of platelet behaviour on von Willebrand factor (VWF) were recorded using customized platelet tracking software. The assay was repeated 3–8 weeks post-transplant in the transplant group and at an interval of >3 weeks in normal healthy volunteers.

Results: Platelet–VWF interactions were markedly reduced in the 10 pre-transplant patients compared with the healthy controls. In seven patients with immediate graft function, dynamic platelet function returned to normal (despite a small decrease in haemoglobin and haematocrit), but remained markedly abnormal in the three patients with delayed graft function (DGF).

Conclusions: Dynamic platelet function returned to normal following transplantation in those with immediate graft function. This early improvement was not observed in those with DGF. There may be important clinical implications, as patients with DGF are more likely to undergo invasive procedures, including transplant biopsies and insertion of central venous catheters.

Key words: chronic kidney disease, delayed graft function, haemostasis, platelets, transplant

Introduction

End-stage renal disease (ESRD) is associated with an increased risk of bleeding, which manifests largely as bleeding into the skin and from mucosal surfaces [1]. Medications contribute to this, as antiplatelet/anticoagulant agents are often indicated and heparin is administered during haemodialysis (HD) to avoid extracorporeal clot formation [2]. Certain anatomical abnormalities predispose to bleeding, such as the presence of uraemic gastropathy [3].

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Platelet dysfunction also contributes to this increased bleeding risk [4].

When healthy endothelium is disrupted and the subendothelial matrix is exposed, von Willebrand factor (VWF) binds to the exposed surface and a coordinated series of events is set in motion to seal the defect. Under arterial shear conditions, platelets are tethered onto the immobilized VWF through the platelet glycoprotein (GP) Ib receptor and roll (translocate) along the surface of the injured vessel wall. The initial tethering also initiates a signalling cascade, which activates the GPIIb/IIIa receptor to bind VWF with high affinity, leading to platelet adhesion and thrombus formation through cross-linking with fibrinogen at the site of injury.

The platelet dysfunction seen in ESRD, termed uraemic platelet dysfunction, is multifactorial. GPIIb/IIIa is dysfunctional in ESRD [5]. There is also abnormal GP expression, leading to hyporesponsive platelets [6]. Other factors include disrupted platelet granule release, depressed prostaglandin metabolism and abnormal platelet cytoskeletal assembly [7].

Abnormal extrinsic factors also play a role, as evidenced by improved platelet function when uraemic platelets are mixed with normal plasma and unimproved function when normal platelets are mixed with uraemic plasma [4]. Although the culprit toxins are not completely defined, guanidinosuccinic acid and methylguanidine, which cause increased nitric oxide production leading to impaired platelet aggregation, are likely key players [8]. Anaemia, common in ESRD, contributes to platelet dysfunction primarily due to rheologic factors [4].

The end result of the above intrinsic and extrinsic factors is dysfunctional platelet aggregation and platelet–endothelial interactions. This has been demonstrated in patients with ESRD using platelet aggregation assays in response to different agonists, adenosine diphosphate (ADP), epinephrine, thrombin and collagen [9, 10].

Successful kidney transplantation reverses the metabolic disarray of ESRD and is therefore likely to completely or partially correct uraemic platelet dysfunction, although published reports are conflicting. Some platelet agonist studies have demonstrated improved platelet aggregation post-transplant with some, but not all, agonists [11–13]. Other groups did not demonstrate increased platelet aggregation with ADP, epinephrine, ristocetin or collagen [14–16].

However, platelet function studies should replicate the *in vivo* environment with flow and shear. Standard platelet function tests, performed in a research capacity, typically measure a single response to a single agonist and are not performed under arterial shear. Therefore they tend not to accurately capture the pathophysiological environment of complex disease states or the *in vivo* environment with flow and shear.

Members of our research team developed the functional assay utilized in this study over a number of years [17]. It is a microfluidic parallel-plate flow chamber coated with immobilized VWF, with a 0.1 mm^2 cross-sectional area that provides uniform, well-defined shear rates (1500/s). Customized image processing quantifies dynamic cellular surface coverage versus time throughout the whole-blood flow assay for a given disease state. Outputs from the device and linked software include instantaneous and mean platelet velocities, periods of motion and stasis and bond dissociation kinetics. It therefore has the capacity to detect subtle changes in platelet behaviour and provides detailed, reliable information regarding platelet function in an environment that replicates the *in vivo* environment.

This assay has been peer reviewed [17–20]. The accuracy of the outputs generated by the platelet tracking software has been validated experimentally [21, 22]. We have also validated the assay clinically: we previously demonstrated the capability of this system to detect subtle changes in platelet function by describing age-related changes in platelet behaviour, differences between term and preterm neonates and changes in platelet function between healthy pregnant and non-pregnant controls [23–25].

The aims of this study were to assess dynamic platelet function in patients with ESRD and to investigate the effect of renal transplantation on platelet function using the Dynamic Platelet Function Assay (DPFA).

Materials and methods

Trial design

Ethical approval was obtained from the Medical Research Ethics Committee of Beaumont Hospital and the Royal College of Surgeons in Ireland. All data and samples were collected in accordance with the Declaration of Helsinki.

Recruitment

Consecutive adults (≥18 years old) scheduled for living donor kidney transplant surgery were recruited. Patients were excluded if they were taking regular antiplatelet medication, regular anticoagulation medication or had a platelet count <125 000/mL pretransplant. Written informed consent was obtained and a numerical trial identifier was assigned. Baseline demographics and laboratory results were recorded.

Delayed graft function (DGF) was defined as the need for dialysis during the first week post-transplantation. A repeat sample and updated clinical details were obtained at 3–8 weeks post-transplantation. Of note, one patient was taking aspirin at the time of the follow-up sampling. This was discontinued shortly afterwards and a third sample was analysed 4 weeks later.

Healthy controls were eligible for recruitment if they self-reported as healthy and were not taking regular antiplatelet medication. They were excluded if they had consumed non-steroidal anti-inflammatory medication in the preceding 2 weeks. A blood sample was obtained at baseline and at follow-up (at least 3 weeks later).

Preparation of blood samples

Venous blood was collected from the antecubital vein using a 20-gauge butterfly needle connected to a citrated Sarstedt monovette syringe (Drinagh, Wexford, Ireland). Blood samples were kept at room temperature with gentle rocking and used within 1 h of phlebotomy. Whole blood cell counts were recorded for each donor, using a pocH-100i Haematology Analyser (Sysmex Corporation, Kobe, Japan).

DPFA

DPFA was performed as previously described [17]. Briefly, custom parallel-plate perfusion chambers were coated overnight with 100 μg/mL VWF, washed with phosphate-buffered saline and blocked with 1% bovine serum albumin for 1 h prior to use. Whole blood was labelled with 1 μM 3,3′-dihexyloxacarbocyanine iodide for 5 min at 37°C prior to perfusion through the chamber at an arterial rate of shear (1500/s). Platelet translocation behaviour was recorded using real-time video microscopy at a frame

rate of 19 frames/s. Image stacks were analysed by a custom designed and validated software package [21].

The assay measurements obtained from this analysis measure the following aspects of dynamic platelet behaviour: tethering to the VWF surface (platelet tracks), rolling across VWF (translocating platelets, translocation distance) and adherence to VWF (adhesion rate, percentage of platelet surface coverage).

Table 1. Baseline demographics in transplant candidates and healthy controls

Demographics	Transplant candidates	Healthy controls
Age (years), mean (range)	40.5 (28–54)	43.6 (28–64)
Gender (male), %	50	44
Cause of ESRD, *n*		NA
IgA nephropathy	5	
SLE	1	
Alport syndrome	1	
ADPKD	1	
FSGS	1	
RRT, *n*		NA
Pre-emptive	2	
Haemodialysis	6	
Peritoneal dialysis	2	
Dialysis vintage (months), mean (range)	11 (0–30)	NA
Medications, *n*		0
Mean (range)	5.5 (0–11)	
Taking ESA	5	
Current smokers, *n*	0	0

IgA, immunoglobulin A; SLE, systemic lupus erythematosus; ADPKD, autosomal dominant polycystic kidney disease; FSGS, focal segmental glomerular sclerosis; RRT, renal replacement therapy; ESA, erythropoiesis-stimulating agent.

Table 2. Laboratory results [mean (range)] on the day of platelet analysis pre- and post-transplant

Laboratory results	Pre-transplant	Post-transplant
Haemoglobin (g/dL)	12.4 (10.9–14.6)**	10.4 (6.6–12.7)**
White cell count ($\times 10^9$/L)	7.5 (3.2–9.9)	7.8 (4.2–12.6)
Platelet count ($\times 10^3$/mL)	248 (201–346)**	284 (242–339)**
INR	1.02 (0.96–1.12)	1.02 (0.93–1.09)
APTT (s)	27.8 (22.8–35.1)	30.5 (25.5–35)
Creatinine (μmol/L)	668.3 (278–1024)**	155.2 (91–248)**
Urea (mmol/L)	18.1 (11.9–25.1)**	8.36 (4.2–11.3)**

INR, international normalized ratio; APTT, activated partial thromboplastin time.
**$P < 0.05$.

Statistical methods

The mean and standard deviation were determined for each measured parameter. The distribution of each measured parameter was determined by Shapiro–Wilk test to inform the choice of a parametric (independent samples *t*-test) or non-parametric test (Mann–Whitney test) when comparing groups. Paired *t*-tests were used to compare individual measurements within groups. Values were considered statistically significant at $P < 0.05$.

Hierarchical cluster analysis using Ward's linkage analysis (Euclidean distance) was used to determine similarity between the transplant recipients, using the difference in pre- and post-transplantation values as input. Spearman and Pearson correlation analysis was carried out on these parameters and significantly correlated variables were not included in the cluster analysis.

Results

Clinical characteristics

Ten patients and nine healthy controls were recruited over a 2-year period (Table 1). One patient had lupus with normal platelet count pre-transplant (235 000/mL).

All 10 patients underwent living donor kidney transplantation, with basiliximab ($n = 9$) or anti-thymocyte globulin ($n = 1$) induction therapy. Maintenance immunosuppression was tacrolimus, mycophenolate mofetil and prednisolone for all patients. Although three patients had DGF initially, all had independent transplant function (serum creatinine 91–248 μmol/L) at the time of follow-up analysis (Table 2).

Platelet function

In the control group, platelet function was normal at baseline and did not significantly change at follow-up. Measures of platelet tethering, rolling and adhesion were reduced in the pre-transplant group at baseline (Table 3, Figure 1).

Seven transplant recipients had a statistically significant improvement in post-transplant dynamic platelet function despite a small drop in mean haemoglobin and haematocrit (which one would expect to lead to reduced platelet–endothelial interactions) (Tables 2 and 3, Figure 2). All of these patients had excellent immediate transplant function.

Platelet interactions did not improve in three transplant recipients (Table 3, Figure 2). Unbiased cluster analysis of the 10

Table 3. Patients with advanced chronic kidney disease have significantly reduced platelet tethering compared with healthy controls

Parameter	Healthy controls ($n = 9$)	Pre-transplant ($n = 10$)	Immediate graft function ($n = 7$)	DGF ($n = 3$)
Platelet tethering tracks (*n*)	806 ± 289**	538 ± 246**	839 ± 348	491 ± 122
Platelet rolling				
Translocation distance (μm)	10.2 ± 2.8	12.2 ± 4.4	13.3 ± 5.4	10.9 ± 3.4
Translocating platelets (*n*)	505 ± 182	372 ± 198	591 ± 295	332 ± 50
Platelet adhesion				
Adhesion rate	0.45 ± 0.07	0.39 ± 0.09	0.46 ± 0.08	0.39 ± 0.07
Platelet surface coverage (%)	14.1 ± 1.5	12.2 ± 2.7	13.9 ± 1.6	12.0 ± 1.1

Dynamic platelet function returns to normal following successful transplantation.
**$P < 0.05$.

post-transplant samples identified the same three patient outliers in terms of dynamic platelet function—KTX04, KTX06 and KTX10 (Figure 2). The three outliers had similar baseline clinical characteristics to the remaining patients, but all three had post-transplant DGF. One patient with DGF (KTX10) was also started on aspirin prior to the follow-up sample (see sections 'Materials and methods' and 'Serial testing').

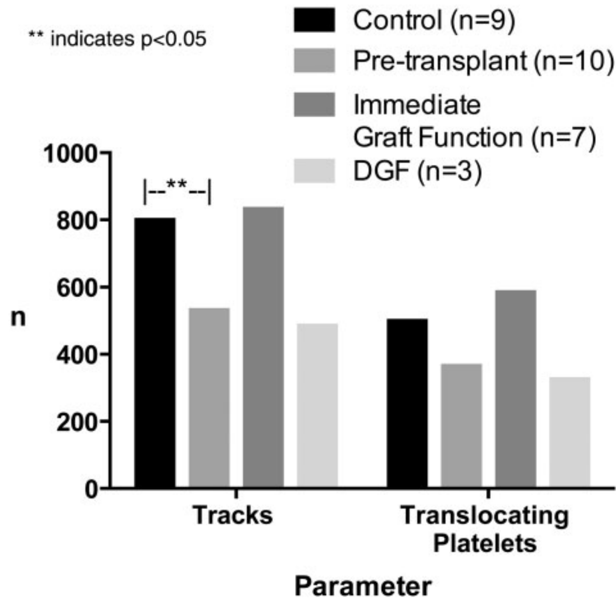

Fig. 1. Platelet tethering (tracks) is significantly reduced in those with advanced chronic kidney disease compared with healthy controls; platelet rolling (translocating platelets) is also reduced in those pre-transplant (P = 0.0470 and 0.1468, respectively). Platelet tethering (tracks) and rolling (translocating platelets) normalize in those with immediate graft function but remain abnormal in those with DGF.

Serial testing

Serial samples were obtained from two of three outliers with a view to better understand platelet function over time. The patient KTX04 was lost to follow-up.

KTX06 had a third sample taken on the second anniversary of the transplant surgery. The transplant function was excellent with a creatinine of 78 μmol/L. The parameters of platelet function had normalized and, when compared with the initial results, were no longer different from those patients with normal function post-transplant (Figures 3 and 4).

KTX10 had a creatinine of 248 μmol/L, haemoglobin of 6.6 g/dL (following a bleed) and was taking aspirin at the time of the follow-up sample. Aspirin was discontinued a few days later. A third sample was analysed 6 weeks after aspirin discontinuation, by which time the haemoglobin had significantly improved (10.1 g/dL) and transplant function was slowly recovering (creatinine 183 μmol/L). Platelet function in the third post-transplant sample was similar to that in the second.

Discussion

The results of the present study demonstrate, for the first time, reduced platelet–VWF interactions in patients with ESRD awaiting transplant using a physiological flow-based assay. Successful kidney transplantation normalized these interactions. This early

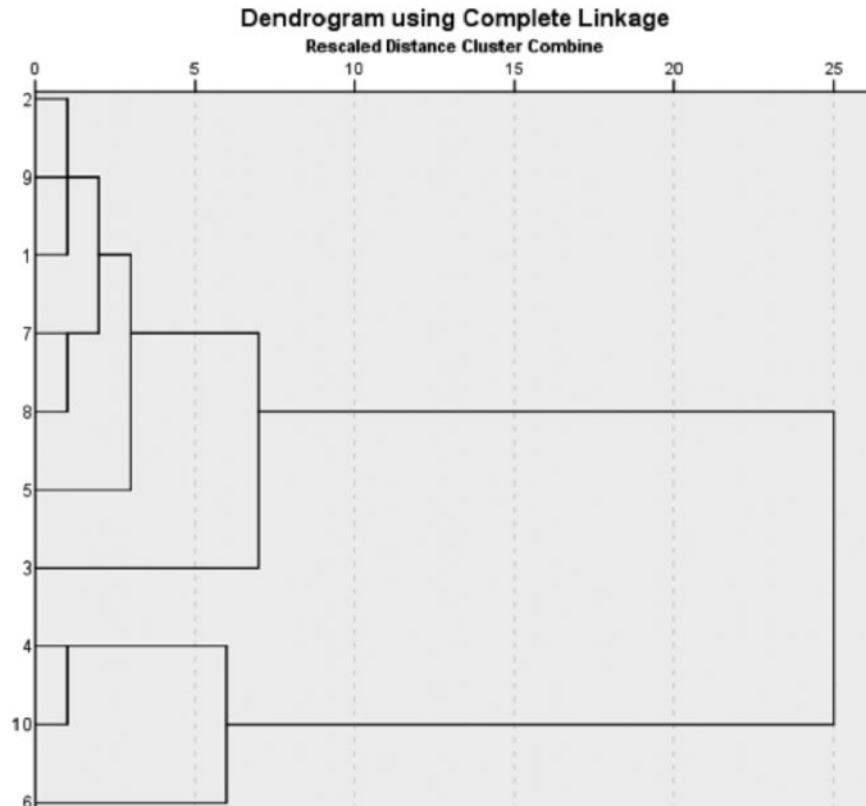

Fig. 2. A hierarchical cluster analysis using Ward's linkage analysis. The figure reads from right to left; two groups quickly separate in terms of similarity for dynamic platelet function. The three outliers, in terms of dynamic platelet function, are patients KTX04, KTX06 and KTX10, all of whom had DGF.

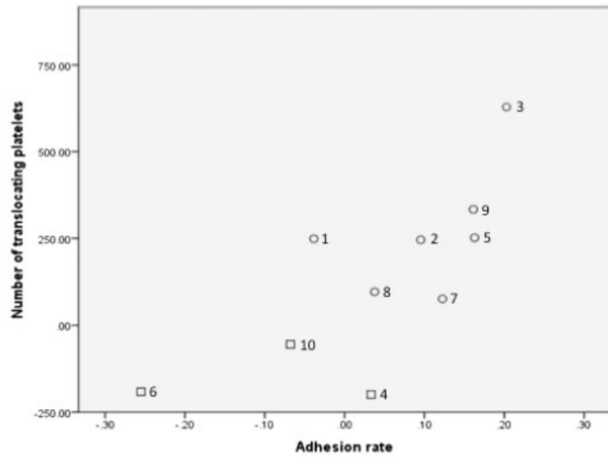

Fig. 3. A scatter plot of two parameters of dynamic platelet function (number of translocating platelets versus adhesion rate) for the 10 transplant recipients. A single value was obtained for each patient by subtracting their post-transplant assay result from their pre-transplant assay result. The scatter plot identified three outlying patients, all of whom had DGF, with no improvement in platelet function post-transplant (represented by the squares in the lower portion of the plot). The circles in the upper portion of the plot represent the seven patients with immediate graft function who demonstrated an improvement in platelet function.

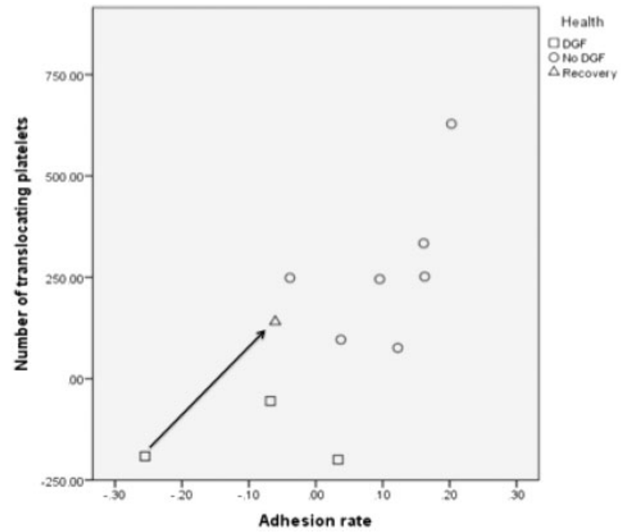

Fig. 4. A scatter plot of two parameters of dynamic platelet function (number of translocating platelets versus adhesion rate) for the 10 transplant recipients. Again, a single value was obtained for patients by subtracting their post-transplant assay result from their pre-transplant assay result. The circles represent those with immediate graft function who demonstrated an improvement in platelet function. Of the three outliers with DGF and persistently abnormal platelet function post-transplant (squares), one had repeat testing 2 years later and demonstrated an improved platelet profile (triangle).

Patient	Gender	Age (years)	Cause of ESRD	DGF	Creatinine at follow-up (μmol/L)
1	Female	50	Focal segmental glomerulosclerosis	No	121
2	Male	34	Polycystic kidney disease	No	174
3	Female	54	IgA nephropathy	No	125
4	Female	30	IgA nephropathy	Yes	111
5	Male	28	IgA nephropathy	No	213
6	Female	37	IgA nephropathy	Yes	79
7	Male	49	Unknown	No	173
8	Male	38	IgA nephropathy	No	217
9	Female	54	Lupus nephritis	No	91
10	Male	31	Alport syndrome	Yes	248

IgA, immunoglobulin A.

improvement was not observed in the three patients who had DGF.

We postulated that results may have been skewed somewhat by KTX10, who was an extreme outlier in many ways and taking aspirin at the time of post-transplant assay. It is known that patients with ESRD can display increased bleeding sensitivity to aspirin compared with healthy controls [26]. However, when repeated 4 weeks later when some of the confounding issues had been resolved (and transplant function was still in the recovery phase), platelet function looked to be similar. Serial sampling of KTX06 demonstrated improvement of platelet function over time in the setting of excellent graft function.

Another research group reported a difference in various platelet parameters in those with DGF post-transplant, although this was a retrospective review of laboratory data. In 232 transplant recipients, a decrease in platelet number and platelet haematocrit was noted in the first 5 days post-transplant, followed by recovery in Days 30–60. Mean platelet volume (MPV) and large platelet ratio (P-LCR) slowly rose from Day 15 onwards and were significantly higher than pre-transplant levels by Days 30–60 and 45–60, respectively. Those 29 patients with DGF had significant differences from the remaining patients during the early days post-transplant; platelet count was lower, while MPV, P-LCR and platelet volume distribution width were higher [27].

Our results suggest that meticulous attention to haemostasis must be given for a considerable time post-transplant in those with DGF, which is usually related to ischaemic injury and, as with any acute kidney injury, takes a variable duration time for recovery. Nephrologists generally pay close attention to haemostasis in patients on dialysis undergoing invasive procedures, with a focus on pre-procedure dialysis, administration of desmopressin and correction of anaemia with erythropoietin/iron. We suggest that similar caution be taken in patients with DGF in the post-transplant setting. Indeed, those with DGF require more frequent invasive procedures such as biopsies and central venous catheter insertion. It was interesting to note that KTX10 had a clinically significant bleed following a transplant biopsy several days prior to the second platelet assay.

It is noteworthy that the role of aspirin, which inhibits platelet aggregation via cyclooxygenase-mediated prostaglandin production, has not been entirely clarified in the post-transplant setting. It is continued post-transplant as secondary prevention in patients with clinically significant cardiovascular disease. It is generally accepted (but not universally enforced) that if there is a clear indication for primary prevention with aspirin, it will be given post-transplant [28]. There is controversy regarding aspirin administration to all post-transplant recipients, irrespective of their cardiovascular risk profile [29, 30]. It is likely that in the future a more personalized approach to anti-platelet therapy will be developed, in which graft function and measures of dynamic platelet function are considered.

We conclude that platelet–VWF interactions are markedly abnormal in patients with ESRD. Excellent, immediate transplant function is associated with normalized platelet function

within weeks of the surgery. DGF is associated with persistence of the abnormalities, but the results for KTX06 suggest this may improve in the longer term in line with renal recovery. This research was hypothesis generating; we now plan to study the hypothesis that platelet function improves in association with renal recovery following transplant surgery by testing serial samples in a cohort of transplant recipients.

Authors' contributions

C.K. was responsible for study design, acquisition and interpretation of data and drafting and revising the manuscript. L.W. was responsible for study conception and design, acquisition and interpretation of data and critical review of the manuscript. D.S. was responsible for acquisition of data and critical review of the manuscript. J.C. was responsible for study conception and design, analysis and interpretation of data and critical review of the manuscript. I.O. and M.K. were responsible for analysis and interpretation of data and critical review of the manuscript. P.J.C and D.K. were responsible for study conception and design, interpretation of data and critical review of the manuscript.

Conflict of interest statement

D.K. has an issued patent for a microfluidic device for assessing object/test material interactions. There is no other potential conflict of interest.

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