

Supplementary information

Bacterial nanocellulose as a corneal bandage material: A comparison with amniotic membrane

Irene Anton-Sales ¹, Justin Christopher D'Antin ^{2,3}, Jorge Fernández-Engroba ^{2,3}, Victor Charoenrook ^{2,3}, Anna Laromaine ^{1*}, Anna Roig ^{1*} and Ralph Michael ^{2,3}

¹ Institute of Materials Science of Barcelona (ICMAB-CSIC). *alaromaine@icmab.es
*roig@icmab.es

² Institut Universitari Barraquer, Universitat Autònoma de Barcelona, Barcelona, Spain.

³ Centro de Oftalmología Barraquer, Barcelona, Spain.

Figure S1: Dehydration rate of BNC hydrogels. Graph showing weight losses of BNC hydrogels (water content >99%) over time when exposed to the air under controlled humidity (45%) and temperature (22° C). Water evaporation took place at a constant speed and after 6 hours of exposure, BNC hydrogels still contained 66% of their initial water content. Data was fitted to a linear regression ($r^2=0.9722$).

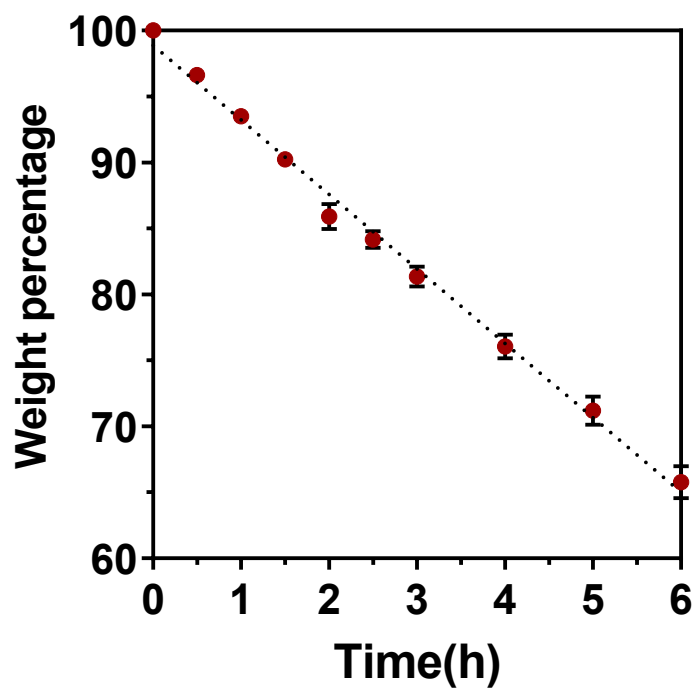


Figure S2: Frontal and slit lamp images of AM and BNC before and after the *ex vivo* stability tests. Dark field images are shown on the first and third columns where integrity of the samples can be visualized. White field photographs were taken with a grid (second and fourth columns) to better visualize the transparency of the materials. Each square of the grid corresponds to one mm². Inserts at the bottom of the grid images correspond to lateral views of the materials captured with a slit lamp. To mark the orientation of the samples a tissue marker was used and for clarity in the pictures, the materials were delineated with a yellow-dotted line.

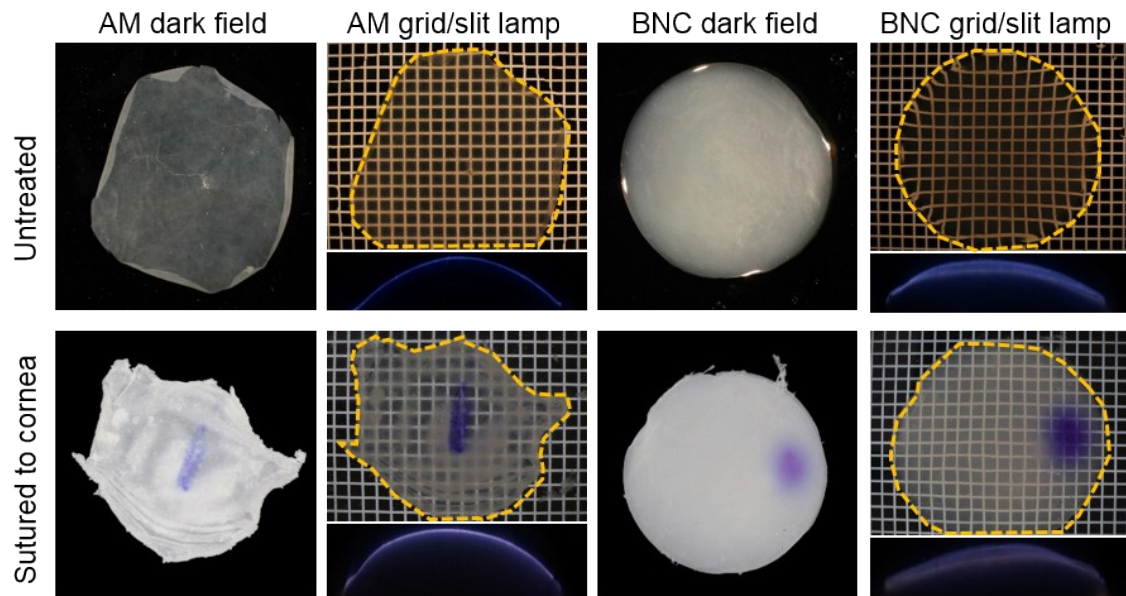


Figure S3: Representative SEM micrographs of the AM and BNC sides expected not to be in contact with the ocular surface. After both *in vitro* and *ex vivo* experiments, the materials did not seem to be degraded and the organic deposits observed on the side placed in contact with the eye surface were not perceived. The only noticeable difference was the presence of elongated crystals on the AM samples of the *in vitro* test (indicated with asterisks). Three samples were imaged for each material at least on three different areas. The mentioned observations were consistent on the three samples that were examined.

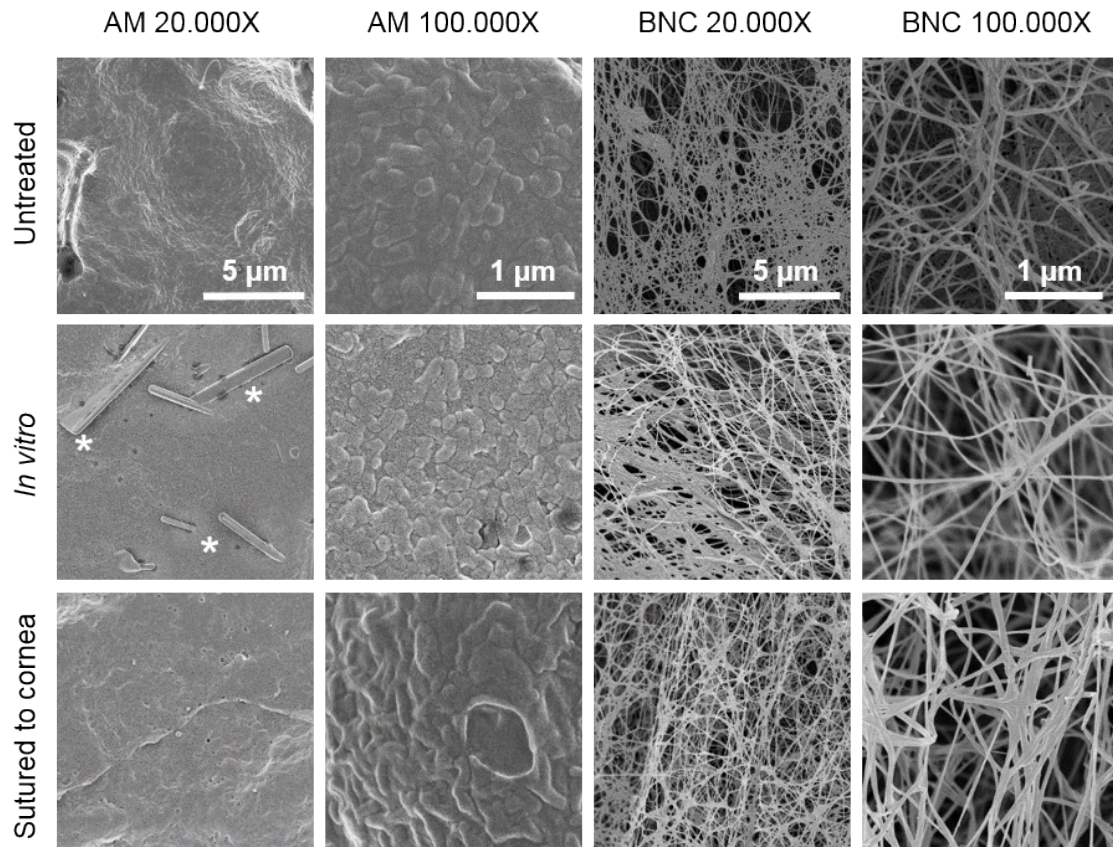
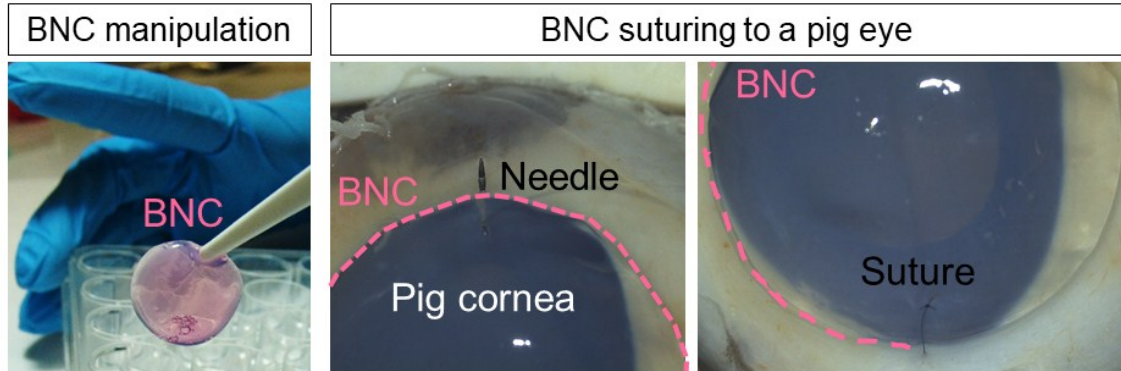


Figure S4: BNC handling and suturing to the ocular surface. BNC hydrogels ($\varnothing=1.6$ cm) were easily manipulated with forceps and maintained their initial shape as illustrated on the left panel. BNC acquired a pink color from the culture medium components. BNC suture process (right panel) to pig eyes was comparable in terms of time and complexity to that of human amniotic membrane. The needle penetrated easily on the material without causing tearing and BNC spreaded and adapted readily on the surface of the cornea.



Experiment	Material characterization	Physiological stability in corneal preservation medium (<i>in vitro</i>)		Physiological stability sutured to cornea (<i>ex vivo</i>)	
		t=0 (untreated)	t= 30 days	t=0 (untreated)	t= 20 days
Number of samples	<i>Different depending on the characterization technique</i>	<i>n= 6 for BNC</i> <i>n= 6 for AM</i>		<i>n= 3 for BNC</i> <i>n= 3 for AM</i>	
Characterization points and techniques	Un treated				
Thickness Micrometre	X				
Thermal stability Thermo-gravimetric analysis	X				
Atomic structure and crystallinity X-Ray Diffraction	X				
Suture stress resistance Suture stretch test	X				
Macroscopic integrity Digital pictures: dark field and slit lamp		X	X	X	X
Optical transparency Digital white field pictures with grid		X	X	X	X
Superficial mass Mass and surface		X	X		
Material absorbance UV-Visible spectrophotometry	X		X		X
Micro and nanostructure Scanning electron microscopy	X		X		X

Supplementary table 1: Summary of the experimental design