Table 3 Association between lens opacification and surgical factors

	IOL opacified			
	No	*Yes*	*Total*	P-*value*[a]
Surgery type				
Routine	121 (58.2%)	87 (41.8%)	208 (100%)	
Combined procedure	3	2	5[b]	>0.99
	($n=124$)	($n=89$)	($n=213$)	
Intra-operational complications				
No	121 (59.0%)	84 (41.0%)	205 (100%)	
Yes	4	3	7[b]	>0.99
	($n=125$)	($n=87$)	($n=212$)	
Complications post-operation				
No	114 (59.1%)	79 (40.9%)	193 (100%)	
Yes	11 (57.9%)	8 (42.1%)	19 (100%)	>0.99
	($n=125$)	($n=87$)	($n=212$)	
Viscoelastic				
Provisc	54 (60.7%)	35 (39.3%)	89 (100%)	
Other	10 (66.7%)	5 (33.3%)	15 (100%)	0.88
	($n=64$)	($n=40$)	($n=104$)	
Anaesthesia				
General	11 (40.7%)	16 (59.3%)	27 (100%)	
Local	113 (61.1%)	72 (38.9%)	185 (100%)	0.07
	($n=124$)	($n=88$)	($n=212$)	

[a]Continuity-corrected χ^2-test or two-tailed Fisher's exact test was used if frequencies were small.
[b]Too few for percentage calculation.

Table 4 Association between lens opacification and serial number

Serial number	*Number of eyes examined*	*Number of eyes opacified*	*Opacification rate (95% CI)*
4JAA00-4N9999	2	0	3% (0–17%)
4PAA00-4T9999	4	0	
4UAA00-4Y9999	11	1	
4ZAA00-439999	0	0	
44AA00-489999	1[a]	0	
49AA00-5D9999	2	0	
5EAA00-5I9999	10	0	
5JAA00-5N9999	25	1	4% (0–20%)
5PAA00-5T9999	24	2	8% (1–27%)
5UAA00-5Y9999	70	55	79% (67–88%)
5ZAA00-539999	35	19	54% (37–71%)
54AA00-589999	26	8	33% (17–53%)
59AA00-6D9999	4	2	
Total	214[a]	88	41% (35–48%)

[a]The total number includes one lens of which the opacity could not be assessed and also another lens of which serial number was unknown.

silicon gasket. The findings support a manufacture-related cause over a patient- or surgical-related cause for opacification. Although no conclusions can be drawn about the nature of this unknown aetiological factor, our data pinpoint it within a narrow time frame of manufacture.

Conflict of interest

The authors declare no conflict of interest.

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Sir,

Effect of lyophilization on the *in vitro* biological activity of bevacizumab

Intravitreal bevacizumab has been effective for vascular endothelial growth factor (VEGF)-mediated diseases of the retina and choroids.[1,2,3] However, repeated injections may be required. An alternative mode of administration would be a biodegradable intravitreal implant[4] of lyophilized bevacizumab, which has not been previously reported.

In an effort to assess the viability of a biodegradable intravitreal implant of lyophilized bevacizumab, we

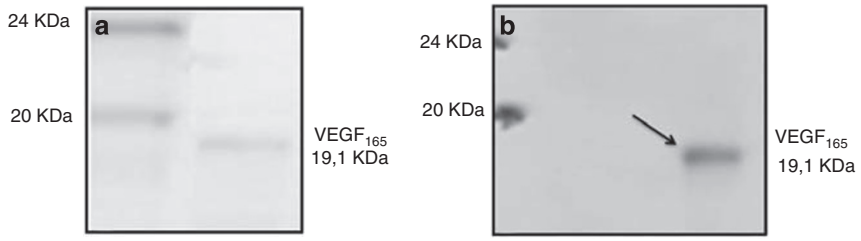

Figure 1 Western blot analysis of $VEGF_{165}$. (a) Right lane: $VEGF_{165}$ is shown on electrophoresis gel stain by Coomassie Brilliant Blue (Sigma-Aldrich) at 19 kDa. Left lane: Trypsinogen (24 kDa) and trypsin inhibitor (20 kDa) were used as controls. (b) Positive immunoreaction of bevacizumab with $VEGF_{165}$ transferred to nitrocellulose membranes was revealed by a chemiluminescent reaction from peroxidase-conjugated goat anti-human secondary antibody (arrow). The corresponding positions of the controls were marked on the left side of the membrane.

evaluated the effect of the lyophilization process of bevacizumab solution on the *in vitro* binding activity of bevacizumab to $VEGF_{165}$.

The commercial solution of bevacizumab (Avastin 100 mg/4 ml; Genentech Inc., South San Francisco, CA, USA) was frozen at −40 °C for 24 h and then lyophilized (E-C Modulyo; E-C Apparatus Inc., New York, NY, USA). It was then stored in dry bottles in a low moist environment. The lyophilized bevacizumab was diluted in Tris-buffered saline at 1 mg/ml and used in western blot analysis.

The $VEGF_{165}$ (Sigma-Aldrich, St Louis, MO, USA) was transferred to nitrocellulose membranes (Transblot 0.45 μM; Bio-Rad Laboratories, Richmond, CA, USA) after denaturation. The blot was blocked by 5% skim milk in Tris-buffered saline with 0.05% Tween (TTBS) for 1 h at room temperature and incubated overnight at 4 °C with primary antibody against VEGF or bevacizumab (Avastin lyophilized) at 1 : 2500 dilution. After washing with TTBS, we incubated blots with peroxidase-conjugated goat anti-human secondary antibody for 30 min at room temperature. After the final wash, we incubated the blot with chemiluminescence reagent (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and the signal was detected with Amersham film (Hyperfilm; GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Gel electrophoresis confirmed the migration pattern of $VEGF_{165}$ (Figure 1a). Subsequently, western blot analysis showed that bevacizumab could still bind to $VEGF_{165}$ *in vitro*. A positive immunoreaction of bevacizumab with $VEGF_{165}$ was revealed by a chemiluminescent reaction (Figure 1b).

This study shows that bevacizumab is still active after freezing and lyophilization, which are essential steps in the development of a biodegradable intravitreal implant. Even deep freezing did not alter the binding activity of bevacizumab to $VEGF_{165}$, which was a concern.[5] Upcoming *in vivo* studies on chick embryos will allow us to definitely test the bioactivity of bevacizumab biodegradable intravitreal implants.

Conflict of interest
The authors declare no conflict of interest.

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Sir,

Acute-angle closure glaucoma as the presenting sign of hypertensive crisis

Severe elevations in blood pressure (BP) are classified as hypertensive emergencies in the presence of acute end-organ damage or as hypertensive urgencies in the absence of acute target-organ involvement.[1,2] We report a