Development of biodegradable PLA/POSS nanocomposites to be used in regenerative medicine as cartilage repair scaffolds

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General motivation of the project

- CARTILAGE injury : also known as ‘THE BEGINNING OF THE END OF A KNEE’

- Elderly people: Quality of life improves=> life expectancy increases!!
  - More elderly people
  - Increment of cardio-vascular and traumatologic diseases.

- Sports: football, basketball, ski…
  - Huge competivity in a powerful market
  - New techniques are needed
    - Decrease the recovery period
    - Guarantee the succes of the treatment

Scaffolding

Gianluigi Buffon, Juventus keeper. He has severe cartilage problems.

Outline

Materials tested for projects

- PLA(*polylactic acid*)/POSS(*polyoligomeric silsesquioxane*) and PLLA (*poly-L-lactic acid*)/POSS
- PHB/Tocopherol (*poly*-3-hydroxybutyrate)
- PHB/PANI (*Polyaniline)*
- PHB - Biological test

EXPERIMENTS

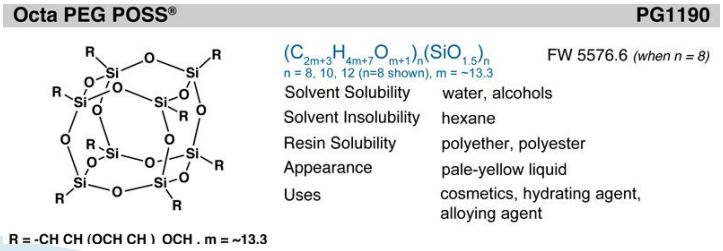
Sample No.	Material	Addition	Conc. of addition w/w %	Solvent	Target type	Voltage	Distance	Pump speed	Comments
CG 6	P(3HB)	Tocopherol	10,00%	TFE	Rotatory drum	10 kV	15cm	0,4ml/h	
CG3	P(3HB)	Tocopherol	10,00%	TFE	Rotatory drum	15 kV	15cm	0,4ml/h	
CG2	P(3HB)	Tocopherol	10,00%	TFE	Rotatory drum	20 kV	15cm	0,4ml/h	
CG7	P(3HB)	Tocopherol	10,00%	TFE	Stable target	10 kV	15cm	0,4ml/h	
CG8	P(3HB)	Tocopherol	10,00%	TFE	Stable target	15 kV	15cm	0,4ml/h	
CG9	P(3HB)	Tocopherol	10,00%	TFE	Stable target	20 kV	15cm	0,4ml/h	
CG13_10	P(3HB)	Tocopherol	5,00%	TFE	Stable target	10 kV	15cm	0,4ml/h	
CG13_15	P(3HB)	Tocopherol	5,00%	TFE	Stable target	15 kV	15cm	0,4ml/h	
CG13_20	P(3HB)	Tocopherol	5,00%	TFE	Stable target	20 kV	15cm	0,4ml/h	
CG25_10	P(3HB)	-	0,00%	TFE	Stable target	10 kV	15cm	0,4ml/h	
CG25_15	P(3HB)	-	0,00%	TFE	Stable target	15 kV	15cm	0,4ml/h	
CG25_20	P(3HB)	-	0,00%	TFE	Stable target	20 kV	15cm	0,4ml/h	
CG4	P(3HB)	Tocopherol	10,00%	Chloroform	-	-	-	-	Failed! Bottle exploded into the heater at 55C
CG5	P(3HB)	Tocopherol	10,00%	Chloroform	-	-	-	-	Failed! Bottle exploded into the heater at 45C
CG10	P(3HB)	Tocopherol	10,00%	Chloroform	-	-	-	-	Failed! Without heating, not dissolved completely.
CG1 / TJK8	PLA Biome	-	-	Chloroform+DMF	-	-	-	-	Just to check the solubility
CG11	PLA Nature	-	0,00%	Chloroform+DMF	Copper grid	TEST 15KV	20cm	TEST 0,8m	Search the best parametres (pump speed, voltage)
CG12	PLA Nature	PEG-POSS	10,00%	Chloroform+DMF	Copper grid	TEST 15KV	20cm	TEST 0,8m	Search the best parametres (pump speed, voltage)
CG14	PLA Nature	-	0,00%	Chloroform+DMF	Rotatory drum	15 kV	20cm	0,8ml/h	
CG15	PLA Nature	PEG-POSS	0,50%	Chloroform+DMF	Rotatory drum	15 kV	20cm	0,8ml/h	
CG16	PLA Nature	PEG-POSS	1,00%	Chloroform+DMF	Rotatory drum	15 kV	20cm	0,8ml/h	
CG17	PLA Nature	PEG-POSS	2,50%	Chloroform+DMF	Rotatory drum	15 kV	20cm	0,8ml/h	
CG18	PLA Nature	PEG-POSS	10,00%	Chloroform+DMF	Rotatory drum	15 kV	20cm	0,8ml/h	
CG18_sh	PLA Nature	PEG-POSS	10,00%	Chloroform+DMF	Rotatory drum	15 kV	20cm	0,8ml/h	A shield was used to avoid fibers stick out of the target
CG19	PLA Nature	PEG-POSS	5,00%	Chloroform+DMF	Rotatory drum	15 kV	20cm	0,8ml/h	
CG20	PLA Nature	PEG-POSS	7,50%	Chloroform+DMF	Rotatory drum	15 kV	20cm	0,8ml/h	
CG21	PLA Nature	-	-	50/50 Chloroform+D	-	-	-	-	Failed! Not dissolved.
CG22	3% PLA Na	-	-	TFE	Copper grid	TEST	10/15/20cm	0,4ml/h	Failed! Electrospray! The PLA concentration has to be raised up to 6 or 12%.
CG23	P(3HB)	-	-	TFE	-	-	-	-	Not enough solution to do the spinning.
CG24	PLA Nature	-	-	75/25 Chloroform+D	Copper grid	TEST	-	-	Fibers.
CG24_B	PLA Nature	-	-	53/47 Chloroform+D	Copper grid	TEST	-	-	Failed! Not fibers.
CG26	6% PLA Na	-	-	TFE	Copper grid	TEST	20cm	0,4ml/h	Failed! There are droplets mixed with the fibers.
CG27	12% PLA N	-	-	TFE	Stable target	15 kV	20cm	0,4ml/h	
CG28	PLA Nature	-	-	50/50 Chloroform+D	-	-	-	-	Failed! It was like a GEL. The PLA and the Chloroform were mixed first, and after the DMF was added.
CG29	12% PLA N	PEG-POSS	1,00%	TFE	Stable target	15 kV	20cm	0,4ml/h	
CG30	12% PLA N	PEG-POSS	5,00%	TFE	Stable target	15 kV	20cm	0,4ml/h	
CG31	12% PLA N	PEG-POSS	10,00%	TFE	Stable target	15 kV	20cm	0,4ml/h	
CG32_10	12% PLA N	-	-	TFE	Stable target	15 kV	20cm	0,4ml/h	Collection time 10 minutes
CG32_20	12% PLA N	-	-	TFE	Stable target	15 kV	20cm	0,4ml/h	Collection time 20 minutes
CG32_30	12% PLA N	-	-	TFE	Stable target	15 kV	20cm	0,4ml/h	Collection time 30 minutes

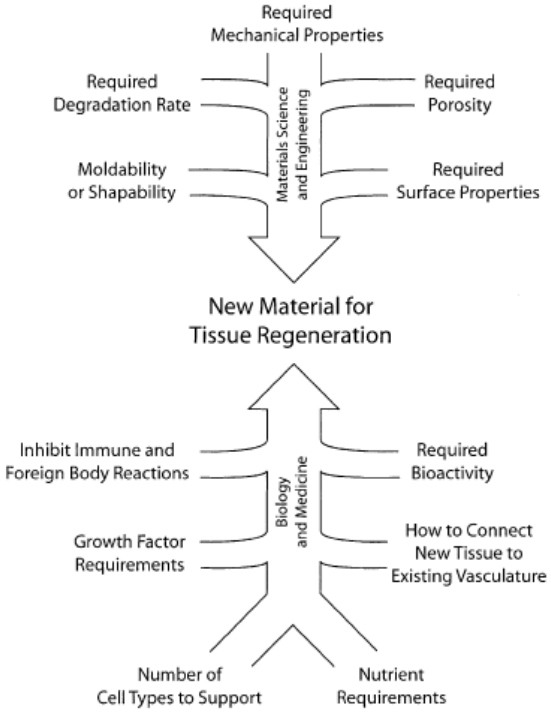
EXPERIMENTS

CG33	12% PLA N	-	-	TFE					
CG33_BC	12% PLA N	-	-	TFE	Water spinning	10 kV	15cm	0,4ml/h	Center. Collection time 25 minutes
CG33_BM	12% PLA N	-	-	TFE	Water spinning	10 kV	15cm	0,4ml/h	Medium. Collection time 25 minutes
CG33_BO	12% PLA N	-	-	TFE	Water spinning	10 kV	15cm	0,4ml/h	Out. Collection time 25 minutes
CG34	Film PLA Natureworks			Chloroform	-	-	-	-	Film made by casting
CG35	Film PLA N	PEG-POSS	1,00%	Chloroform	-	-	-	-	Film made by casting
CG36	Film PLA N	PEG-POSS	2,50%	Chloroform	-	-	-	-	Film made by casting
CG37	Film PLA N	PEG-POSS	5,00%	Chloroform	-	-	-	-	Film made by casting
CG38	Film PLA N	PEG-POSS	10,00%	Chloroform	-	-	-	-	Film made by casting
CG39	Film PLA N	Amino-POS	1,00%	Chloroform	-	-	-	-	Film made by casting
CG40	Film PLA N	Amino-POS	2,50%	Chloroform	-	-	-	-	Film made by casting
CG41	Film PLA N	Amino-POS	5,00%	Chloroform	-	-	-	-	Film made by casting
CG42	Film PLA N	Amino-POS	10,00%	Chloroform	-	-	-	-	Film made by casting
CG43	12% PLLA	-	-	TFE	Copper grid	TEST	20cm	-	Failed! Droplets and fibers! The PLLA concentration must be raised up to 6 or 12%.
CG44	PLLA Pura	-	-	Chloroform+DMF	Stable target	15 kV	20cm	0,4ml/h	
CG45	PHB+PANI	-	-	TFE	Copper grid	TEST	-	-	Failed! Electrospray!
CG46	PHB+PANI	-	-	6%DMF	Copper grid	TEST	-	-	Failed! Electrospray!
CG47_A	P(3HB)	-	-	TFE	Stable spinnin	15 kV	15cm	0,4ml/h	10 min each side.
CG47_50R	P(3HB)	-	-	TFE	Rolling in meta	15 kV	15cm	0,4ml/h	50 min.
CG53_60R	P(3HB)	-	-	TFE	Rolling in meta	15 kV	15cm	0,4ml/h	60 min.
CG53_B	P(3HB)	-	-	TFE	Stable spinnin	15 kV	15cm	0,4ml/h	8 min.
CG53_C	P(3HB)	-	-	TFE	Rolling, new se	15 kV	15cm	0,4ml/h	2 x 10 min.
CG53_D	P(3HB)	-	-	TFE	Rolling, new se	15 kV	15cm	0,4ml/h	2 x 10 min.
CG53_E	P(3HB)	-	-	TFE	Rolling, new se	15 kV	15cm	0,4ml/h	2 x 10 min.
CG53_F	P(3HB)	-	-	TFE	Rolling, new se	15 kV	15cm	0,4ml/h	2 x 10 min.
CG53_WA	P(3HB)	-	-	TFE	Water spinning	15 kV	15cm	0,4ml/h	30 min.
CG53_WB	P(3HB)	-	-	TFE	Water spinning	15 kV	15cm	0,4ml/h	30 min.
CG53_WC	P(3HB)	-	-	TFE	Water spinning	15 kV	15cm	0,4ml/h	30 min.
CG53_WD	P(3HB)	-	-	TFE	Water spinning	15 kV	15cm	0,4ml/h	30 min.
CG53_W	P(3HB)	-	-	TFE	Water spinning	15 kV	15cm	0,4ml/h	30 min. Sample for the SEM
CG48	5% PHBV	-	-	Chloroform					Just to check the solubility
CG49	12% PHBV	-	-	Chloroform					Just to check the solubility
CG50	PHB	-	-	Chloroform					Just to check the solubility
CG52	2% PHBV	-	-	Chloroform					Just to check the solubility
CG51	Film PLA Natureworks		-	Chloroform	-	-	-	-	Film made by casting
CG54	PLLA Pura	PEG-POSS	1,00%	Chloroform+DMF	Stable target	15 kV	20cm	0,4ml/h	
CG55	PLLA Pura	PEG-POSS	2,50%	Chloroform+DMF	Stable target	15 kV	20cm	0,4ml/h	
CG56	PLLA Pura	PEG-POSS	5,00%	Chloroform+DMF	Stable target	15 kV	20cm	0,4ml/h	
CG57	PLLA Pura	PEG-POSS	10,00%	Chloroform+DMF	Stable target	15 kV	20cm	0,4ml/h	
CG58	PLA Nature	-	-	Chloroform+DMF	Water spinning	10-15 kV	15cm	0,4ml/h	To make the hybrid scaffold (casting+spinning). Water conductivity: 588µS. Spinning (10-15KV): 40 min
CG59	16% PLLA	-	-	TFE	Stable target	15 kV	20cm	0,4ml/h	
CG60	20% PLLA	-	-	TFE	Stable target	15 kV	20cm	0,4ml/h	

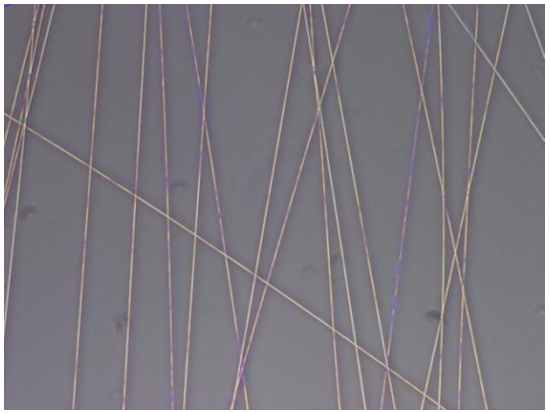
PLA/POSS and PLLA/POSS

- Aim: to develop a new biodegradable nanocomposite for cartilage tissue regeneration
- Materials:
  - PLA (Natureworks)
  - PLLA (Purac)
  - PEG-POSS (Hybrid plastics)
- Process methods:
  - Casting
  - Electrospinning
  - Injection
- Cells: H.M.S.C. differentiated in Chondrocytes (cartilage)
- Why electrospinning ?
  - The most succesful manufacturing method according to the cell culture
- Why PEG-POSS?
  - PEG is a biodegradable polymer
  - POSS is a nonbiodegradable nano-reinforcement expected to change thermical, mechanical and phisical properties of the composite.

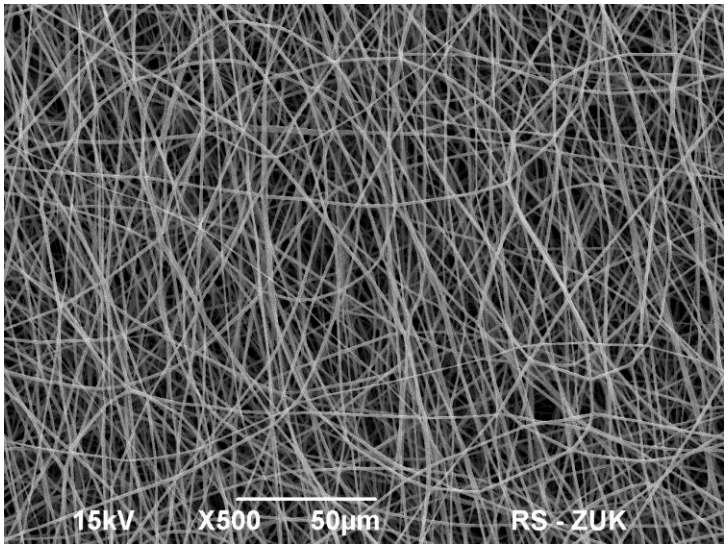


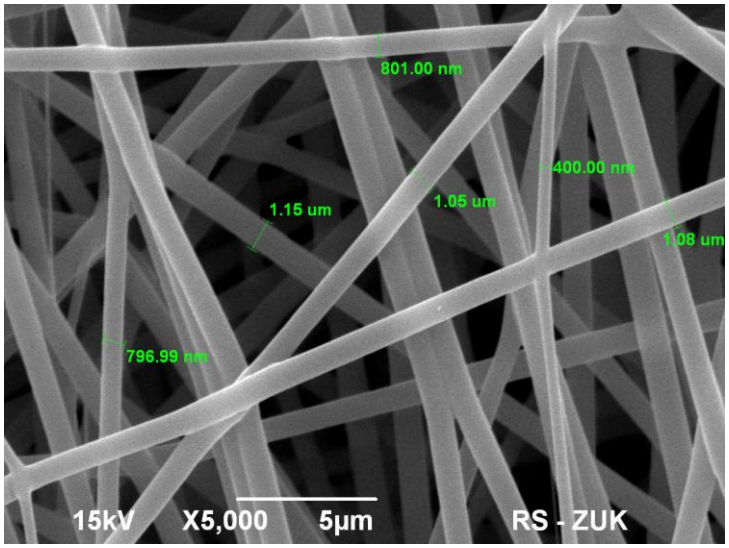


RESULTS



PLA 15KV fibers seen at optical microscope at 10x



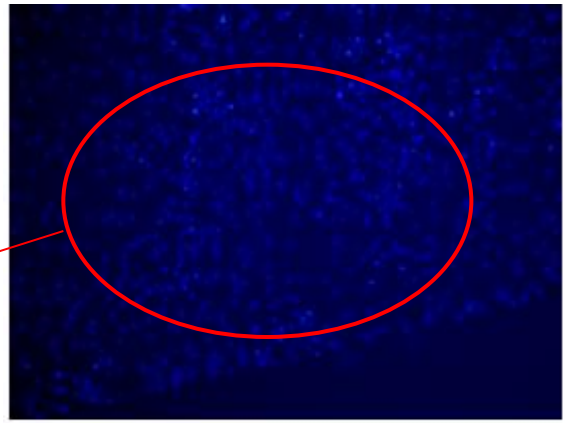


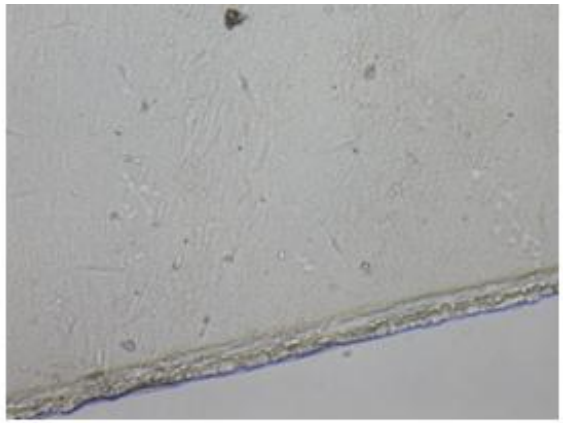
PLA/10%PEG-POSS nanofibers seen at SEM. Left: 500x. Right: 5000x and diameter measured

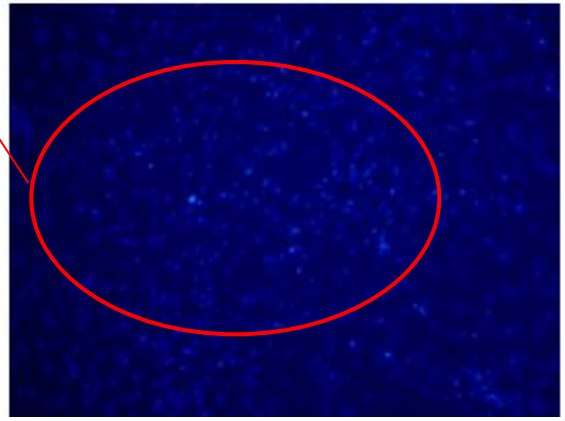
CELL CULTURE

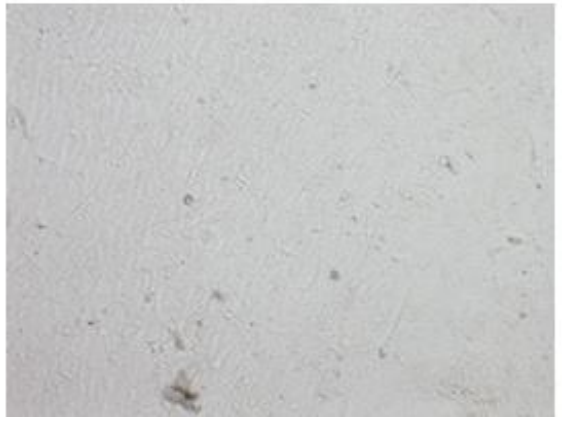
- Results of the H.M.S.C. culture after 2 weeks
  - Sample made by **CASTING** method

Homogeneus cell concentration







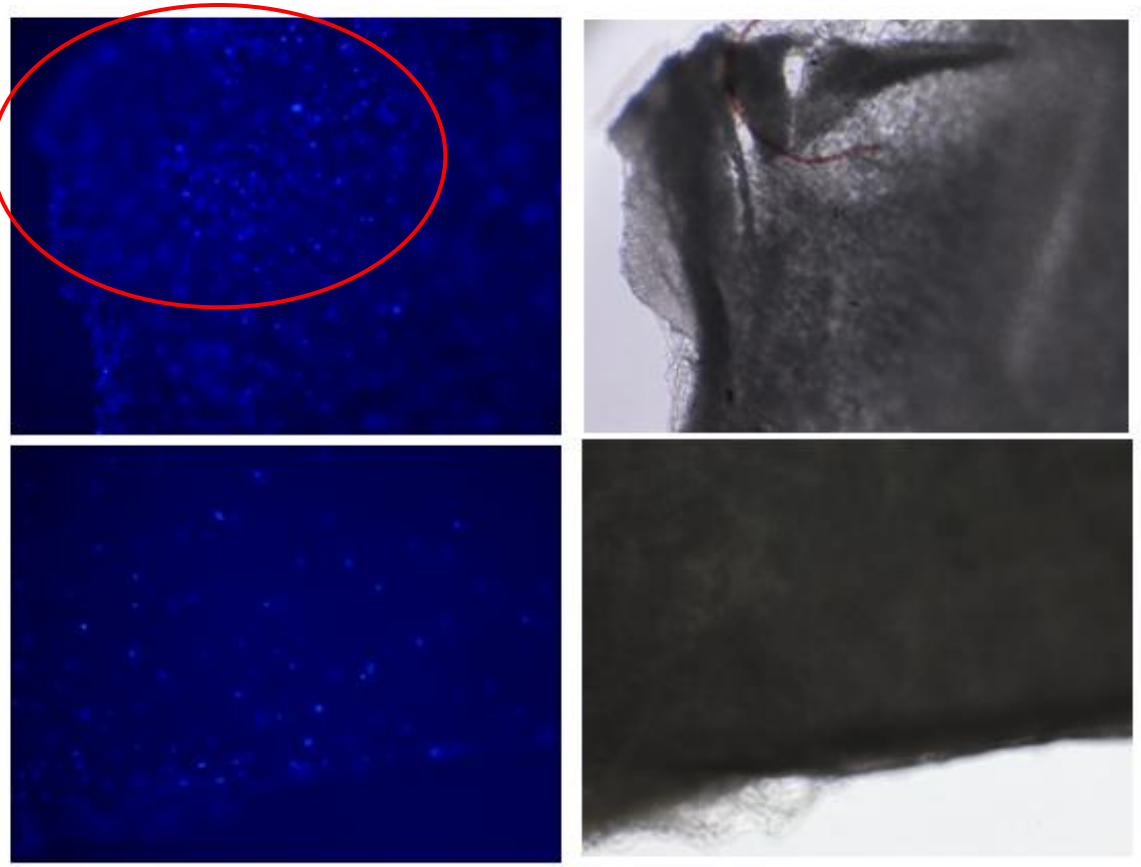


H.M.S.C.: Human Mesenchimal Stem Cells

CELL CULTURE

- Results of the H.M.S.C. culture after 2 weeks
  - Sample made by **electrospinning** method

High cell concentration level zones



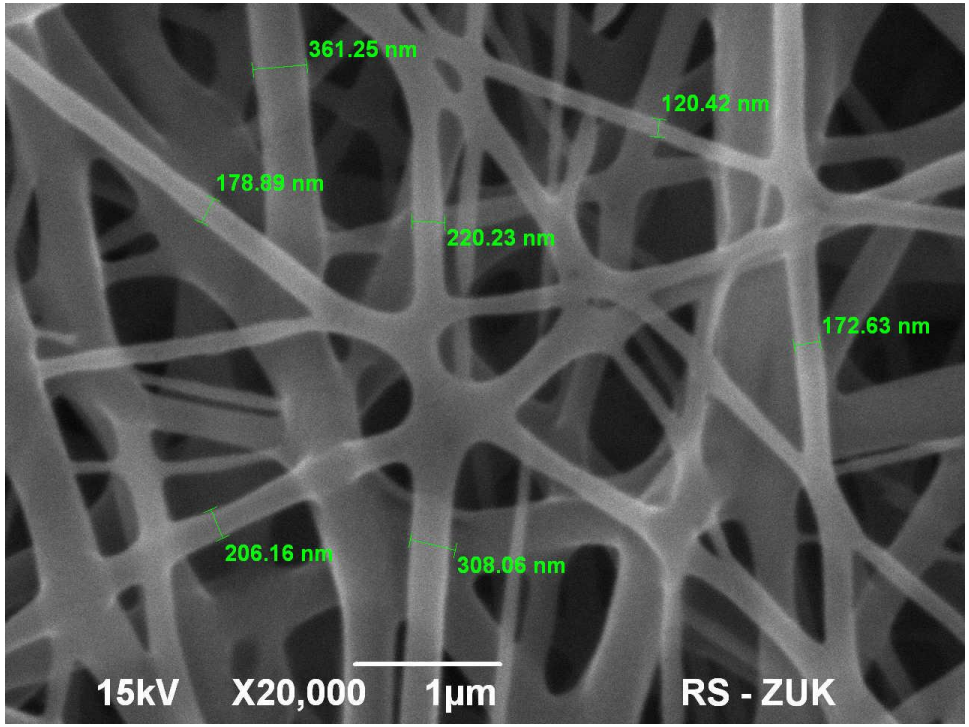
PHB/Tocopherol

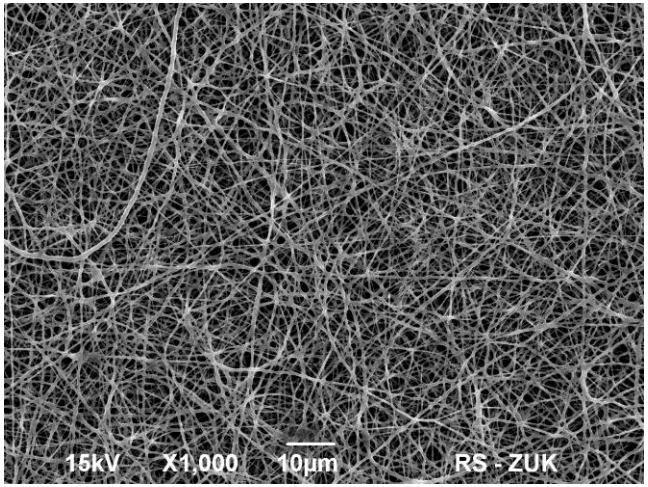
- Aim: to improve the biological properties of scaffolds for bone regeneration using an antioxidant
- Materials:
  - PHB
  - α- Tocopherol (Vitamin E)
  - Bioglass (Main component of the scaffold to fill the gap in the bone), to be packed with the nanofiber net.
- Process method:
  - Electrospinning
- Cells: Osteoblasts (bone)

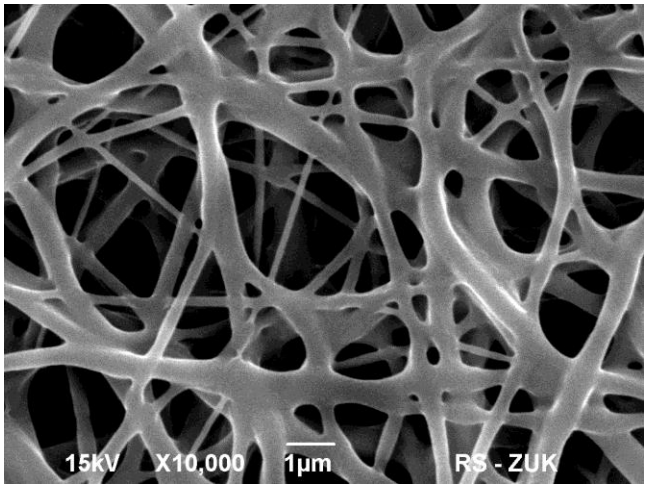
- Why α- Tocopherol ?
  - It is an antioxidant that helps osteoblast cells to grow.
  - Cells don't like Bioglass. Vitamin E covers these particles.

α- Tocopherol (Vitamin E)

RESULTS







PHB/10%Tocopherol nanofibers seen at SEM. Left: 20000x and diameter measured. Right up: 1000x. Right down: 10000x.

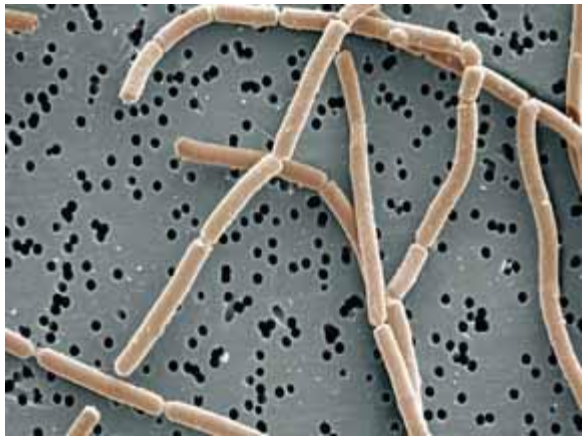
PHB/PANI

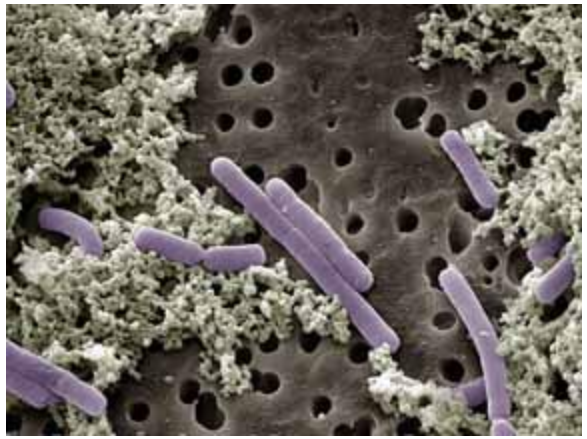
- Aim: to develop scaffolds for nerve regeneration
- Materials:
  - PHB
  - PANI solution (PANI+DBSA+Chloroform)
- Process method:
  - Electrospinning
- Cells: Schwann cells, astrocites, neurones
- Aplications: spine injuries, any other nerve diseases

- Why **PANI**?
  - The PANI is a conductive polymer, suitable for applications where nerve impuls must be transmited.

PHB – Biological test

- **Aim**: to manufacture scaffolds to keep bacteries encapsulated as drug delivery system in cncer therapy
- **Materials**:
  - **PHB**
- **Process method**:
  - **Electrospinning**
- **Bacteries**: lactobacillus bulgaricus (yoghourt)
- **Method**: Some tubular scaffolds were made and bacteries were placed inside





Lactobacillus bulgaricus

SUMARY AND FUTURE CONSIDERATIONS

- About 80 types of materials were produced and analized
- 4 different projects done
- The materials are to be used for biological tests
- The cells tests will give the answer which method of production was the most suitable

THANK YOU FOR ATTENTION !!!