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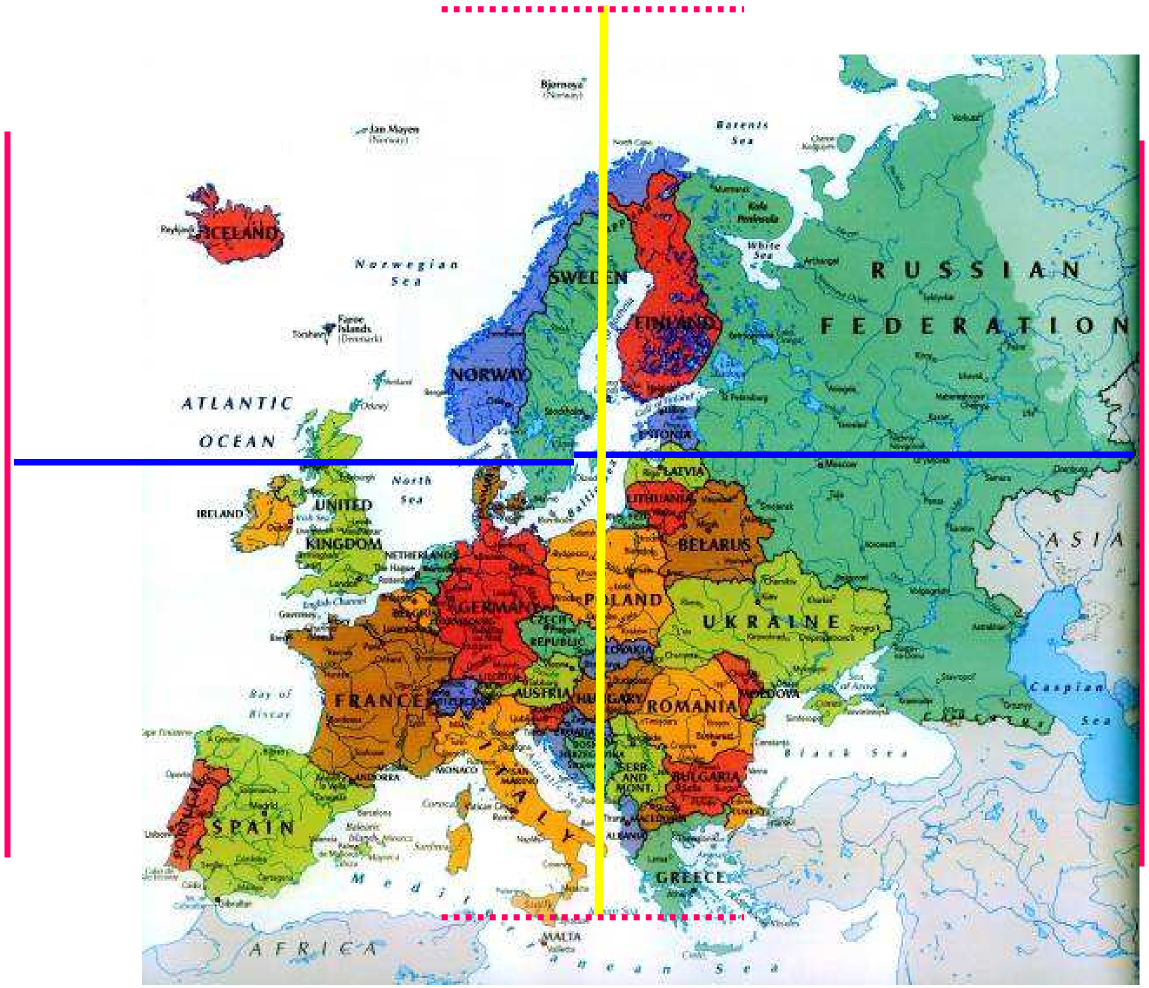
Electrospun materials for bone tissue engineering

PhD. Erika Adomavičiūtė

Kaunas University of Technology, Faculty of Mechanical Engineering and Design, Department of Materials Science

Welcome in the centre of Europe!



According to the French experts in cartography, the geographical centre of Europe is in Lithuania, close to Vilnius (capital of Lithuania).

Lithuania in Europe

Neighbouring states: Latvia, Belarus, Poland, and Russia

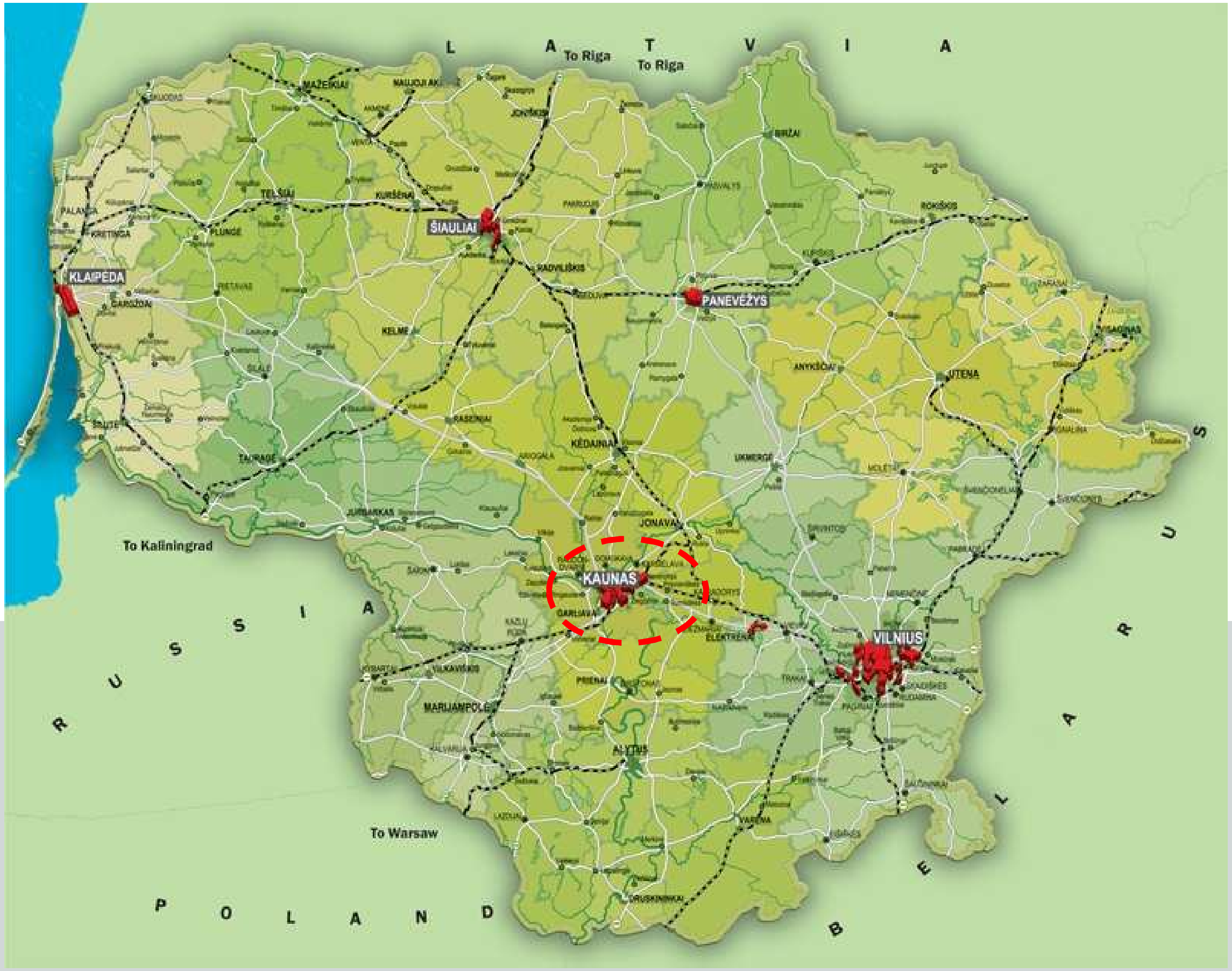
Lithuania is a bridge between East and West, North and South!

Basketball Second Religion

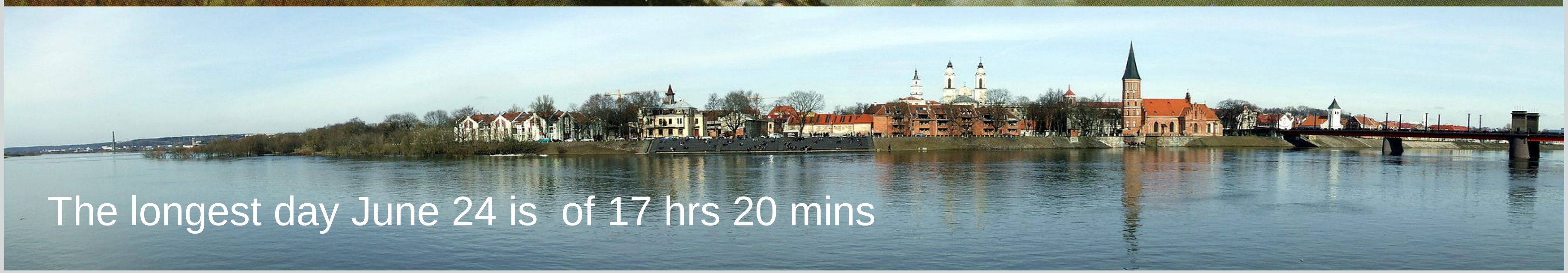
Lithuanian men's basketball team won silver medals won in the European Basketball Championship 2015.

Kaunas

Population in Kaunas ~ 309,000 people (2013)



Kaunas in Summer



Kaunas in Winter

The shortest day December 24 is of 7 hrs 14 mins

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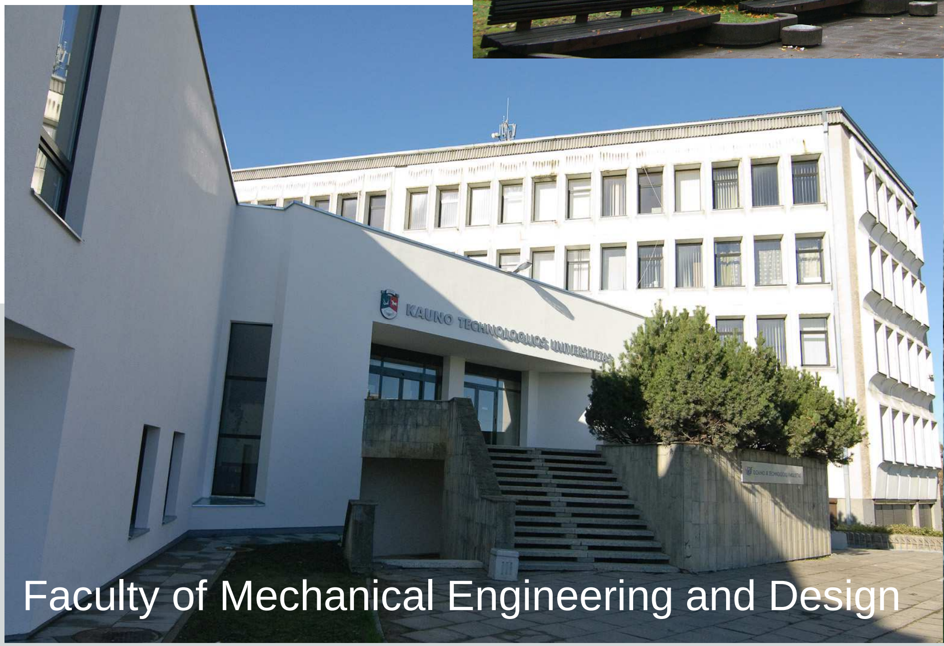
Kaunas University of Technologies (KTU)



Faculty of Mechanical Engineering and Design

KTU Integrated Science, Studies and Business Centre

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KTU Community 2014

11 000
students

1 000
academic staff

775
international students

2 555
graduates

330
PhD students

133 000
alumni

KTU Research AREAS

[redacted]

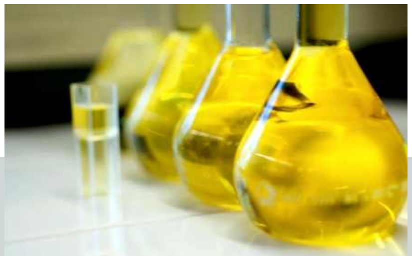
Diagnostic and measurement technologies

[redacted]

New materials for high-tech

[redacted]

Smart environments and information technology



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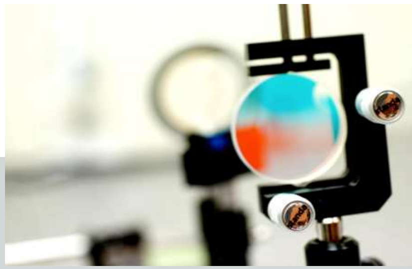
Technologies for sustainable development and energy



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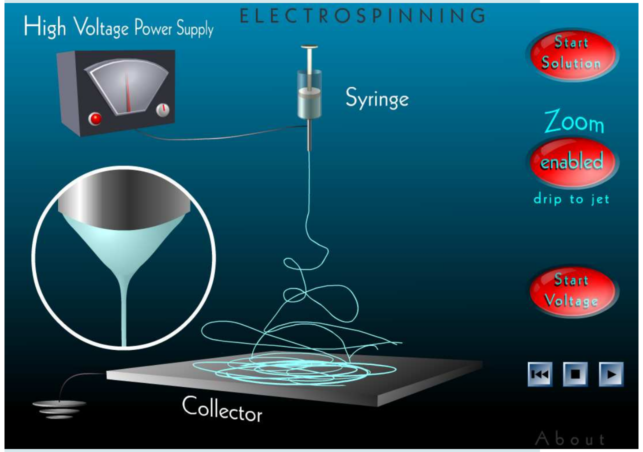
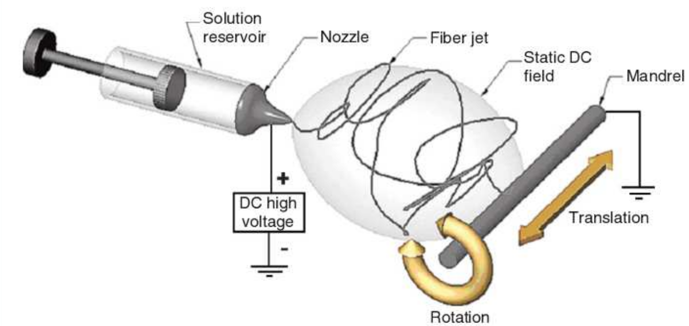
Sustainable growth and social-cultural development

What it is electrospinning?

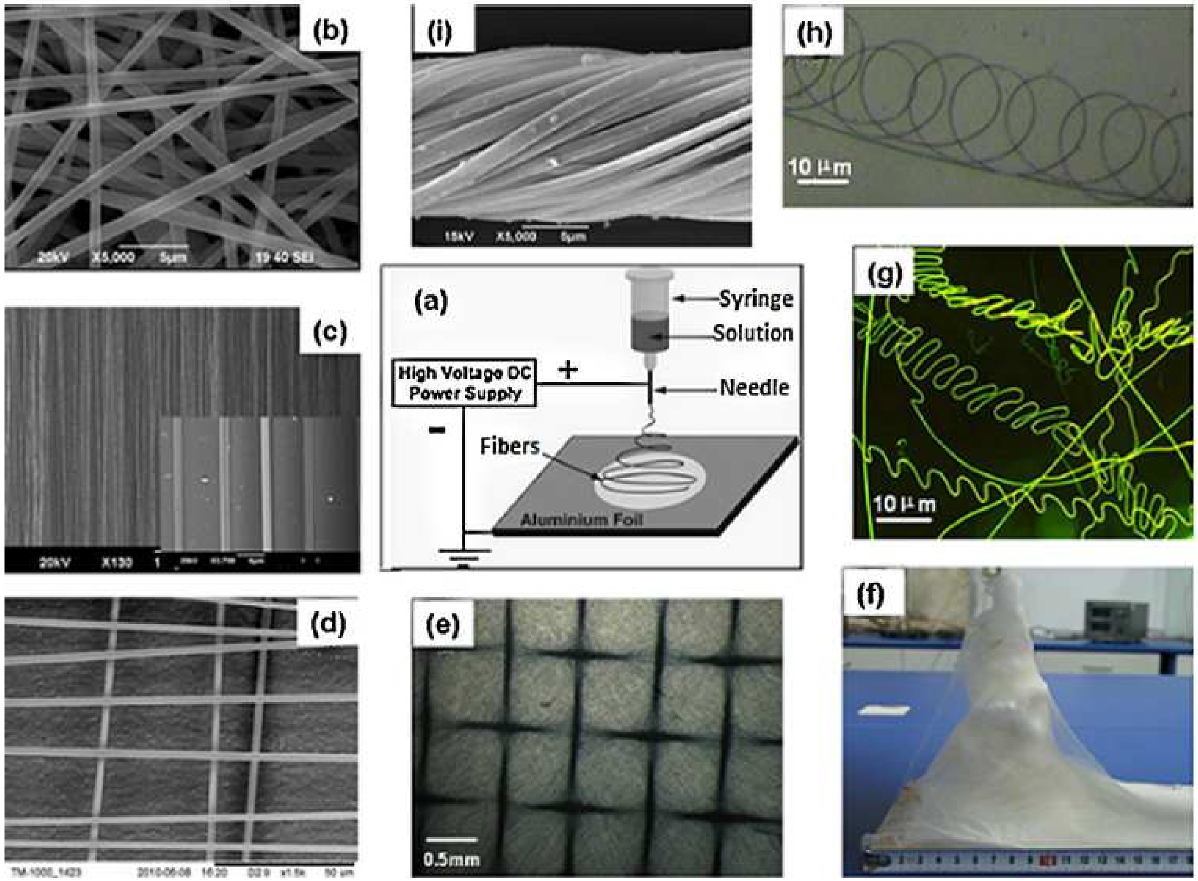


http://nano.mtu.edu/Electrospinning_start.html



Electrospining is a fiber spinning process of generating ultrafine fibers in the nanometer to micrometer scale.

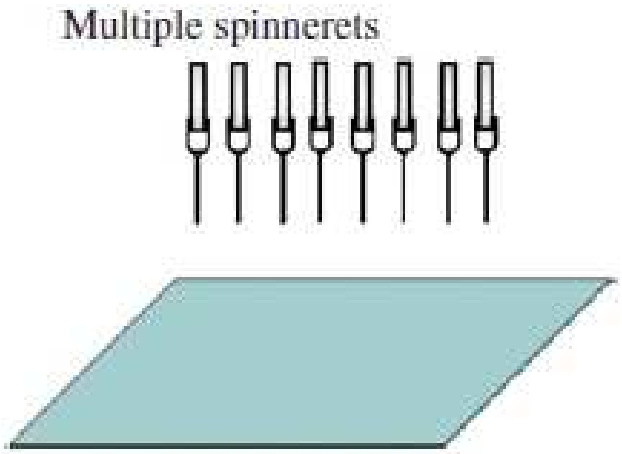
In electrospinning process an electric field is generated between an oppositely charged polymer fluid an a collection of electrode. In mostly cases a polymer solution is added to a glass syringe with capillary tip. An electrode is placed in the solution with another connection made to metal screen. As a power is increased, the charged polymer solution is attracted to scream. Once the voltage reaches a critical value the charge overcomes the surface tension of polymer cone formed on the capillarity tip of the syringe and a jet of ultrafine fibers is oduced. [P.J. Brown and etc. Nanofibers and nanotechnology in textiles]



Electrospun materials

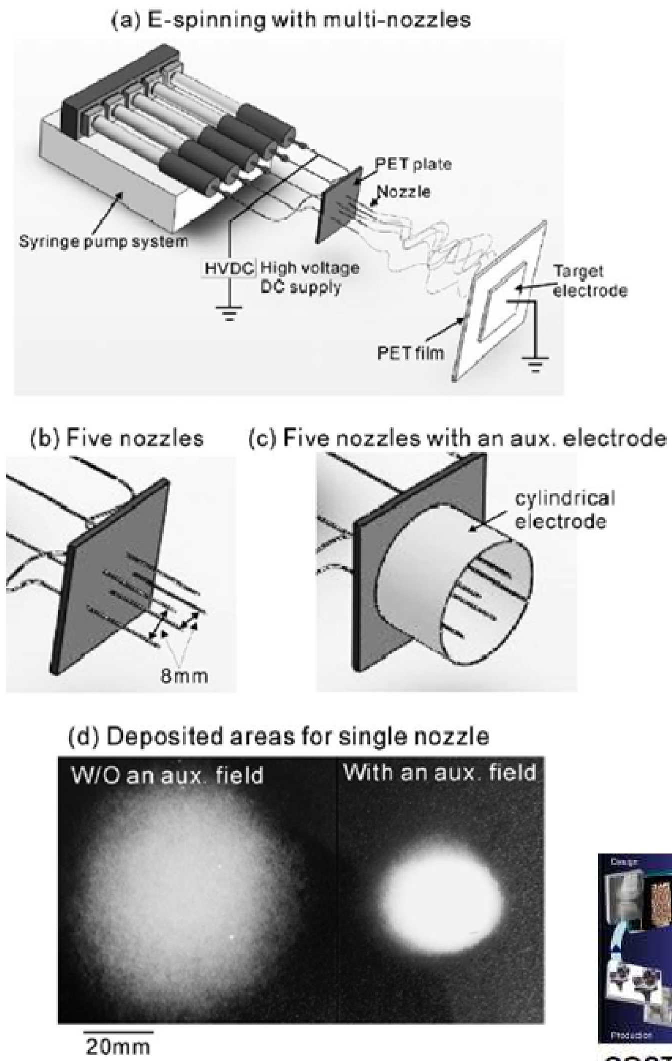
B. Sun with co-authors „Advances in three dimensional nanofibrous macrostructures via electrospinning“ *Progress in Polymer Science*

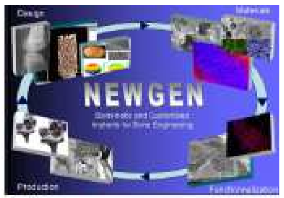
Electrospinning equipments



Electrospinning setup for mass production of electrospun nonwoven mats

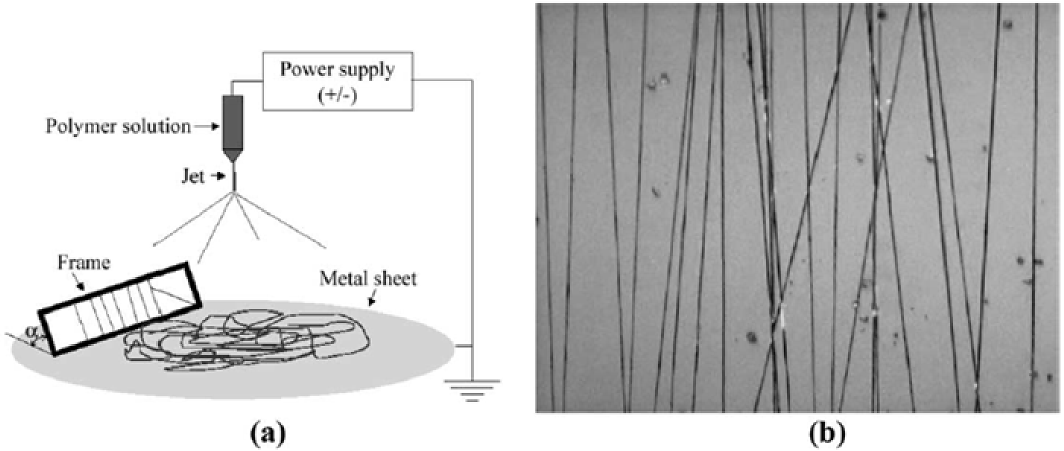
INSTITUTE OF PHYSICS PUBLISHING
Nanotechnology 17 (2006) R89–R106



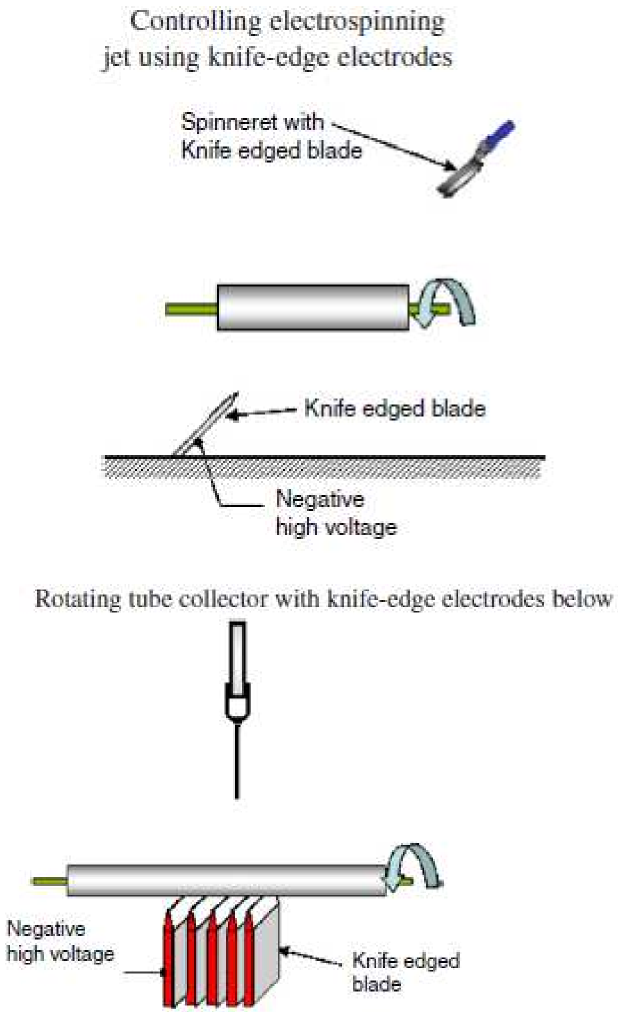


COST Action MP1301

Electrospinning equipments

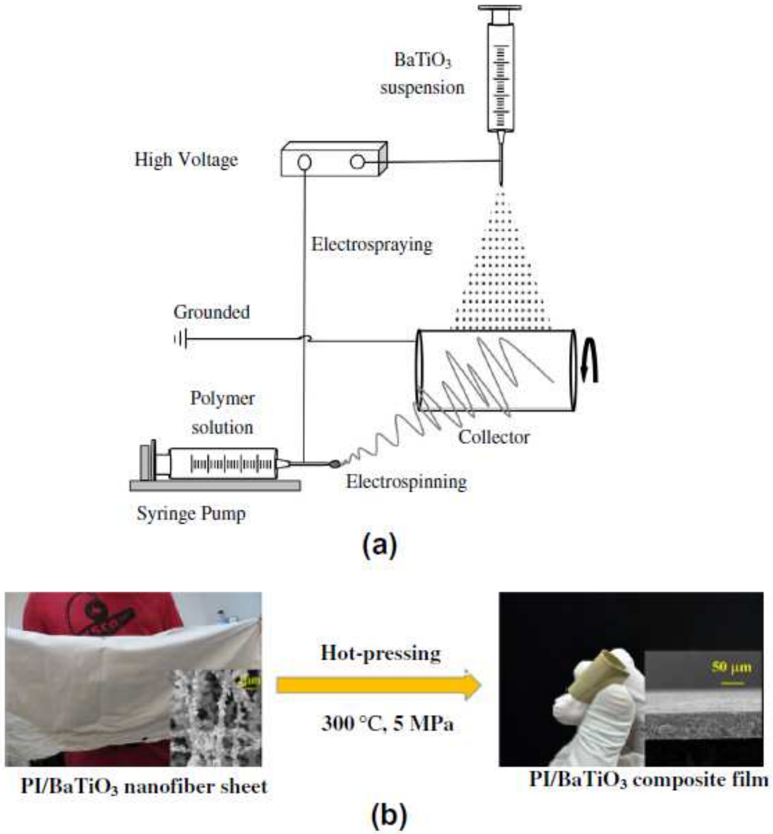


Electrospinning setup for aligned fibres

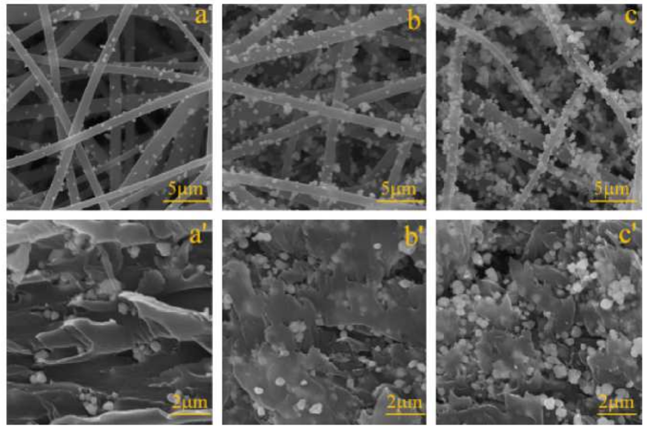


INSTITUTE OF PHYSICS PUBLISHING
Nanotechnology 17 (2006) R89–R

Electrospinning equipments



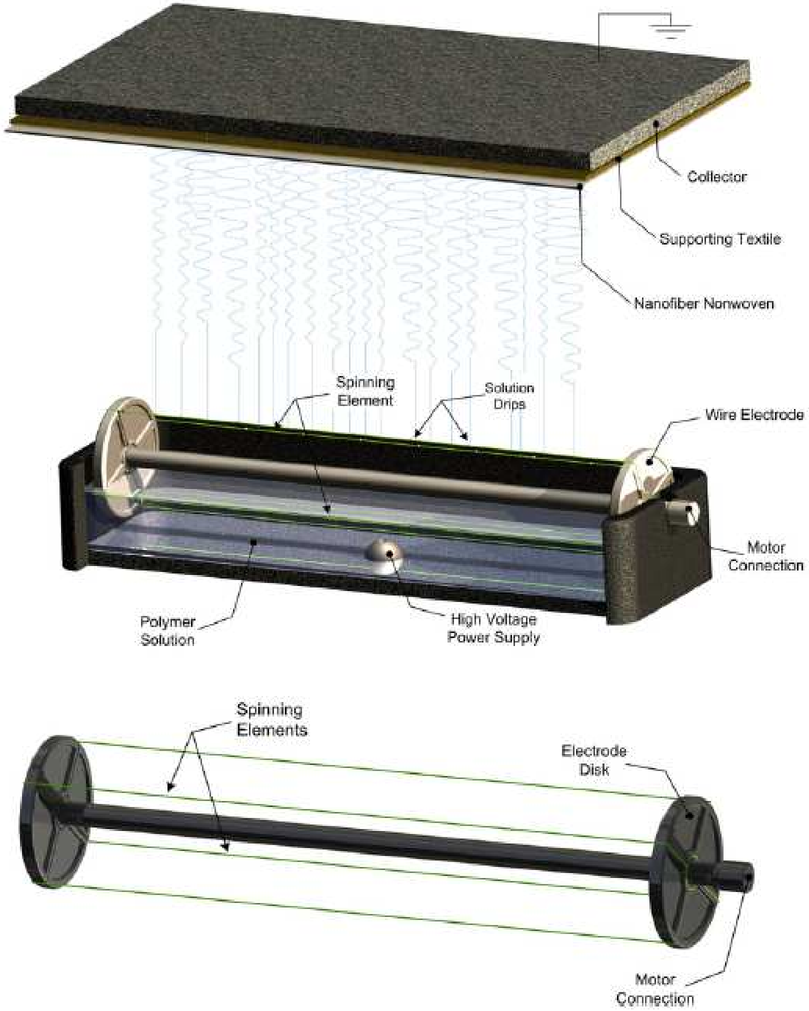
Electrospinning and electrospraying processes



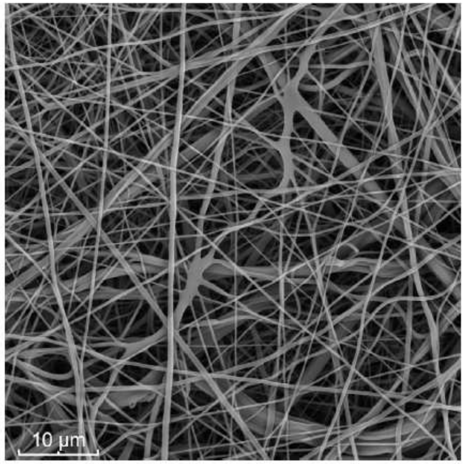
The SEM images of PI/$BaTiO_3$ composite nanofibrous (a–c) and cross-section of the composites (a′–c′) with $BaTiO_3$ content of 10 vol% (a), 30 vol% (b), 50 vol% (c).

Y.Ding European Polymer Journal 49(2013)2567-2571

Electrospinning equipments



Scheme of the electrospinning process (at the top) using rotating electrode with cotton cord spinning elements (at the bottom).



SEM image of the PUR nanofiber mat produced by electrospinning process.

W. Sambaer et al. / Chemical Engineering Science 66 (2011) 613–6.

Why electrospinning in scaffold formation?

Tissue engineering scaffolds should have the following characteristics:

- Porosity for cell migration;
- Balance between surface hydrophilicity and hydrophobicity for cell attachment;
- Mechanical properties comparable to natural tissue to withstand natural loading conditions;
- Degradation capability so that it gets completely reabsorbed after implantation;
- Nontoxic byproducts;
- 3D matrix [P.J. Brown and etc. Nanofibers and nanotechnology in textiles]

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Why electrospinning in scaffold formation?

Electrospun nonwoven materials have unique characteristics:

- large specific surface area (up to 100 m 2/g);
- high porosity (up to 95%);
- diameter of fibers 10-1000 nm.

Due large surface area these materials is capable to absorbing fluids very efficiently; wide variety of size and shape of material may be electrospun.

By mimicking the size-scale of natural ECM components such as type I collagen, nanofibrous scaffolds provide an advantageous microenvironment that enhances cellular attachment, proliferation, and in some cases, promotes terminal differentiation of stem cells [J. Mater. Chem., 2010, 20, 8776–8788]

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Electrospun polymer for bone tissue engineering

Table 1. Summary of electrospun nano-microfibers system produced for the bone reconstruction

[J-H Jang et. Al/Advanced Drug Delivery Reviews 61 (2009) 1065-1083]

Composition		Fiber diameter	Assays	Remarks
Synthetic polymers	PLA (*L*- and *DL*-type)	141–2140 nm	MC3T3-E1	Effect of osteogenic factors and fiber size
	PCL	20–5000 nm	BMSC, *in vivo* (rat)	Tissue engineering
	PHB, PHBV, blend	2300–4000 nm	SaOS-2 & L929	
Natural polymers	Collagen I	50–1000 nm	hMSC	
	Chitosan	200 nm	MG63, *in vivo* (rabbit)	Bone formation at 4 weeks
	Silk fibroin	217–610/183–810 nm	MC3T3-E1	
	Silk fibroin	700 nm	BMSC	Poly(ethylene oxide) (PEO) addition
Polymer blends	PCL–gelatin	tens of nm–1000 nm	BMSC	Cell penetration with gelatine addition
	PLLA–gelatin	190–390 nm	MC3T3-E1	Enhanced cell responses on blends
	PCL–heparan sulfate	–	BMSC	Osteogenic differentiation
Inorganics	Bioactive glass	84–630 nm	Production, bone bioactivity, rBMSC	Excellent bone bioactivity and BMSC responses
	Bioactive glass	320 nm	Production, osteoblast adhesion	FN-introduction, enhanced cell adhesion
	Hydroxyapatite and fluoro-hydroxyapatite	240–1550 nm	Production, dissolution	Reduced dissolution by fluorine addition
	Hydroxyapatite	10,000–50,000 nm	Production	Microfibers
	Hydroxyapatite	200–500 nm	Processing	
	Silicate	–	*In vitro* (MG63)	Apatite forming ability
Composites/hybrids	Gelatin–hydroxyapatite	200–400 nm	Production, osteoblasts	Enhanced osteoblastic differentiation
	Collagen–hydroxyapatite	75–160 nm	Production, osteoblasts	
	Chitosan–hydroxyapatite	~214 nm	hFOB	PEO addition
	PCL–$CaCO_3$	760–900 nm	Mechanical test, *in vitro* (hFOB)	GBR membrane application
	PLLA–hydroxyapatite	~1000–2000 nm	Production, MG63	Surfactant introduction
	Siloxane–gelatin	40 to 670 nm	Production, MC3T3-E1	Hybridized structure, Ca requirement
	PCL–HA–collagen	~189–579 nm	hFOB	
	PCL–βTCP	200–2000 nm	Osteoblast responses	Better cell adhesion due to βTCP
Surface functionalized	PCL	~250 nm	Production, osteoblasts, PDL fibroblasts	Apatite mineralized, higher osteogenic responses
	PLLA	200–2200 nm	Production	NaOH-treatment
	PDLLA	–	Production	$Ca(NO_3)_2$ addition
	PLLA, PLLA–collagen	287–364 nm	hFOB	Mineralization with collagen
	PLGA, PLGA–PEG	–	Fibroblast adhesion	Amination, RGD-immobilization
Drug/gene delivery	PLA, PCL	–	Antibacterial effects	Antibiotic delivery

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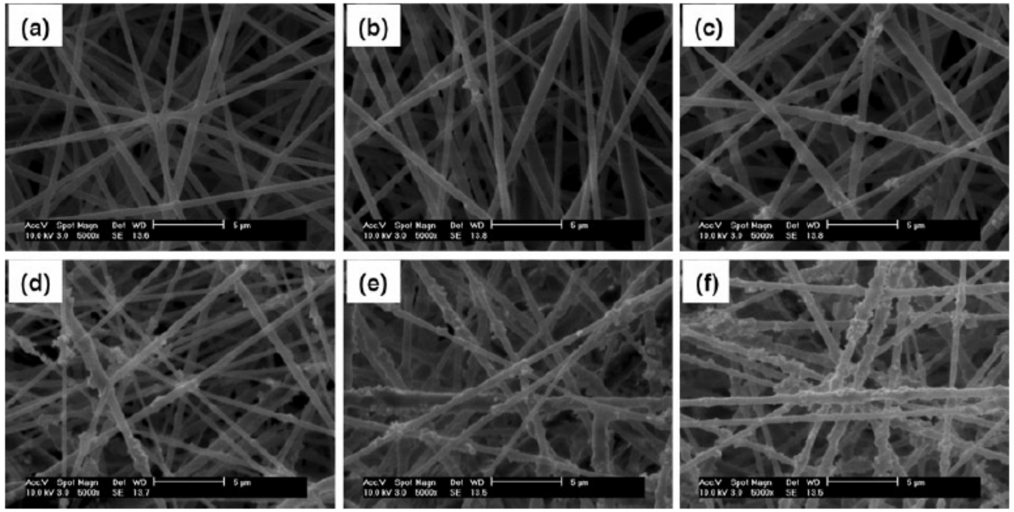
Poly(lactide-co-glycolide)/hydroxyapatite nanofibrous scaffolds fabricated by electrospinning for bone tissue engineering

Lihong Lao, Yingjun Wang, Yang Zhu,
Yuying Zhang & Changyou Gao

J Mater Sci: Mater Med (2011) 22:1873–1884
DOI 10.1007/s10856-011-4374-8



SEM images a) PLGA; b) PLGA/0,5 Hap; c) PLGA/2.5 Hap; d) PLGA/5 Hap; e) PLGA/10HAp and f) PLGA/15HAp nanofibrous scaffolds

J Mater Sci: Mater Med (2011) 22:1873–1884
DOI 10.1007/s10856-011-4374-8

COST Action MP1301

Poly(lactide-co-glycolide)/hydroxyapatite nanofibrous scaffolds fabricated by electrospinning for bone tissue engineering

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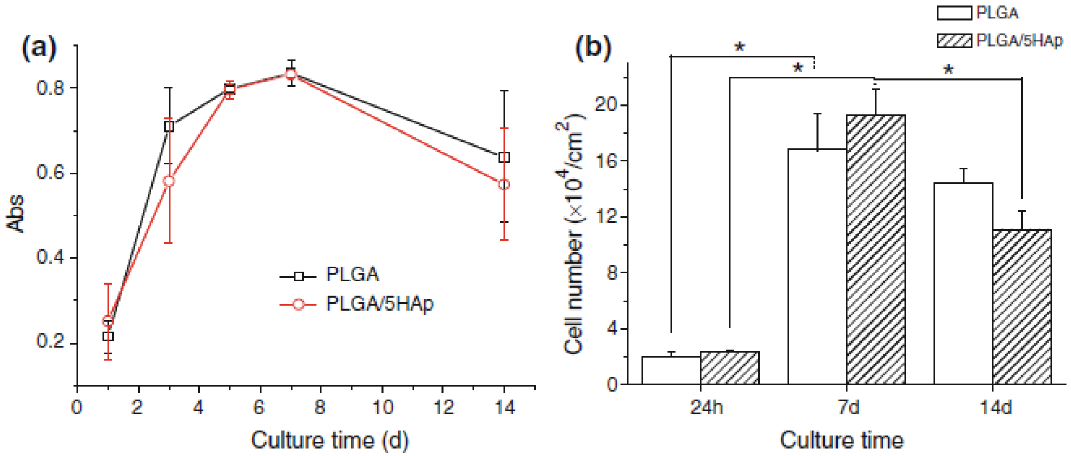
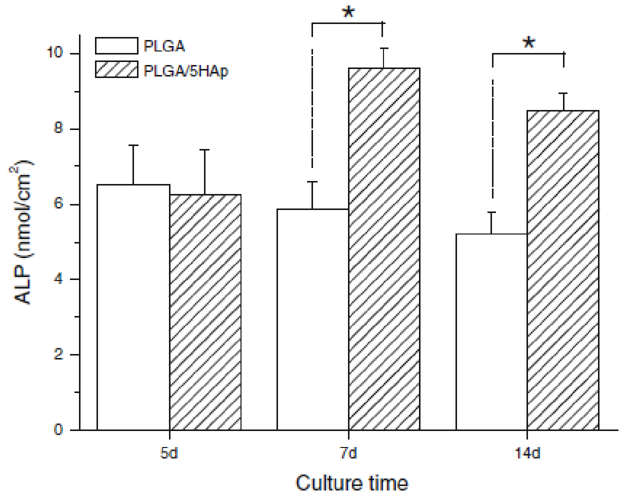


a) Viability, b) proliferation of MC3T3-E1 osteoblasts on the control PLGA and PLGA/5HAp nanofibrous scaffolds

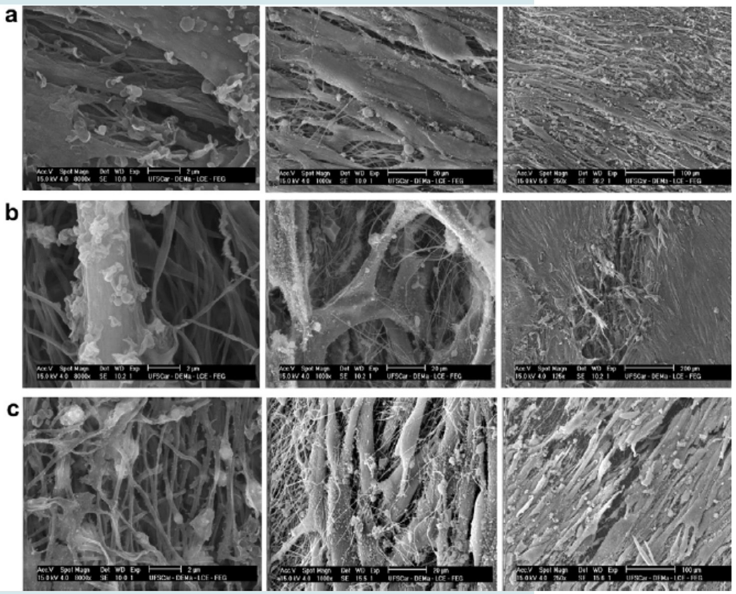


ALP activity* of MC3T3-E1 osteoblast seeded on the control PLGA and PLGA/5HAp nanofibrous scaffolds after 5,7 and 14 days culture.

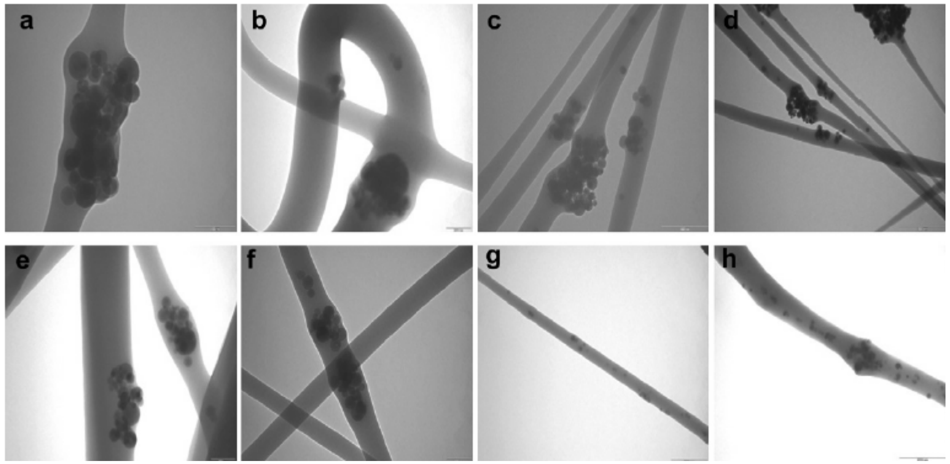
ALP activity* *is an enzyme secreted by osteoblasts and acts as one of the markers to confirm the osteoblastics phenotype and mineralization*

Influence of the microstructure and mechanical strength of nanofibers of biodegradable polymers with hydroxyapatite in stem cells growth. Electrospinning, characterization and cell viability

Wilson A. Ribeiro Neto, Ildeu H.L. Pereira, Eliane Ayres, Ana C.C. de Paula, Luc Averous,
Alfredo M. Góes, Rodrigo L. Oréfice, Rosario Elida Suman Bretas
Polymer Degradation and Stability 97 (2012) 2037e2051

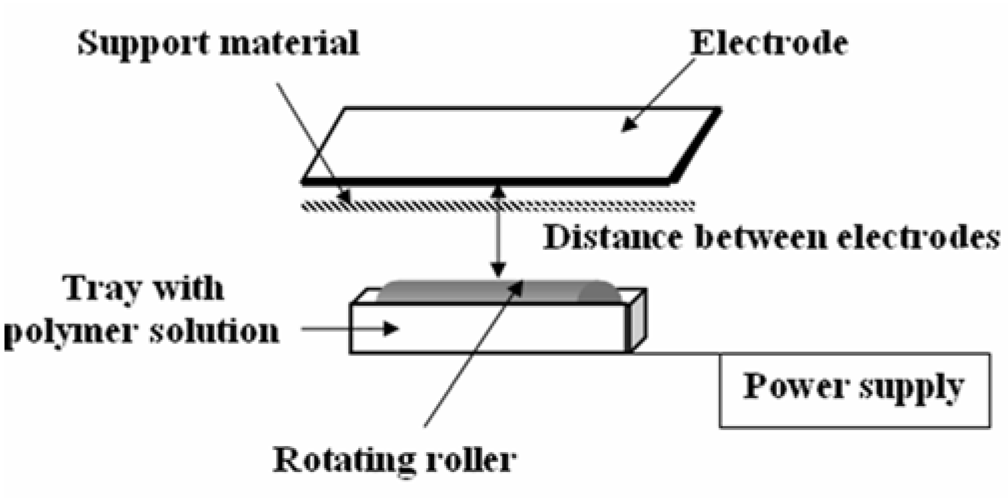


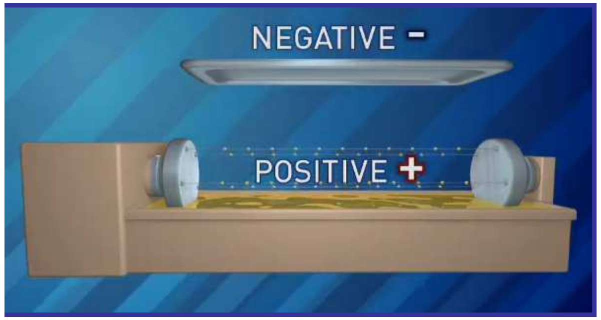
h-ASC after 5 days in substrates of nanofibers of: (a) PLA 7%; (b) PLA-nHA 1%; (c) PLA-nHA 5%. Polymer Degradation and Stability 97 (2012) 2037e2051

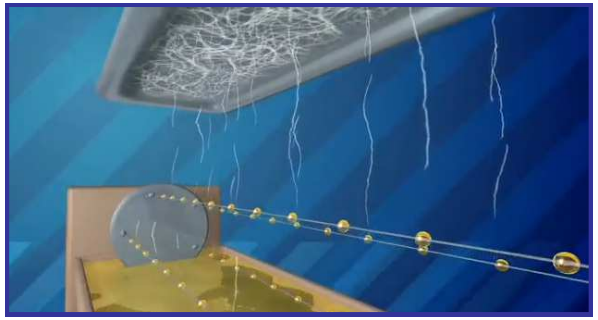


TEM micrographs of the nanofibers of: (a),(b) PLA-nHA 1%; (c),(d) PLA-nHA 5%; (e),(f) PCL-nHA 1%; (g),(h) PCL-nHA 5% Polymer Degradation and Stability 97 (2012) 2037e2051

Electrospinning equipment in Kaunas University of Technology







NANOSPIDER ™ (Elmarco)

Electrospinning equipment in Kaunas University of Technology

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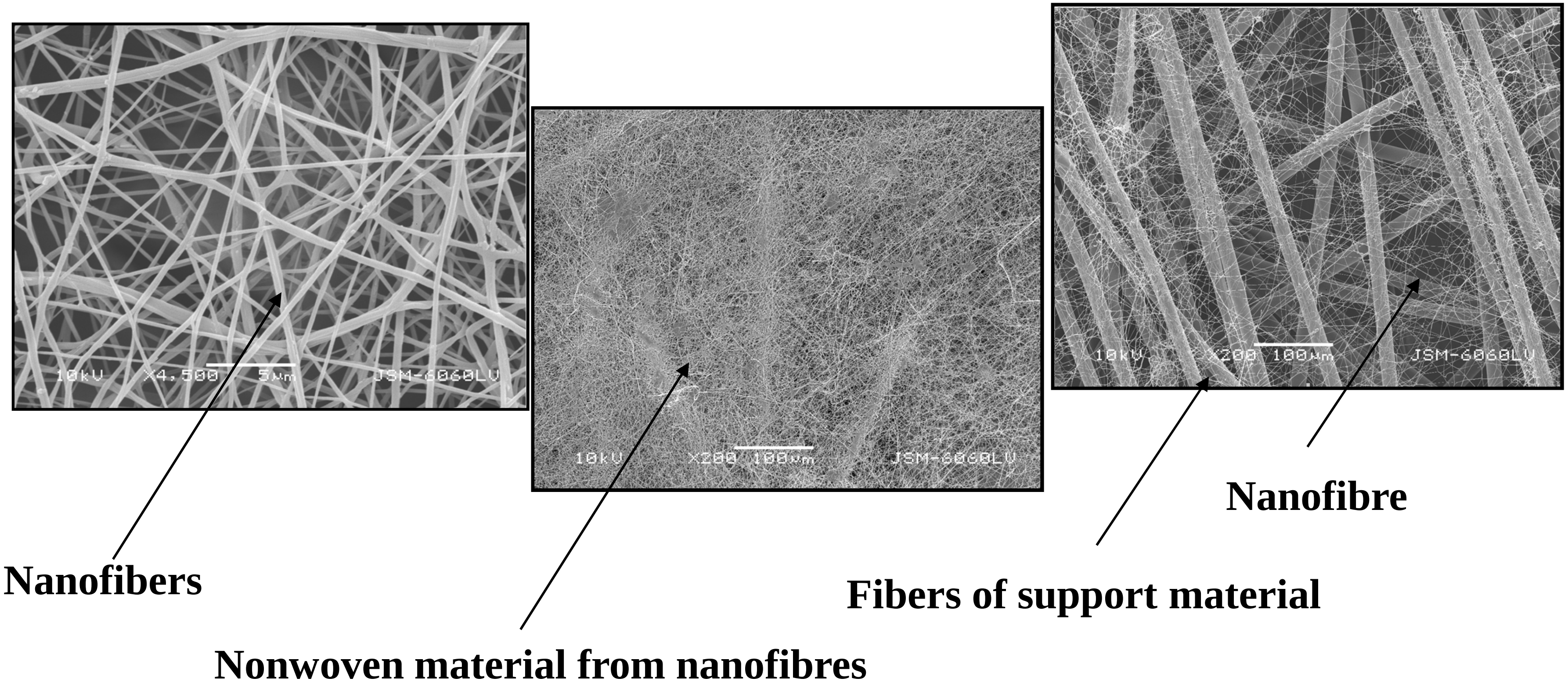
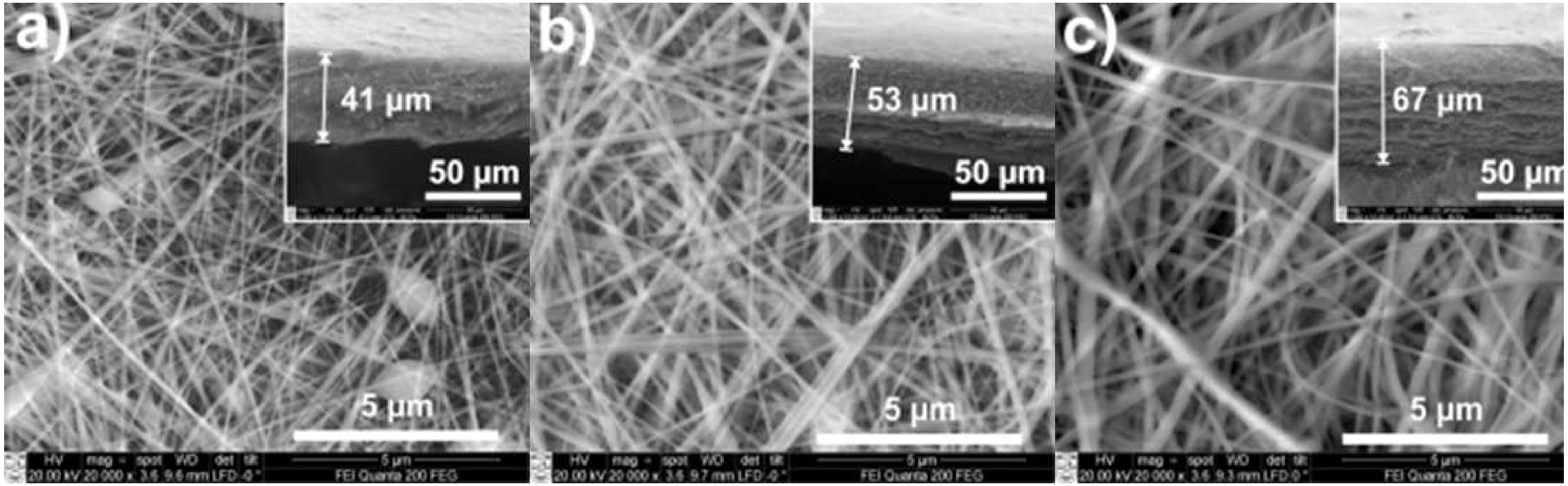
What is formed by electrospinning process?



SEM images of nonwoven materials from nanofibres (Kaunas University of Technology)

What is formed by electrospinning process?



The SEM images of electrospun PVA mat structure (scale bar 5 µm) deposited from different PVA concentration solutions (also different resulting viscosities): a) 11 wt.%, b) 13 wt.%, c) 15 wt.%. The inset demonstrates SEM images of electrospun mats cross-sections, arrows indicate thickness (scale bar 50 µm). Average of thickness a) 41.3±1.6 µm, b) 53.3±3.4 µm, c) 66.7±1.3 µm [E. Adomavičiūtė. T. Tamulevičius, L. Šimtonis and et.c Materials Science, 2015, No 1]

MATERIALS

POLY(VINYL ALCOHOL) (PVA) Sigma Aldrich Mowiol 10-98 Mw – 61000
HYDROXYAPATITE (Hap) Sigma Aldrich

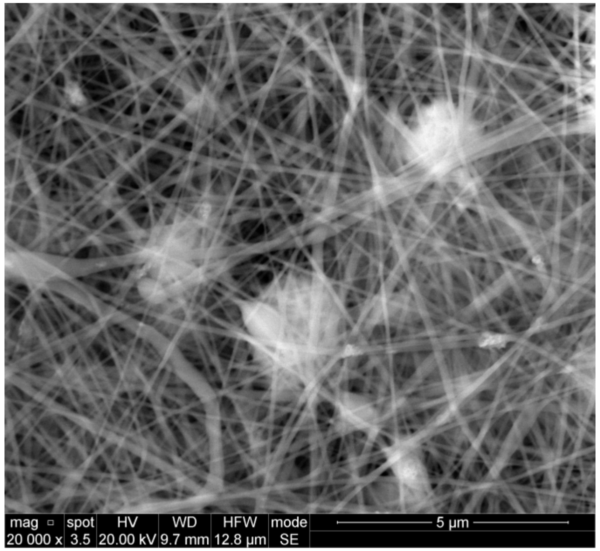
By magneting stirring for 4 hours at 80 °C was prepared PVA solution with concentration 13%. In PVA solution was added 1,5 %, 3% anf 5% amount of Hap powder.

METHODS

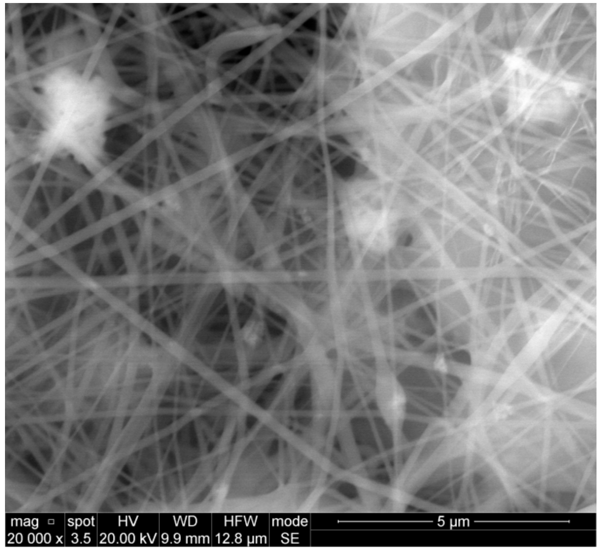
Electrospinning equipment Nanospider TM (Elmarco). Applied voltage 70 kV, distance between electrodes 13 cm.

The structure of electrospun materials estimated by SEM Quanta 200 FEG .

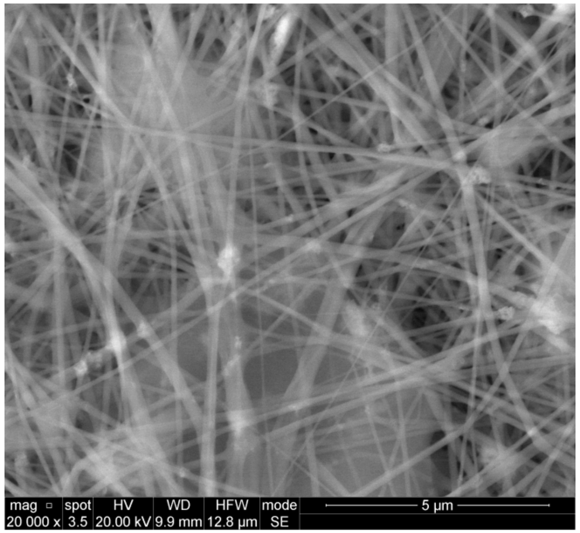
The structure of electrospun nonwoven materials with HAp particles



PVA with 1,7% amount HAp

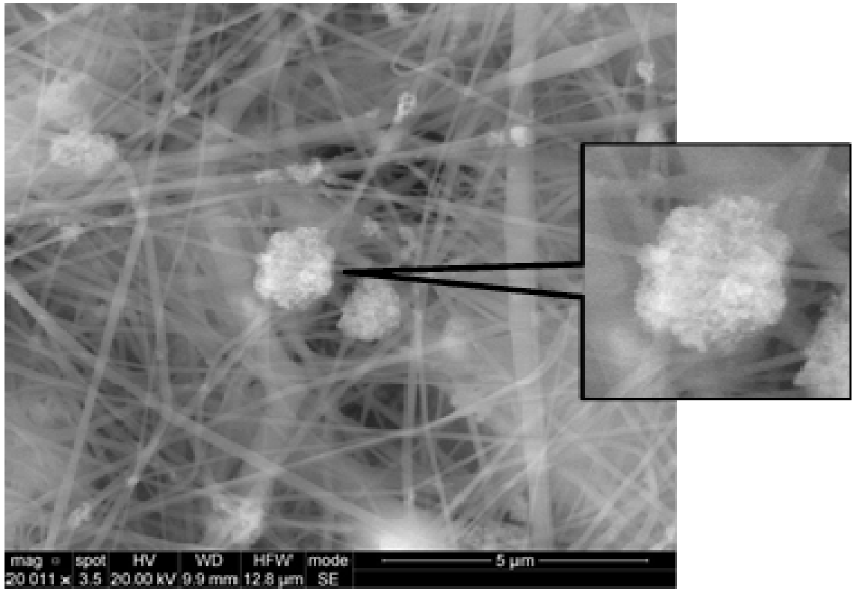


PVA with 3,3% amount HAp

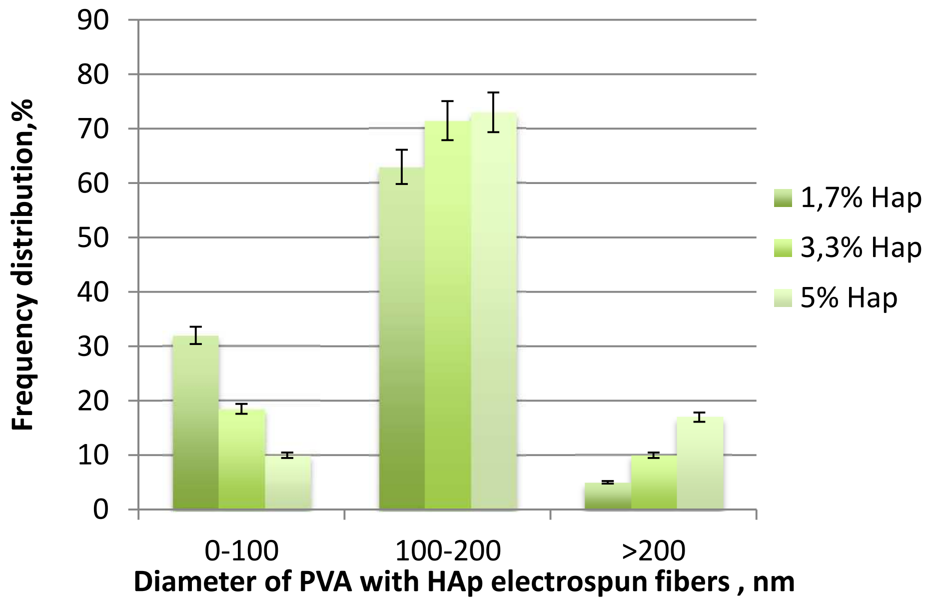


PVA with 5% amount HAp

The structure of electrospun nonwoven materials with HAp particles



Electrospun nonwoven mat with HAp particles



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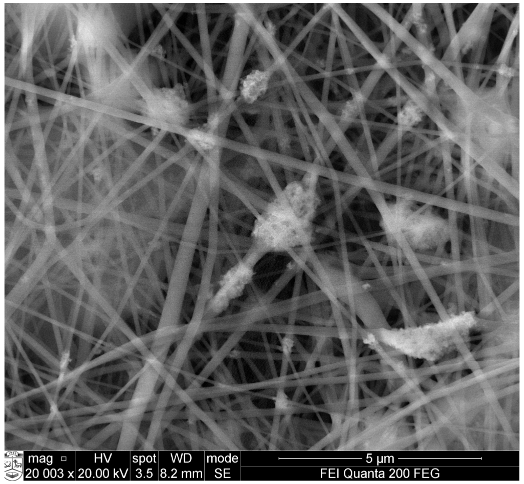
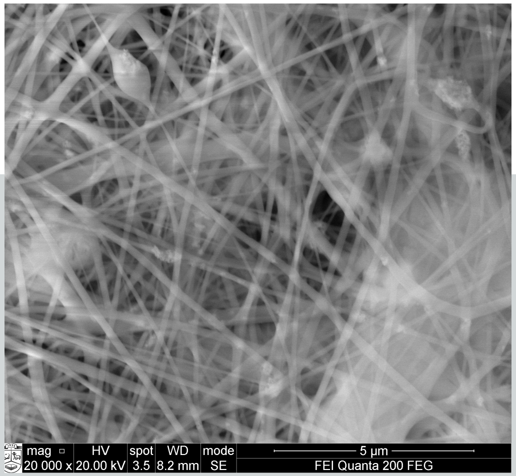
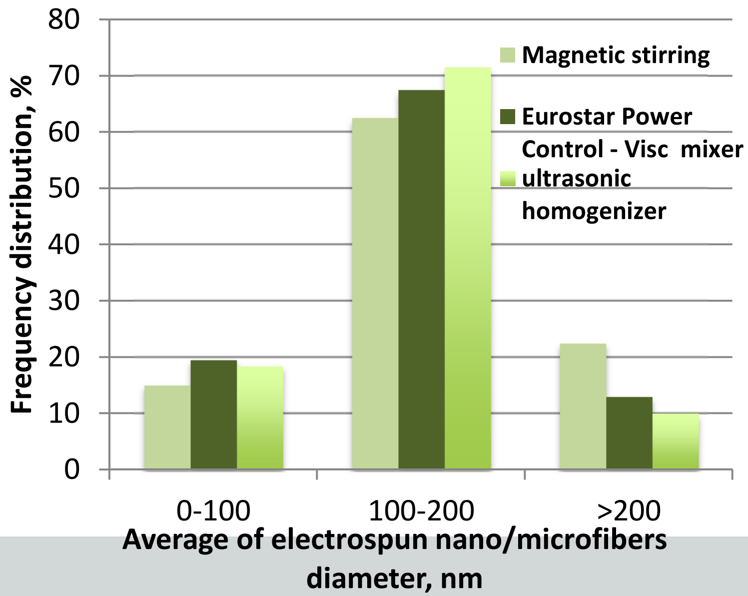
Electrospun materials with HAp particles



SEM image of electrospun PVA mat with 5% HAp (polymer solution was mixed 1 hour by mixer)



SEM image of electrospun PVA mat with 5% HAp (polymer solution was mixed 2 min by ultrasonic homogenizer)



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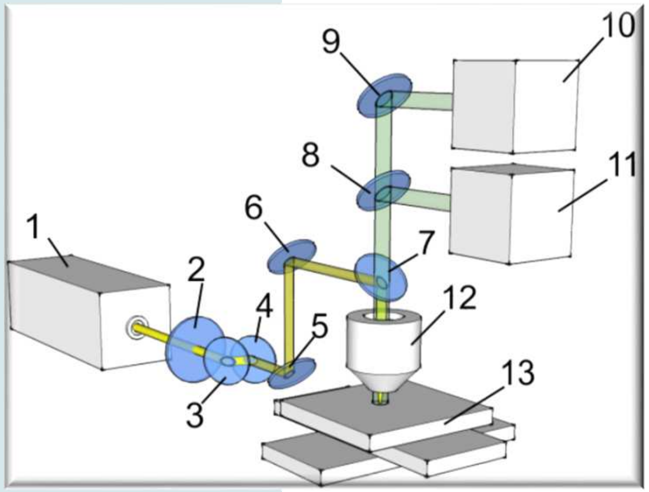
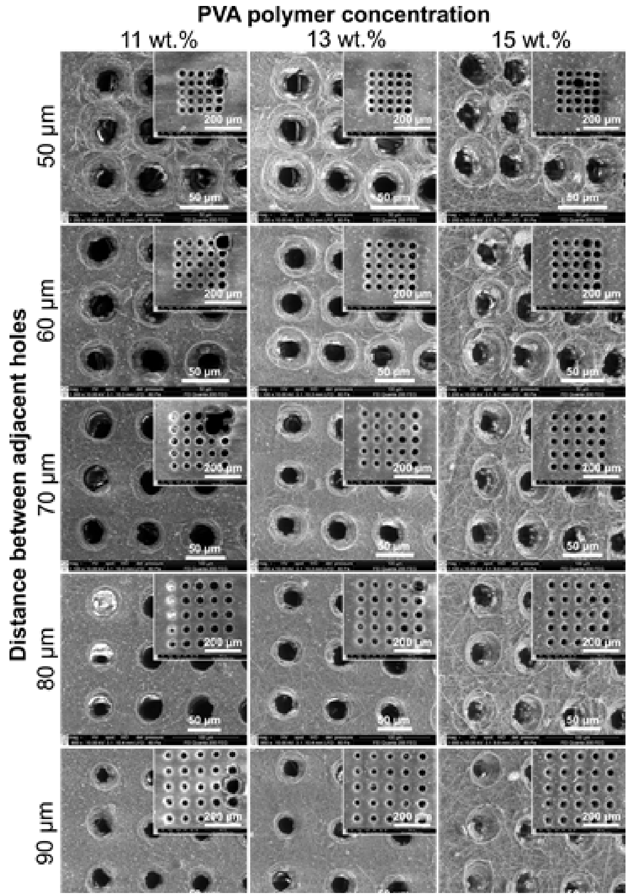
Disadvantage of electrospun nonwoven mats for scaffolds preparation *is too small pore size*

Designed pore networks can be created in electrospun scaffolds via laser ablation.



Principal scheme of ultrafast laser micro structuring system FemtoLab. 1 – femtosecond laser Pharos, 2 – λ/2 wave plate, 3,4 – Brewster angle polarisers (2, 3, 4 - attenuator), 5, 6, 7, 8, 9 – dichroic mirrors, 10 – CCD camera, 11 – LED illumination, 12 – objective, 13 – XYZ stage

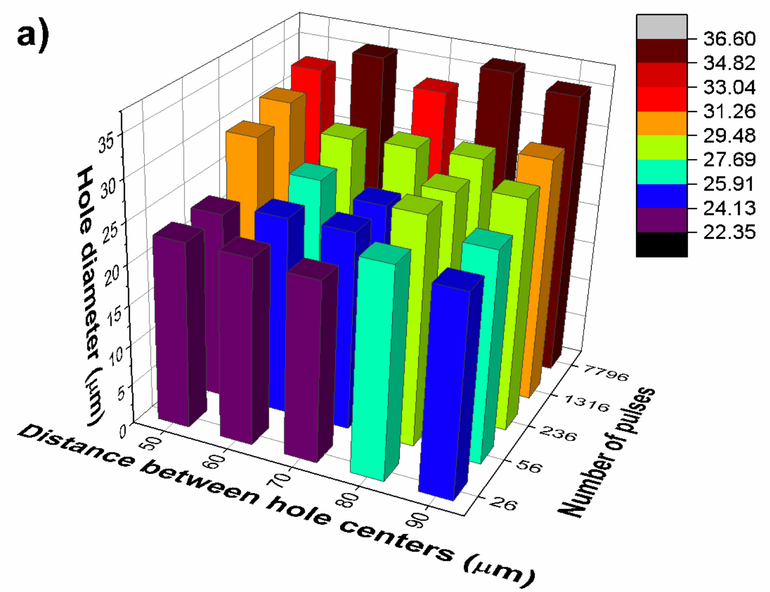


E. Adomavičiūtė, T. Tamulevičius, L.Šimatonis and et.c Materials Science (Mediagotyra) 2015, No 1.

Microstructuring of Electrospun Mats Employing Femtosecond Laser

Table 2. Average hole diameters with one standard deviation indicated in brackets of the laser processed electrospun mats obtained from the SEM images

PVA concentration, C (w/w%)	Number of pulses	Average hole diameter (μm)				
		Distance between the adjacent points (μm)				
		50	60	70	80	90
13	26	23.1 (1.0)	23.1 (2.4)	22.4 (1.3)	26.1 (1.3)	25.0 (2.0)
	56	23.2 (2.6)	24.5 (3.8)	24.6 (1.3)	28.4 (1.1)	26.2 (2.7)
	236	29.6 (2.4)	26.0 (2.2)	24.6 (1.9)	27.9 (1.1)	28.8 (1.6)
	1316	31.2 (2.7)	28.2 (2.5)	28.6 (2.0)	28.9 (1.8)	30.4 (1.4)
	7796	32.8 (2.4)	35.7 (2.3)	32.8 (2.0)	36.6 (8.0)	35.2 (2.1)
15	26	27.2 (3.3)	26.9 (1.9)	28.5 (0.9)	27.2 (3.2)	24.3 (2.6)
	56	27.5 (1.9)	28.4 (2.8)	29.7 (1.7)	28.6 (1.6)	27.3 (2.1)
	236	31.9 (3.5)	29.3 (2.6)	32.3 (3.4)	25.6 (1.7)	26.4 (2.9)
	1316	29.9 (1.9)	34.1 (7.0)	32.7 (2.8)	30.4 (1.9)	31.8 (2.6)
	7796	35.4 (2.6)	36.6 (2.7)	33.8 (0.5)	31.7 (1.9)	33.8 (2.4)



The dependence of diameter of laser micro-structured holes upon applied number of pulses and distances between the adjacent holes for mats electrospun from 13% concentration of PVA solutions

E. Adomavičiūtė, T. Tamulevičius, L.Šimatonis and et.c Materials Science (Mediagotyra) 2015, No 1.

Electrospinning for bone tissue engineering

Conclusions

❖ The structure of electrospun materials due a large surface area-to-volume ratio and due similarity to native ECM make this materials ideal candidates for scaffolds formation.

❖ The structure of electrospun materials depends from technological parameters and polymer solution properties: polymer type; solvent time; viscosity, electric conductivity, and additional particles insertion way.

❖ In order to get smaller HAp particles with more uniform distribution in nonwoven mat is better to use ultrasonic homogenizator.

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Conclusions

❖ From electrospun polymer solution with higher amount of HAp particles a mat with thicker nano-microfibers is formed.

❖ The electrospun amount of Hap particles in polymer solution depends from concentration (viscosity) of electrospun solution.

❖ Increase diameter of pores in electrospun nonwoven materials is possible by employing femtosecond laser.

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Thank YOU for attention!