BACTERIAL NANOCELLULOSE

a sofisticated biomaterial

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OUTLINE

BACTERIAL NANO CELLULOSE

- Production
- Properties
- Biomedical Applications
- Non-Biomedical Appls

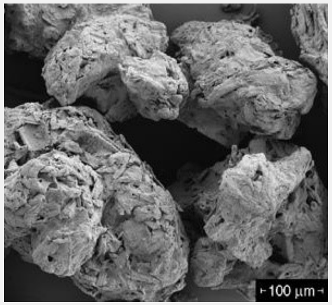
FUNCARB RESEARCH GROUP

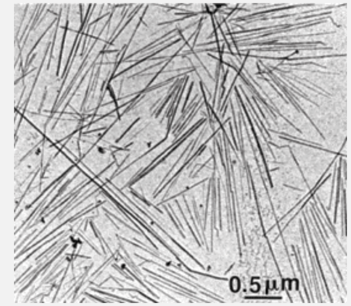
- Aims & Scopes
- Research Activities
- BC production & Applications

BCTECHNOLOGIES

- Innovation
- Entrepeneurship
- Market entry

PLANT CELLULOSE



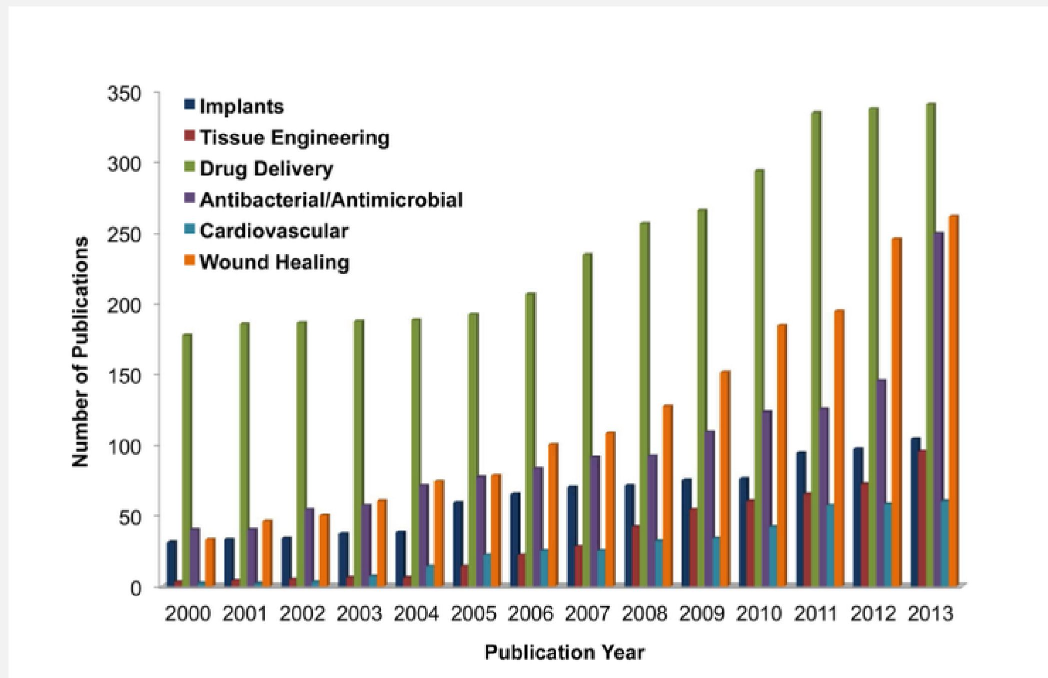


Microcrystalline cellulose

Microfibrillated cellulose

Cellulose whiskers

Cellulose derivates



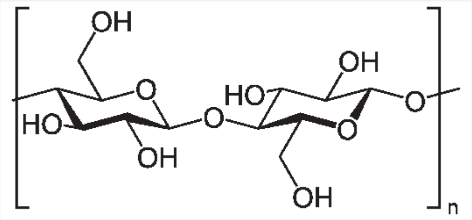
PLANT CELLULOSE

Food

Pharmaceutical

Cosmetics

Tissue Engineering



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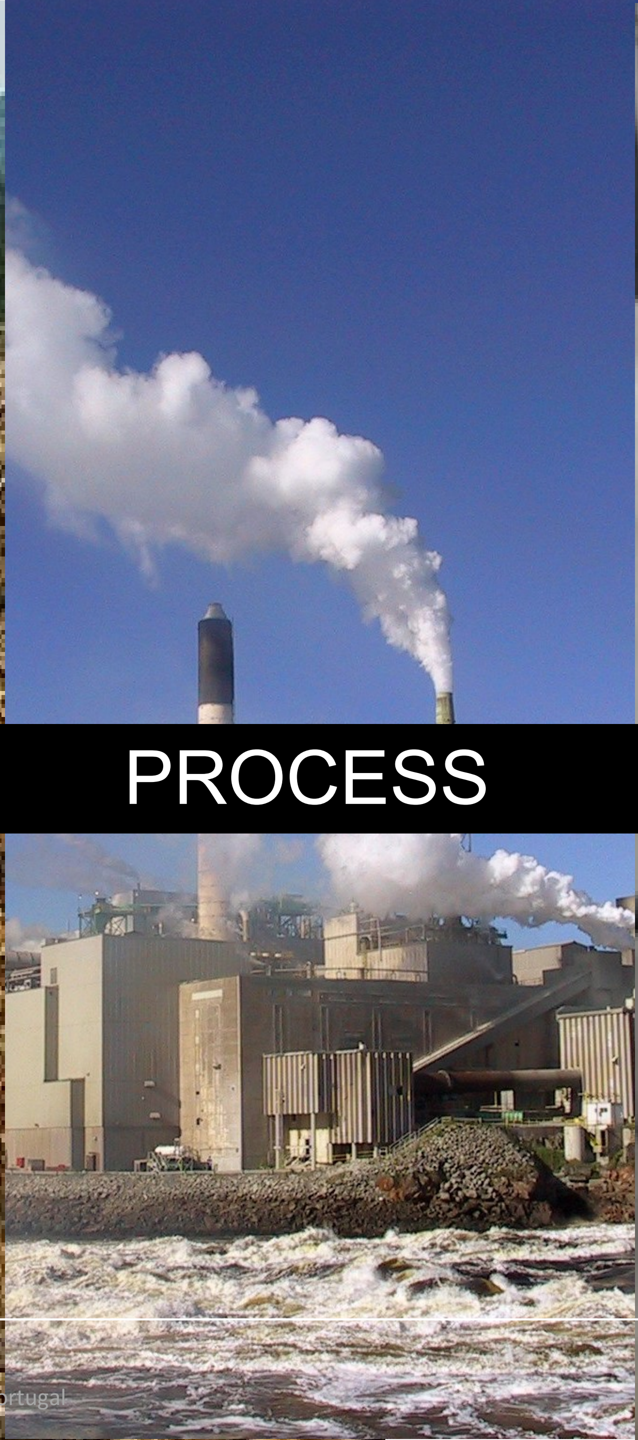
RAW MATERIALS

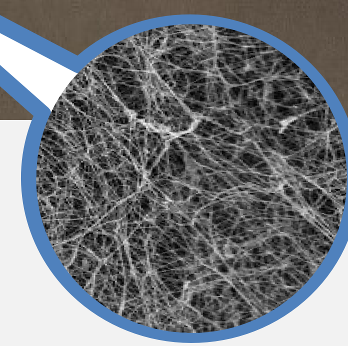


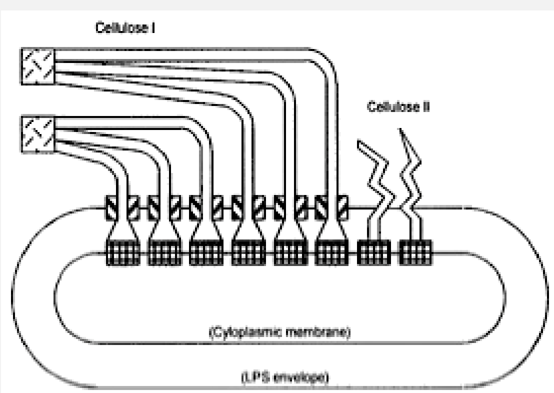
PROCESS

PROGRESS

Komagataeibacter sucrofermentans (Gluconacetobacter xylinus)

BACTERIAL NANOCELLULOSE



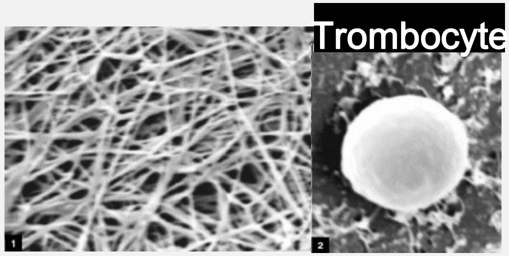


Iguchi, *et al*. 2000. *J. Mat. Sci*. 35:261-270;
Czaja, *et al*. 2006. *Biomat*. 27:145-151;
Klemm, *et al*. 2001. *Prog. Polym. Sci*. 26:1561-1603.

BNC: PROPERTIES

PURITY

- Cellulose is the only synthesized biopolymer
- Biodegradable and recyclable

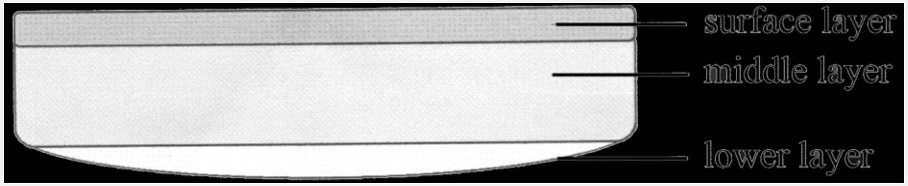


HIGH CRYSTALLINITY

- 60-90% CI

ULTRAFINE FIBER NETWORK

- Cross-sectional dimensions of 3-4nm by 70-100nm; length 1-9 μm



HIGH MECHANICAL STRENGHT

- Young Modulus of 15-35GPa

Klemm, *et al.* 2001. *Prog. Polym. Sci.* 26:1561-1603; Klemm, *et al.* 2005. *Angew. Chem Int*. Ed. 44:3358-3393; White & Brown, Jr. 1989. In: Cellulose and Wood - Chemistry and Technology, p.573-590.; Klemm, *et al.* 2001. *Prog. Polym. Sci.* 26:1561-1603

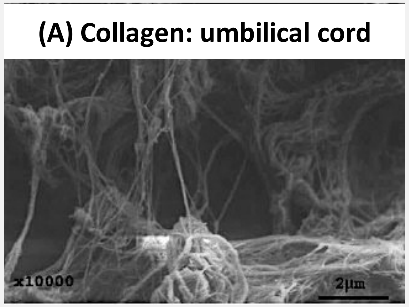
BNC: PROPERTIES

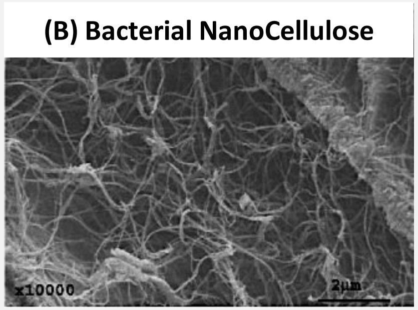
HIGH ABSORBENCE CAPACITY

- Remarkable capacity to hold water (up to 200 times its dry mass)

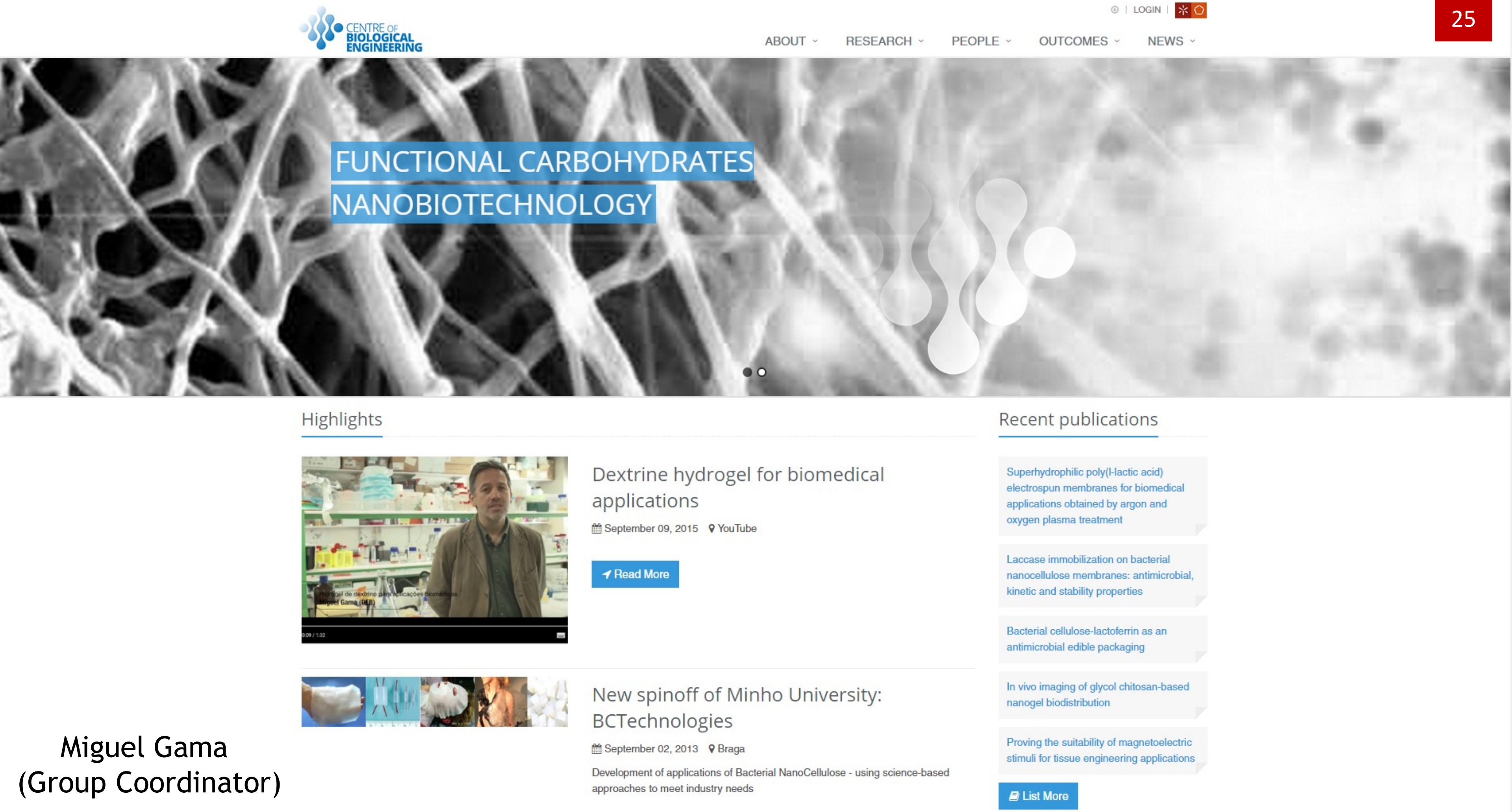
IN SITU MOLDABILITY AND IN/EX SITU MODIFICATION

NATURAL MIMIC OF THE ECM





Klemm, *et al.* 2001. *Prog. Polym. Sci.* 26:1561-1603; Klemm, *et al.* 2005. *Angew. Chem Int*. Ed. 44:3358-3393; White & Brown, Jr. 1989. In: Cellulose and Wood - Chemistry and Technology, p.573-590.; Klemm, *et al.* 2001. *Prog. Polym. Sci.* 26:1561-1603



Miguel Gama
(Group Coordinator)

RESEARCH | Biotechnology & Biomedical Engineering

AIMS | Development of new Biomaterials & tolls for biomedical applications

MATERIALS | Natural polysaccharide

Controlled/Targeted Drug Delivery

Dextrin
Chitosan

Hydrogels ,
Self-assembled nanogels

Improved biocompatibility and resorption properties

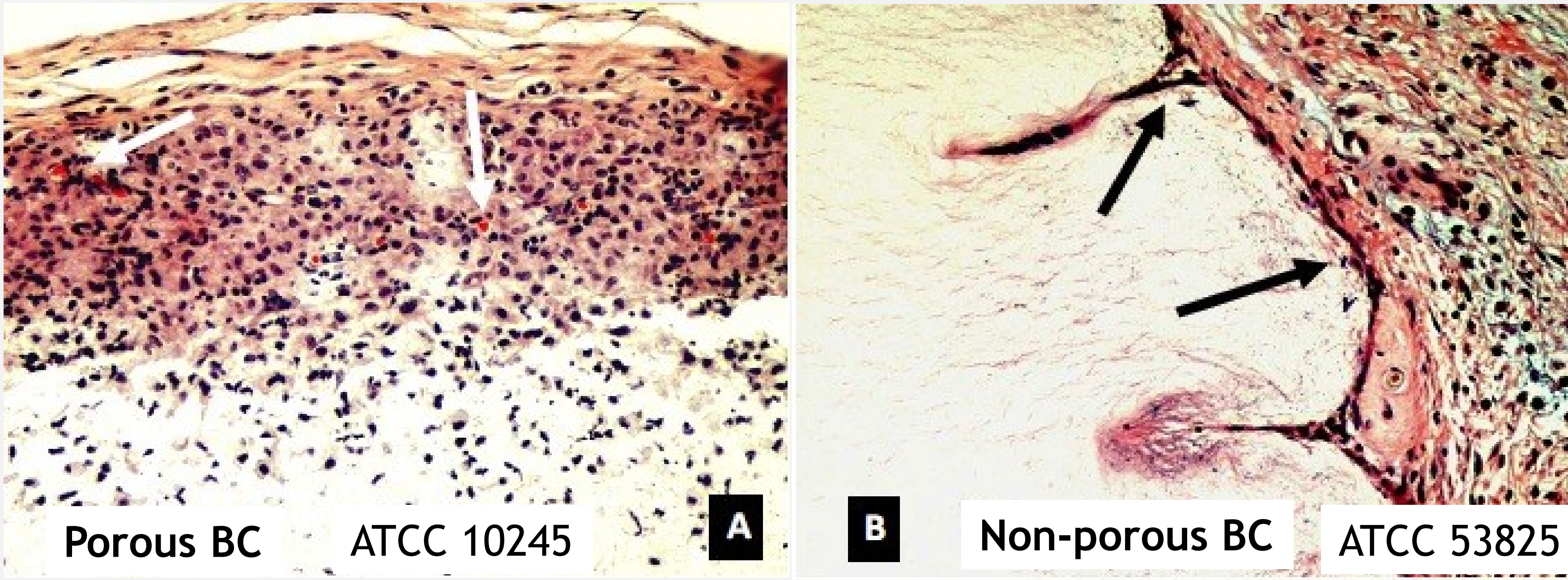
Bacterial
NanoCellulose

Artificial Vascular Prosthesis
Tissue Engineering of:

Cartilage,
Nerve,
Bladder

Novel BNC production and modification techniques

BIOMEDICAL APLICATIONS



BC subcutaneous implants, in mice, demonstrate the excellent biocompatibility. Furthermore, the effect of porosity on the proliferation of cells inside the implant was analysed using different BC producing strains: vascularization of the more porous material was observed.

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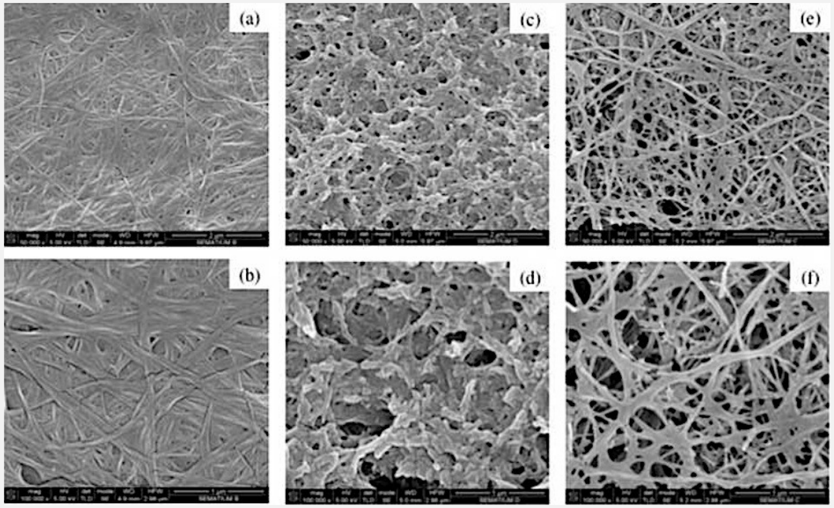
Engineering porosity by nitrogen plasma treatments

Surface modification	Autoclaved (Y/N)	Oxygen (%)	Carbon (%)	Nitrogen (%)	O/C	N/C
BC	N	45.20	54.79	0.01	0.82	0.0001
BCP	N	38.31	55.79	5.90	0.69	0.1057
	Y	36.66	59.75	3.59	0.61	0.0600

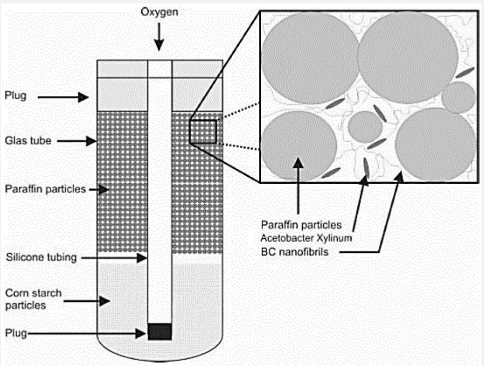
X-ray photoelectron spectroscopy (XPS)
scanning electron microscopy (SEM).
Adhesion of microvascular (HMEC-1), neuroblast (N1E-115)
and fibroblast (3T3) cell lines

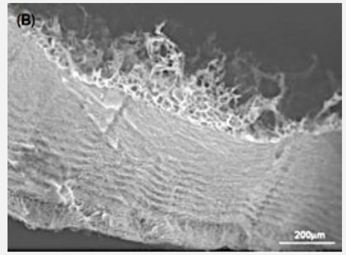
- Nitrogen plasma treatment allowed to increasing the concentration of functional groups on BC surface;
- Surface modifications was stable over time;
- Plasma treatment allowed improved adhesion of endothelial and neuroblast cells to the material;
- The modified material showed enhanced porosity

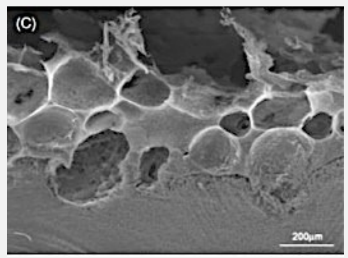


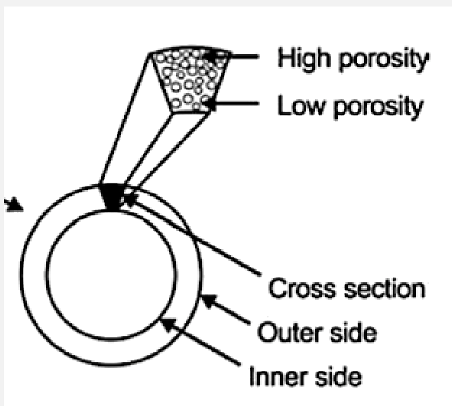
Andrade et al. 2010. *Journal of Biomedical Materials Research* Part A. 92A, (1):9–17.

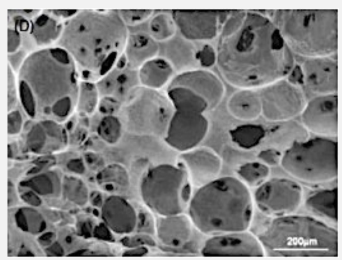
TAILORING MICROPOROSITY





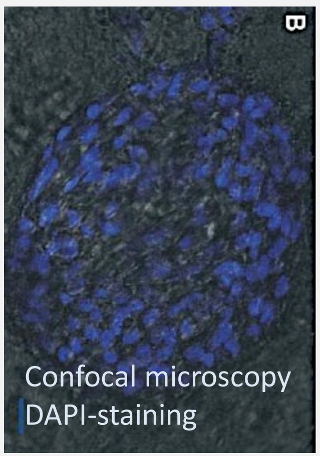






- **+ Microporosity and pore interconnectivity**
- **+/- Non-homogeneous porosity (no control)**
- **- Poor mechanical properties**

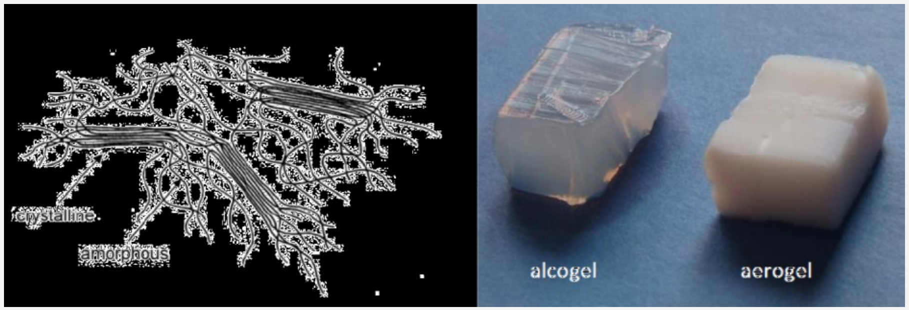
Human smooth muscle cells

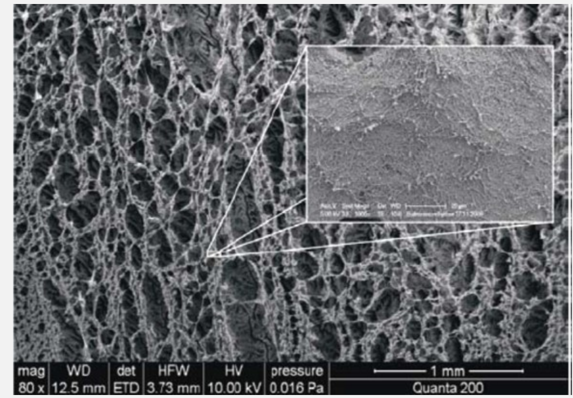


Bäckdahl et al. 2008. *J Tissue Eng Regen Med.* 2(6):320-330

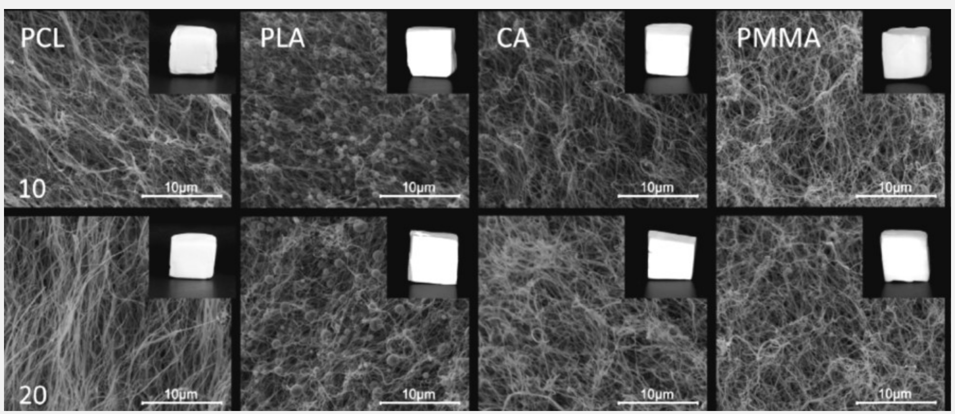
AEROGELS FROM BACTERIAL CELLULOSE

controlled release matrices





Loading bioactive
scCO2 drying



biocompatible polymers:
polylactic acid (PLA),
poly-caprolactone (PCL),
cellulose acetate (CA),
poly(methyl methacrylate) (PMMA),

Liebner *et al*. 2010. *Macromolecular Bioscience*. 10(4):349-352
Haimer *et al*. 2010. *Macromolecular Symposia*. 294(2):64-74
Pircher *et al*. 2014. *Carbohydrate Polymers*. 11:505-513

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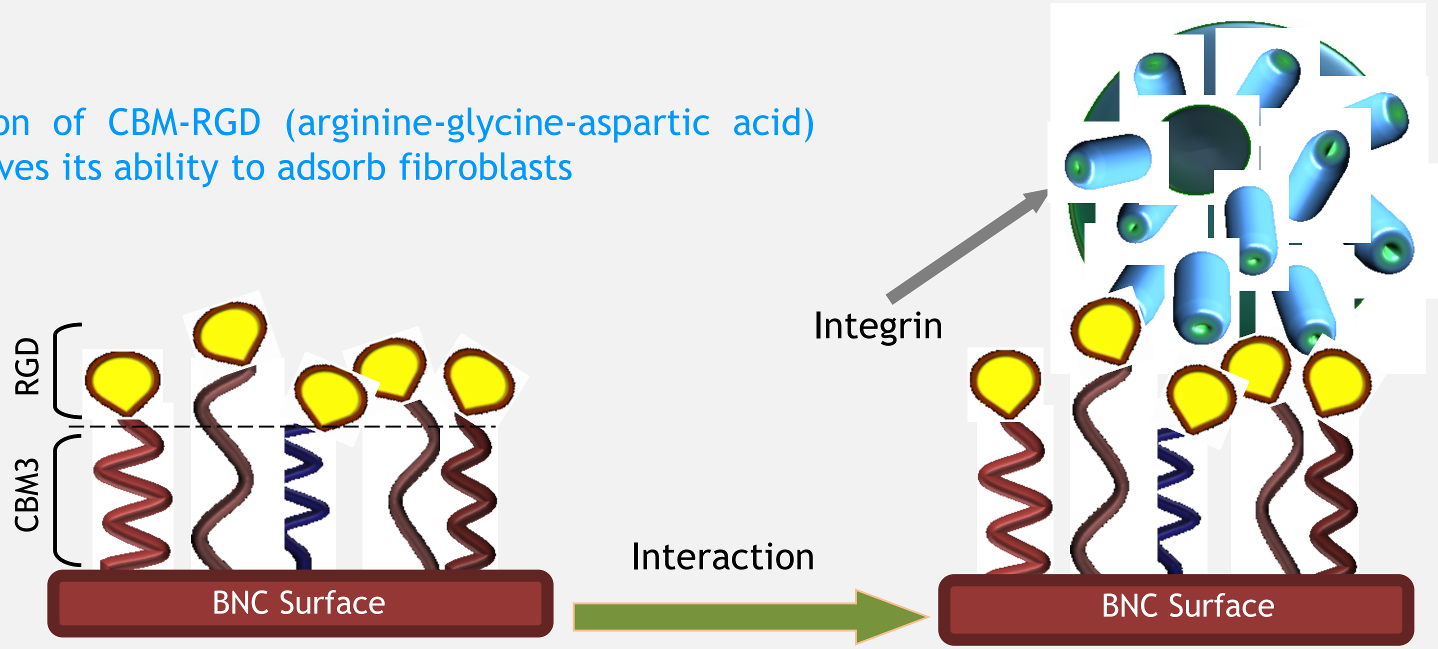
➢ Surface-activation of BNC with CBMs (Carbohydrate Binding Modules) conjugated and bioactive peptides for biomedical applications:

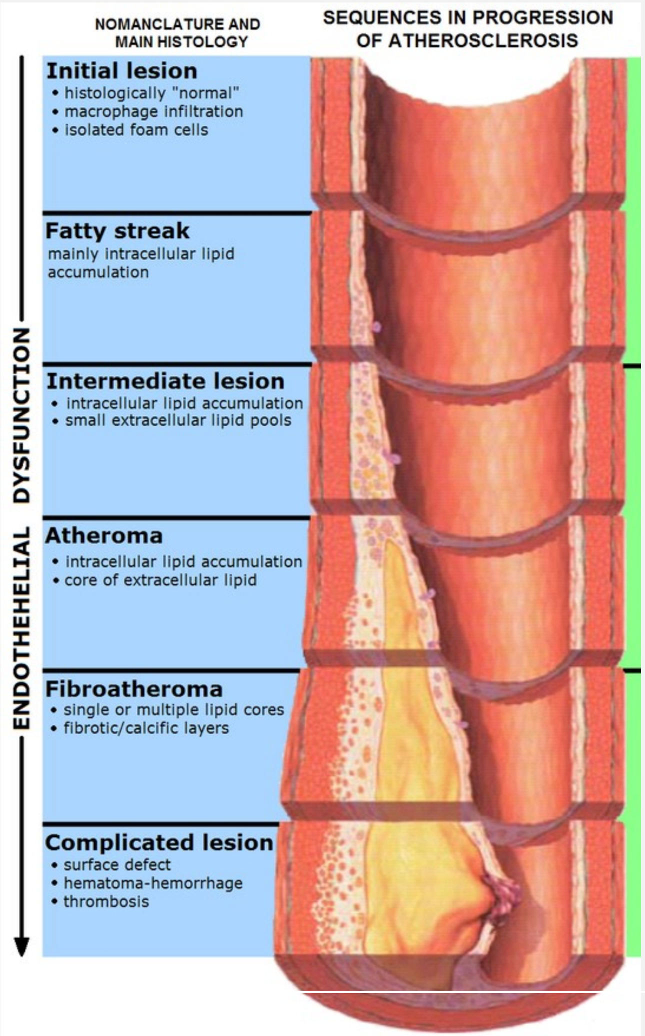
- **CBM-RGD or GRGDY** and **CBM-LL37** to improve cell adhesion and angiogenesis

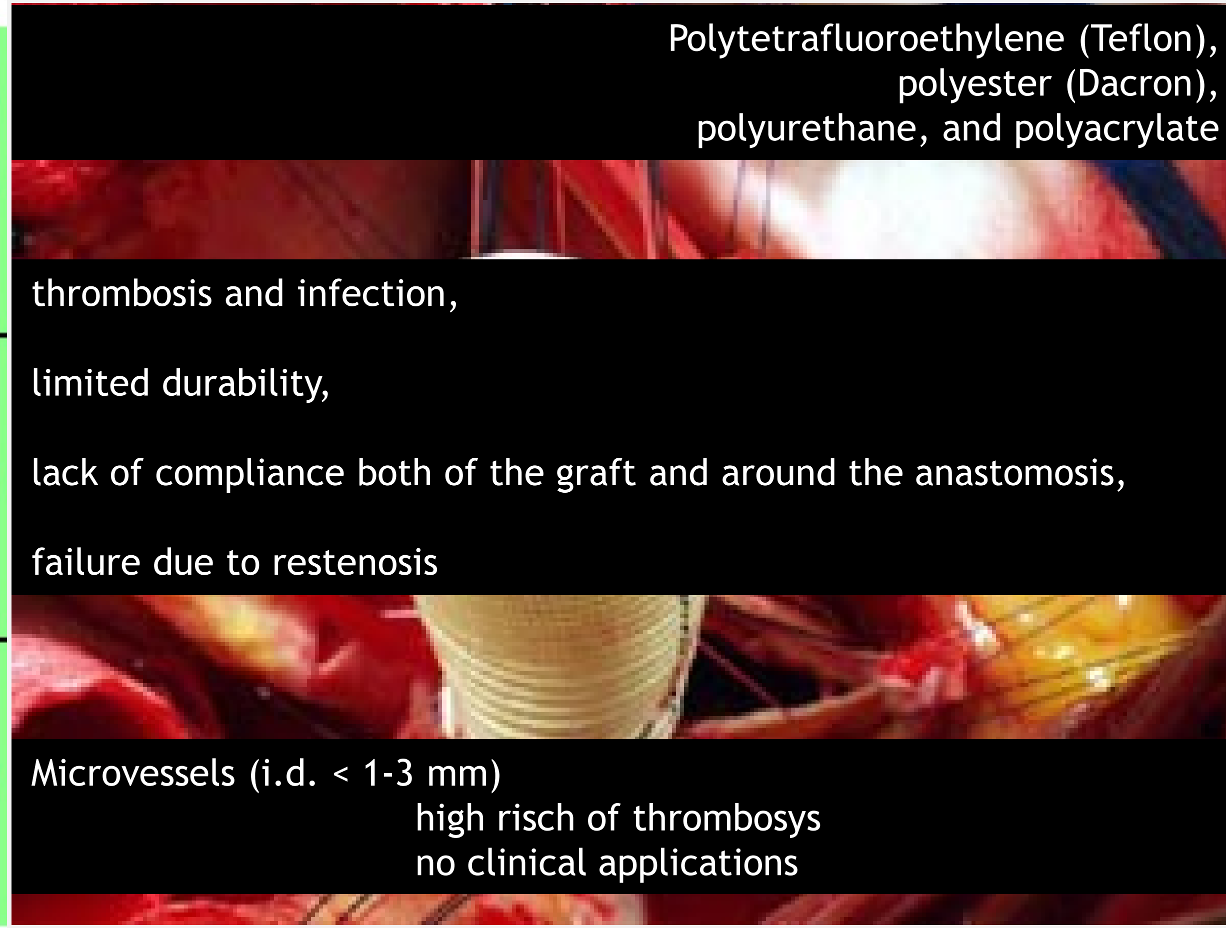
- The adsorption of CBM-RGD (arginine-glycine-aspartic acid) onto BNC improves its ability to adsorb fibroblasts



Andrade *et al*. 2010. *J. Biomed. Mater. Res.:* A. 2010 Jan;92(1):9-17.
Andrade et al. 2010. *Acta Biomaterialia* 6:4034-4041

TISSUE ENGINEERING BLOOD VESSELS





Polytetrafluoroethylene (Teflon),
polyester (Dacron),
polyurethane, and polyacrylate

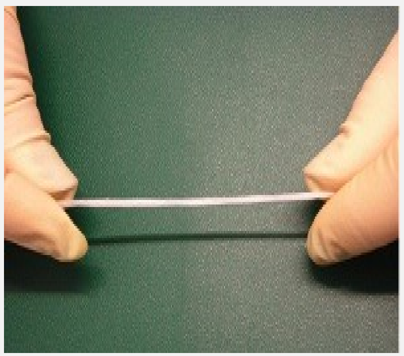
thrombosis and infection,

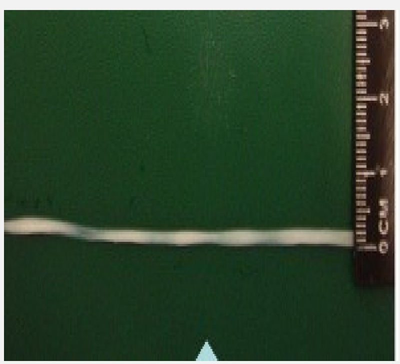
limited durability,

lack of compliance both of the graft and around the anastomosis,

failure due to restenosis

Microvessels (i.d. < 1-3 mm)
high risch of thrombosys
no clinical applications

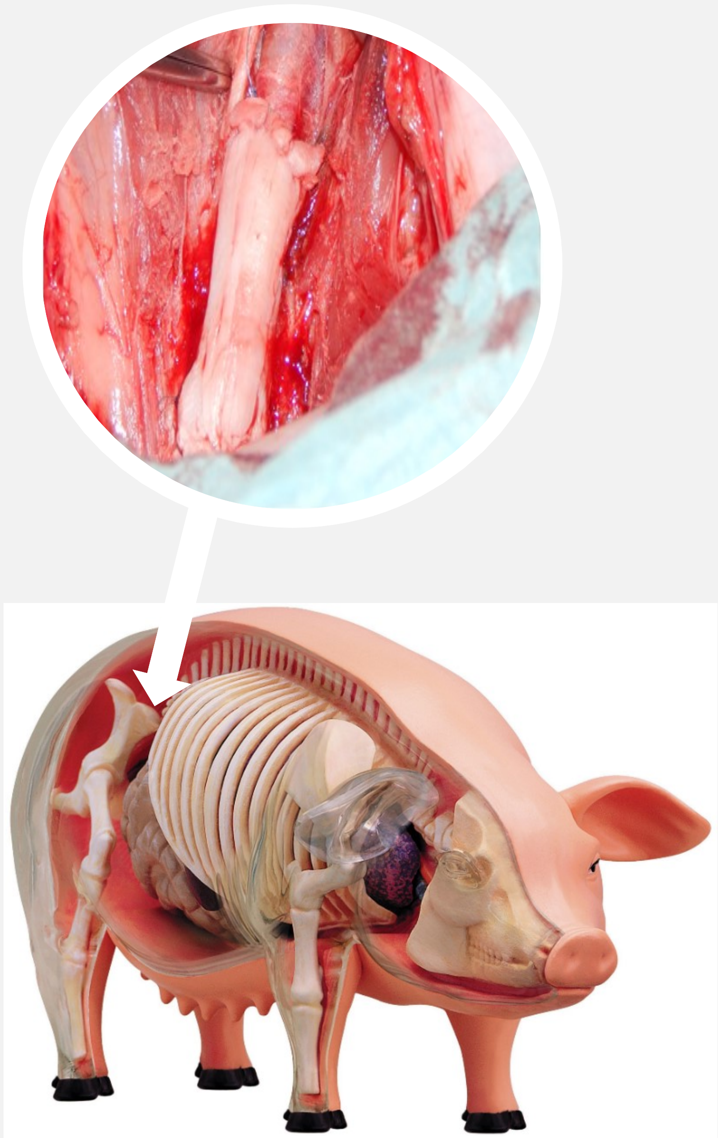




Surgery at ICBAS' facilities
(Instituto de Ciências Biomédicas Abel Salazar)

In vivo vascular grafts replacement in pig:
(4mm internal diameter BC tubes)

Functionallity, tissue integration, hemocompatibility, endothelialization



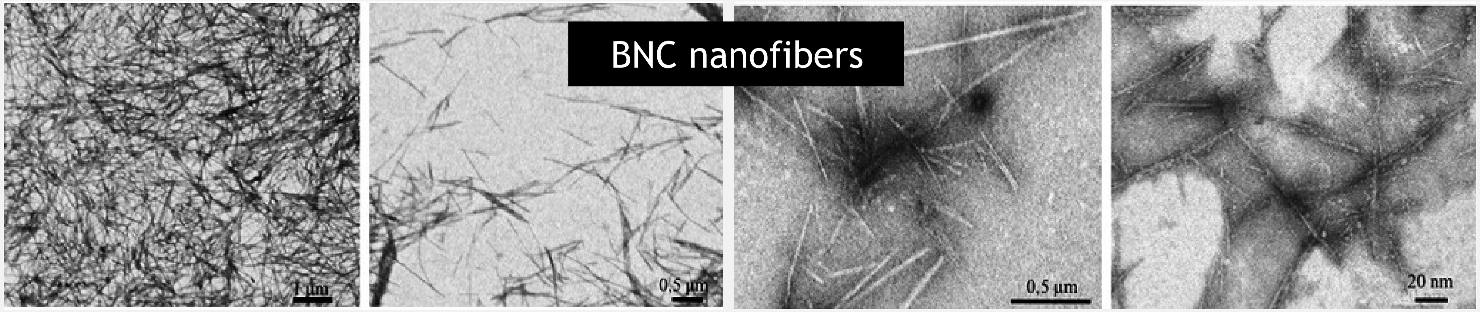
Surgery at ICBAS' facilities
(Instituto de Ciências Biomédicas Abel Salazar)

Patency: 1 month
Endothelization of the luminal wall
Neovascularization

• A major concern with fibres (ex. asbestos) is their carcinogenic potential;
• The cytotoxicity of a nanomaterial is many times cell-specific

Inflammatory response of BNC implants may yield BNC nanofibers

The toxicity of BNC nanofibers must be evaluated if considering biomedical aplications



Moreira, S.; Silva, N.B.; Almeida-Lima, J.; Rocha, H.A.; Medeiros, S.R.; Alves, C. Jr.; Gama, *F.M.Toxicol Lett*. 2009. Sep 28;189(3):235-241.

Evaluation of cellulose nanofibres **mutagenicity** by *Salmonella* reversion assay

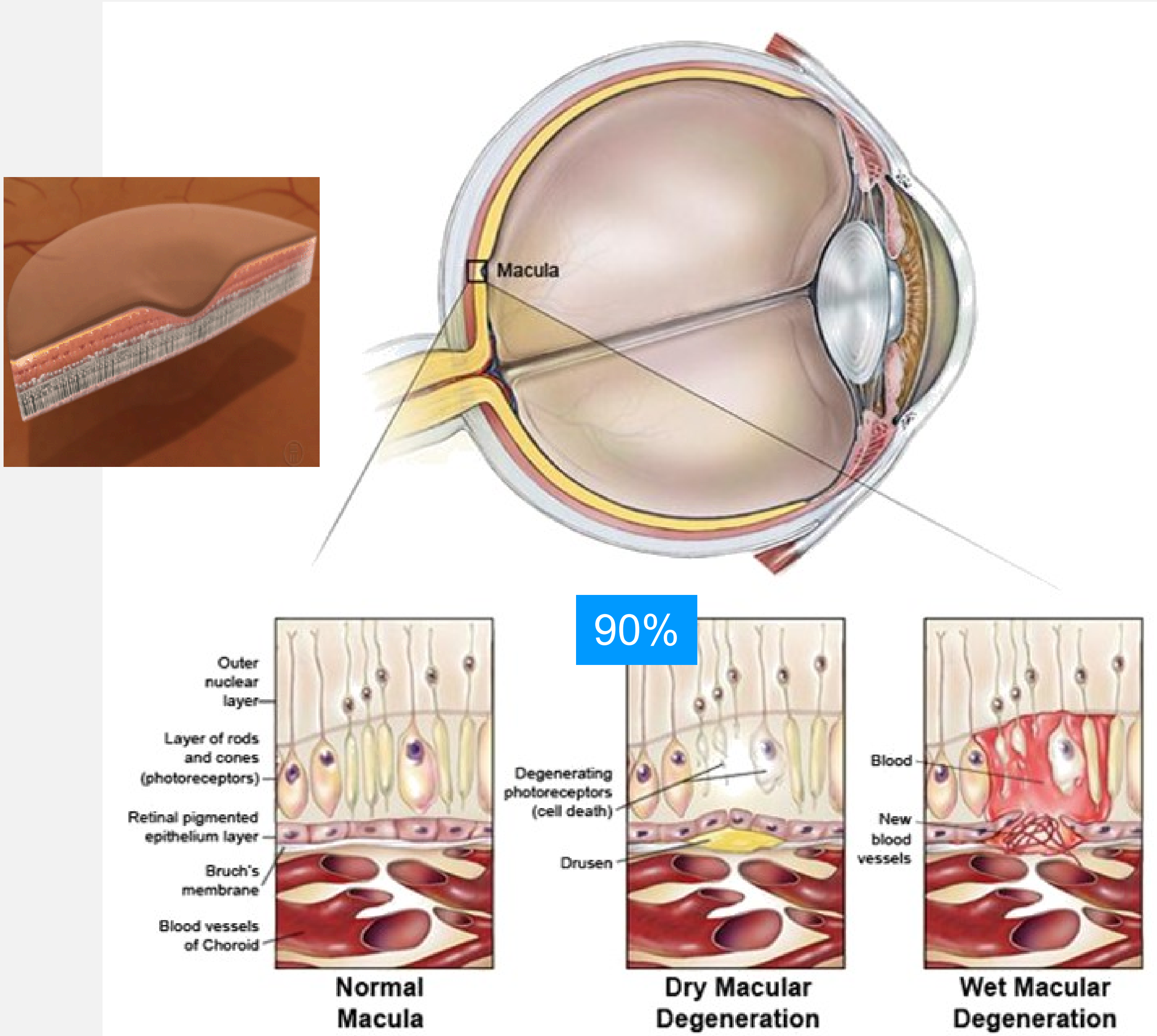
Proliferation assays (Chinese Hamster Ovary CHO or mouse embryo fibroblast 3T3)

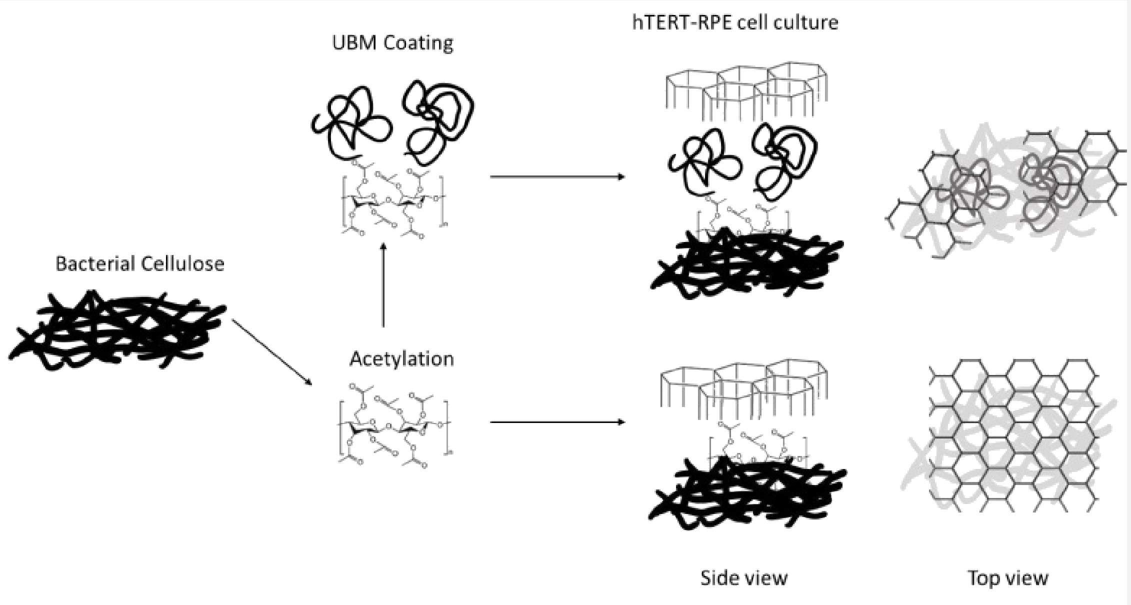
Evaluation of cellulose nanofibres **genotoxicity** by single cell gel assay (comet assay)

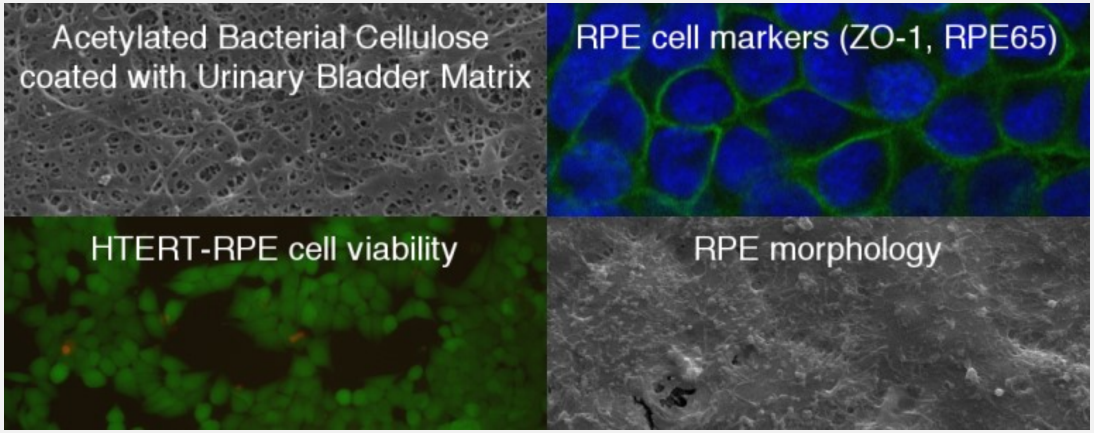
- BC nanofibers do not present a mutagenic behaviour
- Proliferation was only 15–20% lower in the presence of NFs
- BC nanofibers do not cause detectable DNA alterations

Moreira, S.; Silva, N.B.; Almeida-Lima, J.; Rocha, H.A.; Medeiros, S.R.; Alves, C. Jr.; Gama, *F.M.Toxicol Lett*. 2009. Sep 28;189(3):235-241.

Age-related macular degeneration (AMD or ARMD)







Gonçalves *et al*. 2016. *Colloids and Surfaces B: Biointerfaces*. 139:1-9.
Gonçalves *et al*. 2015. *Biomacromolecules*. DOI: 10.1021/acs.biomac.5b00129.

WOUND DRESSINGS

Czaja *et al*. 2006. *Biomat*. 27:145-151.
Czaja et al. 2007. Springer; pp. 307-321

(a) A moist environment for tissue regeneration;
(b) Significant pain reduction;
(c) the specific microbial cellulose nano-morphology which appears to promote cell interaction and, tissue re-growth;
(d) significant reduction of scar formation; and,
(e) easy and safe release of wound care materials from the burn site during treatment.

OVERVIEW

Cell adhesion & Biocompatibility

•Surface chemical modifications:
(ex situ)
-Trimethyl ammonium betahydroxy propyl-BC,
-Diethyl aminoethyl-BC,
-Aminoethyl-BC
-Carboxymethyl-BC (CM-BC)

-Adhesive proteins
(collagen type I, collagen type IV, fibrin, fibronectin or laminin)

-Arg-Gly-Asp (RGD)

-Phosphorylation and sulfation
(mimick glucosaminoglycans of cartilage tissue *in vivo*)

Porosity and biodegradability

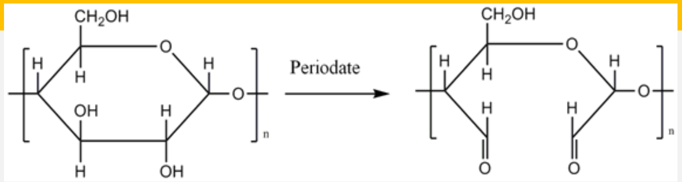
•Bulk modifications (in situ):

- N-acetylglucosamine (GlcNac)
- Chitosan

-Porogens
(starch, paraffin)

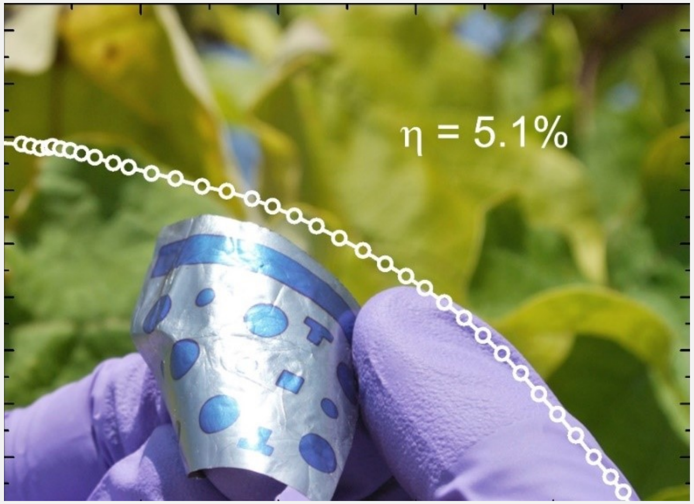
• Surface chemical modifications (ex situ):
- Periodate oxidation

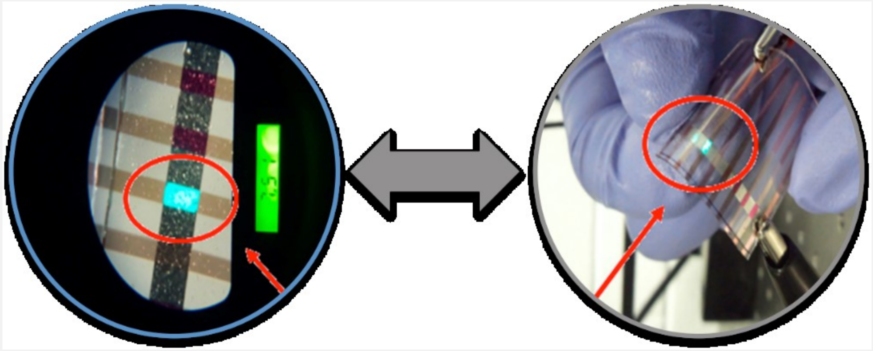
- Aerogels

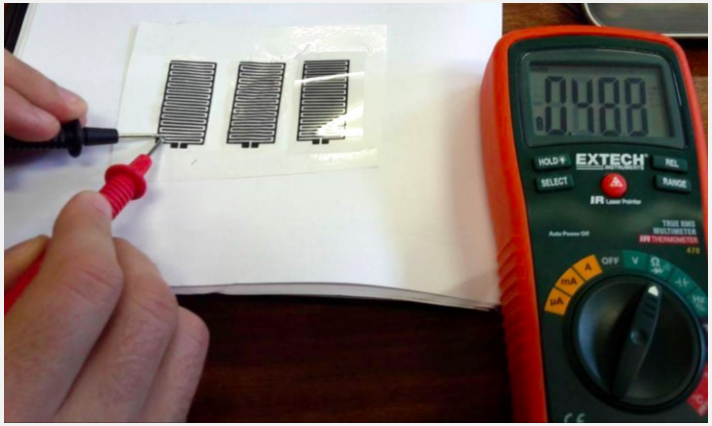


Ciechanska, D. 2004. *Fibr. Text. East. Europe*. 12(4):69-72
Li *et al*. 2009. *Mat. Sci. Engin*. 29:1635-1642
Lee *et al*. 2001. *App. Environ. Microbiol*. 67(9):3970-3975
Ogawa *et al*. 1992. *Int. J. Biol. Macromol*. 14(6):343-347

e-DEVICES







OVERVIEW

- **Excellent in vivo biocompatibility**
- **No foreign body reaction**
- **Nanofibrilar 3D structure (mimics extracelullar matrix)**
- **High mechanical strength & Cristallinity** (Young Modulus of 15-35GPa; 60-90% CI)
- **High water retention** (up to 200 times its dry weight)
- **High shape retention**
- **In situ moldability /modification**

- **Not degradable in human body**
- **Low porosity** (Low cell and tissue ingrowth)
- **Strain variability**
- **High cost**

HIGH PRODUCTION COSTS

NICHE MARKETS (HIGH VALUE-ADDED)

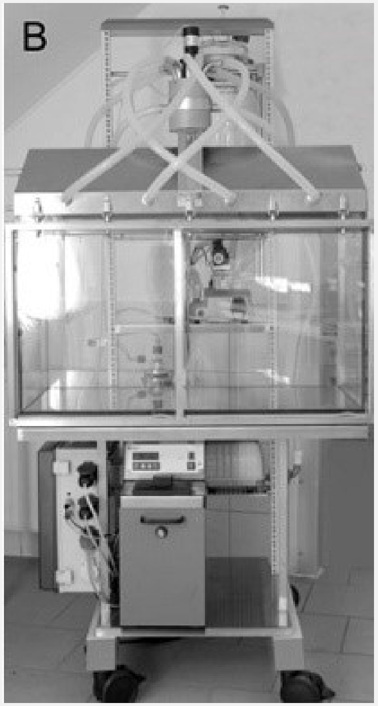
INDUSTRIAL BNC PRODUCTION

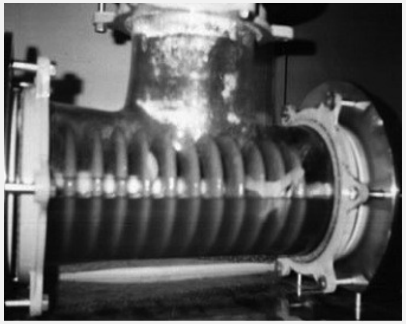
STRATEGIES

• **Specific fermentation media:**
- Agro-industrial wastes,
- Defined media,

• **Over-producing mutant strains**

• **Bioreactors:**
-Air-lift
- Agitated systems
- Membrane bioreactors (silicone rubber)
- Film bioreactors (horizontal disks)
- Aerosol bioreactor





http://www.bi.go.id/sipuk/en/?id=4&no=52323&idrb=46501

Miguel Gama

Fernando Dourado

BC Bacterial Cellulose TECHNOLOGIES

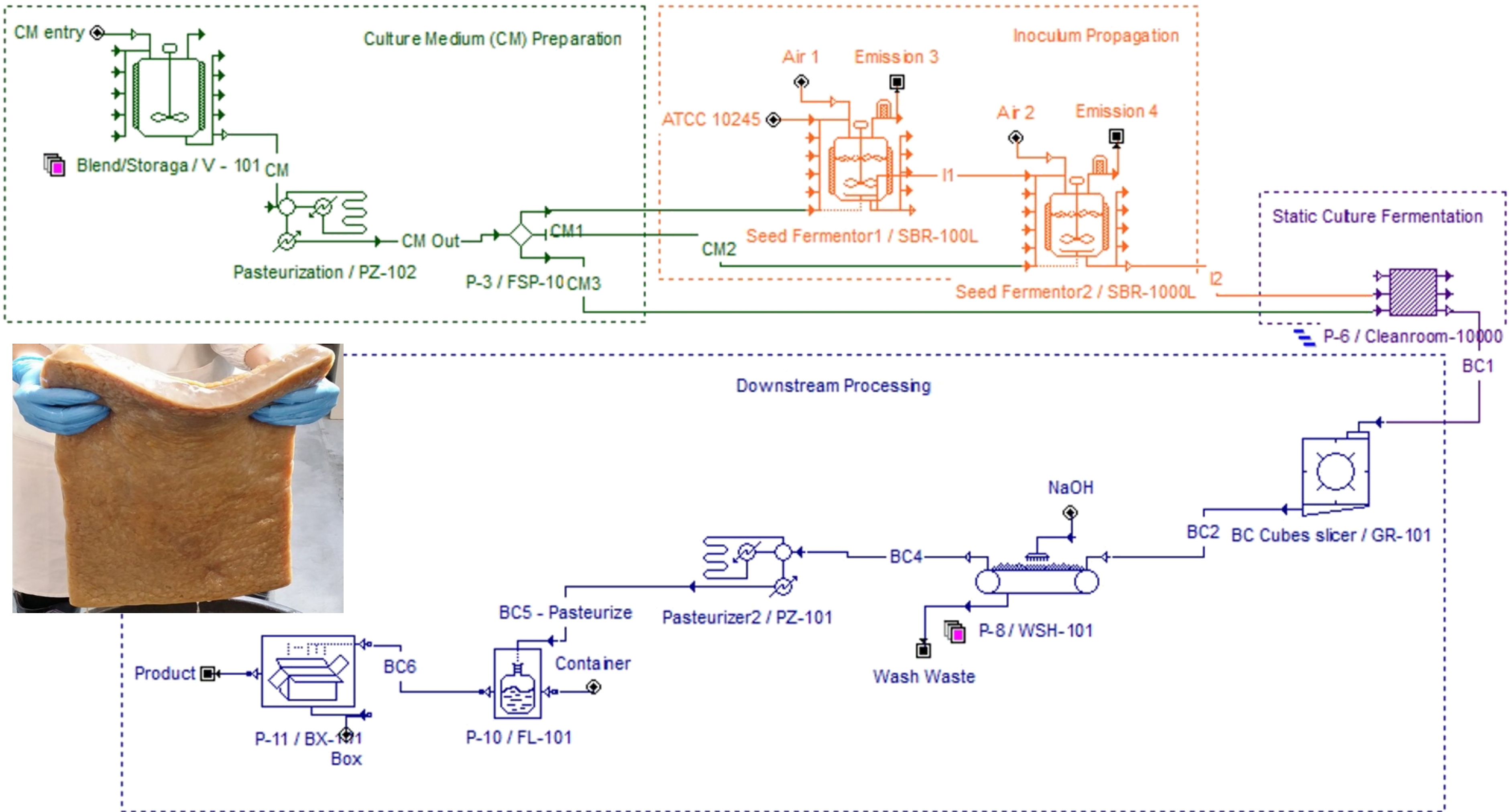
To identify new ideas & inovative oportunities, whith high added-value in the:

Roadmap

- Food (food ingrediente/additive)
- Biomedical (small calibre artificial blood vessels, wound dressings)
- Composites (bio-plastics, electronic displays, pulp & paper)

and create complimentary sinergies with industry, to market new products based on bacterial cellulose.

Bacterial Cellulose Technologies



Bacterial Cellulose Technologies

Fibrous Tofu

Fibrous Falafel

"Mousse" (milk/yogurt)

Low calorie fibrous Gummies

Fibrous Meat Burger

Novel Food Regulation

(EC) No 258/97: defines "**novel food**" as a food or food ingredient that does not have a significant history of consumption within the European Union before 15 May 1997.

Novel Food Additives

(EC) No 1331/2008, Article 3(2)(a): defines "food additives" as "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods".

Bacterial Cellulose Technologies

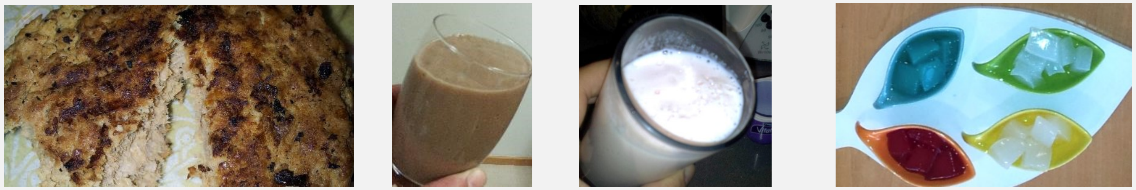
Industrial BNC Production

Biomedical applications

Electronics

Footwear (BNC eco-leather)

Food pplications



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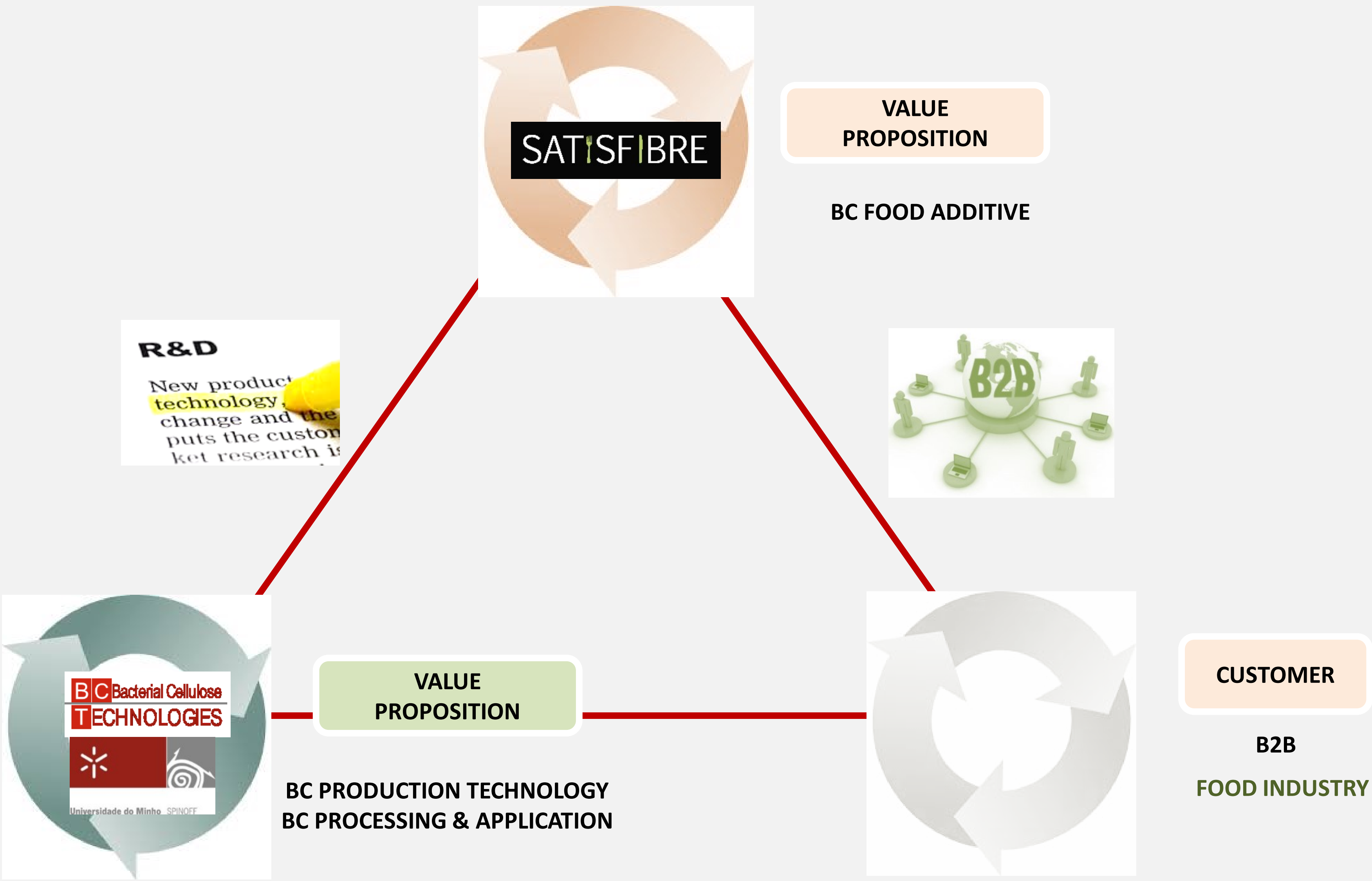
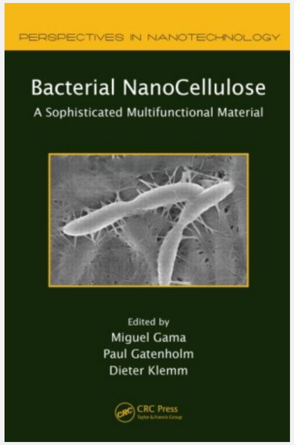
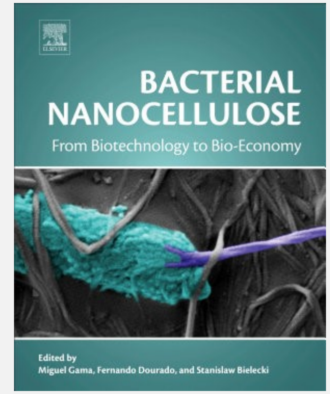
Bacterial Cellulose Technologies





Miguel Gama, Paul Gatenholm, Dieter Klemm
CRC Press, 2012



Francisco Gama Fernando Dourado Stanislaw Bielecki
Elsevier, 2016

1st International Symposium on Bacterial NanoCellulose,
New Orleans, USA

2nd International Symposium on Bacterial NanoCellulose,
Gdańsk, Poland

Thank you for your attention