

## Magnetized Bacterial Nanocellulose (MBNC) for Neuroendovascular Reconstruction of Brain Aneurysms

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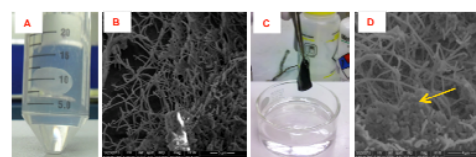
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**Statement of Purpose:** Current treatments for brain aneurysms involve clipping and neuroendovascular coil embolization, including stent-assisted coiling in an attempt to occlude the aneurysmal defect from the parent artery. Such treatments are invasive and traumatic, not suitable in most patients with increased risks. In 2006, Drs. Tigno and Armonda introduced an alternative method that sought to use scaffold stents with their inherent thrombogenicity coupled to stem cell derivatives from the arterial wall and the need to create a local, focal attraction force of cells to the abluminal side of the stent scaffold for an *in situ* reconstruction of the tunica media<sup>1</sup>. In 2009, Drs. Allain and Tigno introduced for the first time, a multi-functional nanostructured bioactive coating designed to render an asymmetric region of the stent scaffold magnetic and biomimetic<sup>1,2</sup>. The bioactive coating utilizes bacterial nanocellulose (BNC) as a platform for both magnetic attraction and cell attraction and proliferation.

**Methods:** Bacterial Nanocellulose was obtained from a culture of *A. xylinum* bacteria after 15 days of growth at 30°C. The magnetization of the BNC was realized through the reaction of Iron III and Iron II and heating up to 84°C. Ammonium hydroxide was added to the mixture in order to precipitate out super paramagnetic iron oxide nanoparticles (SPION). Subsequently, magnetic bacterial nanocellulose (MBNC) was coated with PEG to improve its biocompatibility. All samples were characterized before and after magnetization using scanning electron microscopy (SEM). Cytotoxicity and biocompatibility were evaluated using Porcine aortic smooth muscle cells (PASMOC). Preliminary cellular migration assays demonstrated the behavior between MBNC and cells labeled with paramagnetic nanoparticles. Human aortic smooth muscle cells (HASMCs) were magnetized through passive uptake, in which cells were incubated with the 6 µg/mL SPION solution. Electroporation was optimized for cell viability using: 16 µg/mL, 500 V/cm, 100 µs, and 4 pulses. Prussian blue staining, SEM and TEM techniques were used to determine the concentration of nanoparticles inside the cells.

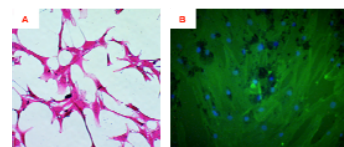
**Results:** Thick pellicles of BNC were grown as a translucent and sticky material composed of micro fibrils that bundle together to form long intertwined ribbon-shaped fibrils. BNC pellicles harvested in our experiments round with a diameter of approx. 2 cm (Fig. 1A, 1C).

After completion of the magnetization procedure, the samples exhibited a paramagnetic behavior. Observation at the macroscale shows changes in the MBNC; it looks darker, had a smaller diameter, and was more rigid compared to BNC (Fig. 1B). An effective magnetic attractive force was observed using a magnet of 6 Gauss. SEM data clearly indicated the location of nanoparticles attached to the BNC fibers (Fig. 1D, yellow arrow).



**Figure 1:** A) BNC B) SEM image of BNC C) MBNC C) D) SEM image of MBNC.

Internalization of SPIONs by HASMCs and their distribution was revealed using Prussian blue staining for iron oxide content (Fig. 2A). The process for passive uptake and electroporation were positive for iron content. However, the passive uptake  $60.57 \pm 1.12$  pmol/cell and electroporation  $21.13 \pm 0.96$  pmol/cell respectively showed better results in terms of cell viability. Visualizations of samples through conventional optical and fluorescence microscopy allowed the detection of adequate cellular proliferation and viability (Fig. 2B).



**Figure 2.** A) Iron uptake by HASMCs B) HASMCs growing on MBNC

**Conclusions:** Synthesis of MBNC impregnated with magnetic nanoparticles was successfully demonstrated. A viable, resilient membrane coat resulted from the first biocompatible coating for neuroendovascular application using a stent scaffold. Cell viability and minimal cytotoxicity was achieved. Cell migration tests and examination of cellular magnetic attraction confirmed the viability of MBNC as a multi-functional coating.

### References:

1. J.P. Allain, J. Tigno and R. Armonda in ed. Kateb and Heiss, The Textbook of Nanoneurosurgery, Taylor & Francis, 2013].
2. Patent Pending, J.P. Allain, J. Tigno, R. Armonda, L. Reece, Z. Yang WIPO/PCT # WO2013/052934 A2