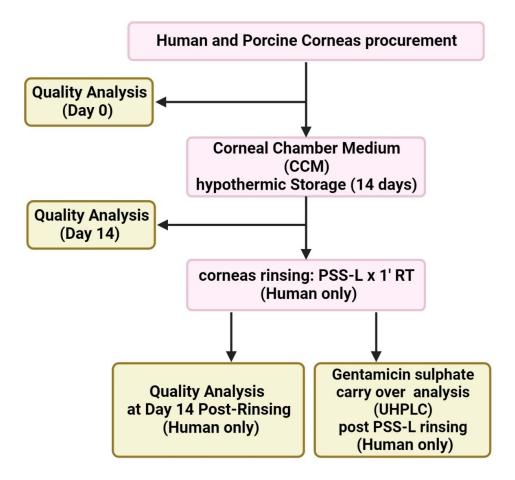
Supplementary Figure 1: Flow chart of the experimental protocol adopted in this study. Quality analysis at Days 0, 14 and 14^{PR} (Post-Rinsing) consisted in specular microscopy analysis (ECD, HEX%, CV%), light microscopy analysis (ECD, endothelial mortality %), and corneal transparency measurement. Gentamicin sulphate carry over analysis was performed on UHPLC analysis on homogenised 8.25 mm corneal buttons. Created with BioRender.com.



Supplementary Table 1. Grading system for Evaluation of the Reactivity Zone Before and After NRU Viability Assay (28).

Grade	Reactivity	Description of Reactivity Zone	
0	None	No detectable zone around or under specimen	
1	Slight	Some malformed or degenerated cells under specimen	
2	Mild	Zone limited to area under specimen	
3	Moderate	Zone extending specimen size up to 1.0 cm	
4	Severe Zone extending farther than 1.0 cm beyond specimen		

Supplementary Table 2: Endothelial cell density (ECD, both light microscopy and specular microscopy), endothelial mortality, endothelial morphology score, HEX%, CV% and transparency of human corneas stored 14 days in Eusol-C 2-8°C (Day 14) and rinsed with PSS-L at the end of the storage (Day 14^{PR}: post-rinsing). The number of samples are indicated in parenthesis. Values are expressed as mean data \pm SD. * *repeated measures ANOVA , followed by post hoc Bonferroni test; † Friedman test for multiple

comparison of not-normally distributed data; § Friedman test, followed by post hoc Nemenyi test.

Parameter	Time-point	Human corneas (n)	
ECD Light Microscopy (cell/mm ²)	Day 0	2103 ± 360 (11)	
	Day 14	2085 ± 408 (11)	
	Day 14 ^{PR}	2126 ± 356 (11)	
<i>p</i> for multiple	<i>p</i> for multiple comparison *		
ECD Specular Microscopy (cell/mm ²)	Day 0	2218 ± 352 (7)	
	Day 14	2263 ± 322 (7)	
	Day 14 ^{PR}	2253 ± 324 (7)	
<i>p</i> for multiple	0.9660		
Mortality (%)	Day 0	1.2 ± 1.2 (15)	
	Day 14	3.1 ± 3.3 (15)	
	Day 14 ^{PR}	4.0 ± 3.6 (15)	
<i>p</i> for multiple	e comparison +	0.6703	
HEX (%)	Day 0	55.3 ± 2.6 (7)	
	Day 14	51.9 ± 4.3 (7)	
	Day 14 ^{PR}	53.4 ± 4.5 (7)	
<i>p</i> for multiple	0.3813		
CV (%)	Day 0	38.9 ± 3.6 (7)	
	Day 14	41.3 ± 3.1 (7)	
	Day 14 ^{PR}	39.1 ± 2.3 (7)	
<i>p</i> for multiple	0.3813		
Transparency (%)	Day 0	71.7 ± 3.2 (15)	
	Day 14	62.9 ± 4.1 (15)	
	Day 14 ^{PR}	64.5 ± 4.2 (15)	
<i>p</i> for multiple	0.0017		
Da	< 0.0001		
Day	0.0055		
Day 1	4 vs Day 14 ^{PR §}	0.3102	

aterial which has been supplied by the author(s) BMJ Open Ophth

Supplementary Table 3. *In vitro* cytotoxicity test by direct contact in BALB 3T3 cell line according to ISO 10993-5: effect on % viability of Corneal Chamber Medium (CCM) and PSS-L medical devices. The number of replicates is reported in parenthesis. *According to the Supplementary Table 1, the achievement of a numerical grade greater than 2 is considered a cytotoxic effect; **According to the ISO 10993-5, a sample is cytotoxic when the percentage of cell viability is lower than 70%.

	ССМ	PSS-L
BALB 3T3 cell viability (%), mean ± SD	88.4 ± 0.4 (4)	97.6 ± 2.0 (3)
Morphological grade*, median; IQR	0; 0 (4)	0; 0 (3)
Cytotoxicity test result**	Not cytotoxic	Not cytotoxic