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The combination of meltblown technology and electrospinning – The influence of the ratio of micro and nanofibers on cell viability

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ABSTRACT

This study describes the production, testing and characterization of biodegradable scaffolds for bone tissue, which consist of the exact ratio of meltblown microfibers and nanofibers produced through the electrostatic field. All fibrous materials were produced from polycaprolactone. Three kinds of materials were prepared in the experiment with the same area density and with different well-defined ratio of microfibers as a mechanical component and nanofibers as a cells adherent component. All prepared materials showed optimum porosity of the inner structure for cell proliferation and in comparison to the materials with nanofibers they had good mechanical properties. Important structural properties and homogenity of each material were observed by electron microscopy and analyzed by image analysis. The effect of various ratios of microfibers and nanofibers on adhesion and proliferation of osteoblasts *in-vitro* was characterized.

1. Introduction

Electrospinning is a technology used for the production of polymer nanofibrous materials from polymer solutions or melts. This technique is thoroughly described in literature [1] and the nanofibrous materials prepared by electrospinning are suitable for tissue engineering as scaffolds [1,2]. The structure of nanofibrous mats is appropriate for cell adhesion and proliferation [2–4]. However, mechanical properties of these mats are often not adequate. The mechanical properties can be improved by a combination of electrospinning with another nonwoven technology which produces microfibers, typically 1–7 μm in diameter [6]. The meltblown technology produces fibers from polymer melt by extrusion through a die with small orifices [5].

This article is based on our previous research [7], where the basic development of the first composite materials for bone tissue engineering had been presented. These materials consist of nanofibers and microfibers with confirmed mechanical functionality and with excellent performance properties for cell proliferation [8–10]. The aim of this follow-up study is to describe the rate of cell viability, depending on the ratio of micro and nanofibers in the composite. The results have shown that the cell viability increases with the increasing content of nanofibers in the composite while maintaining the required structural properties. Based on the obtained results we can therefore determine the critical value of the smallest possible content of nanofibers in the composite. On the other hand, the composite shows mechanical instability beyond this critical value of nanofibers.

2. Experimental part

2.1. Materials

Poly-ε-caprolactone (PCL; Mw 45,000; Sigma Aldrich), absolute ethanol (Penta Chemicals) and chloroform (Penta Chemicals) were used for the composite materials production.

2.2. Scaffold fabrication

Solution of 16 wt% polycaprolactone (PCL) in chloroform/ethanol (9:1) was prepared for the electrospinning process. The scheme of the production equipment set-up in optimal conditions is shown in Fig.1. The set-up was composed of a meltblown device (J&M Laboratories), an electrospinning device (a multi needle spinner and a countervailing pressure cylinder) and computer-controlled pumps. The meltblown extruder screw rotated at 3–15 rpm for 30–120 min, respectively (depending on the ratio of micro- and nanofibers in the sample). 100 g of polymer per sample was always extruded. Air velocity was 20 ms^{-1} at 200 mm from a

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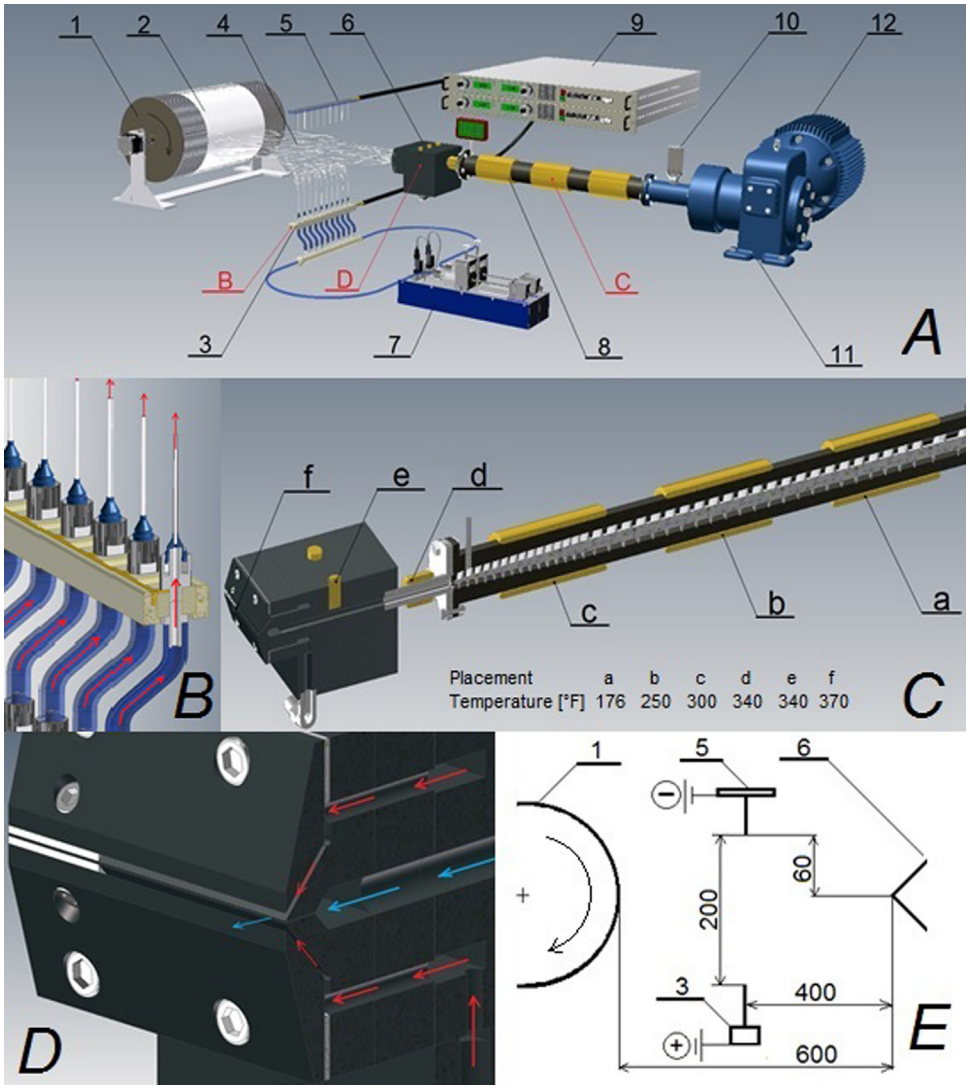


Fig. 1. The scheme of the combination of meltblown technology and electrospinning: the scheme of the overall set-up (A); detail of the multi needle spinning electrode (B); the meltblown die and extruder with heating zones – the optimal temperature set-up (C); the detail of meltblown die (D); the device proportion description in millimeters (E).

meltblown die. The meltblown die length was 100 mm. The needle spinner had adjustable spacing and a number of needles. There were 10 needles with a diameter of 1.2 mm with the spacing of 25 mm. The polymer dosage was 70 ml/h. The spinner was charged up to 35 kV positive and the collector 20 kV negative. Fibers were deposited on an intercepting drum collector which rotated at 4 rpm. The study compares three materials containing nanofibers with the numerical ratio in the composite: 1) 26% (ME26); 2) 55% (ME55); 3) 71% (ME71). The ratio represents the number of nanofibers relative to the total number of all fibers (micro and nano). The ratio was calculated from three SEM images for each sample. The ratio of micro and nanofibers in the composite was only influenced by changing the meltblown extruder rotation speed. Production and structural variables for all three created samples are shown in Fig. 2. During all experiments, the ambient temperature and relative humidity were set to 23 °C; 45%.

A: 1 – drum collector, 2 – composite fiber layer, 3 – multi needle spinner, 4 – airstream with fibers, 5 – needle collector, 6 – meltblown die, 7 – pumps, 8 – extruder, 9 – high voltage sources, 10 – hopper, 11 – transmission, 12 – engine.

2.3. Morphology characterization

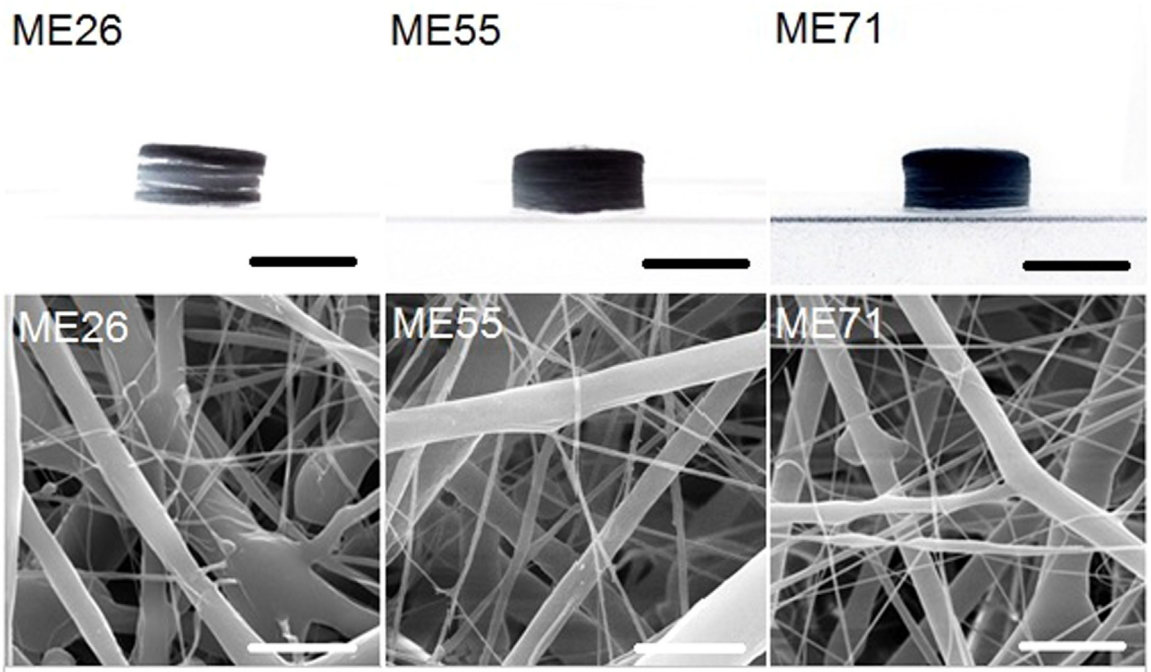
The dry samples were sputter coated with gold (5 nm) and observed by a scanning electron microscope (SEM, TescanVega 3SB). The biocompatibility of the material, cell proliferation and ability of the cells to migrate into the structure of scaffold were tested *in-vitro* by means of MG-63 osteoblasts.

2.4. In-vitro testing of MG-63 osteoblasts

Human osteoblasts–MG63 (ATCC) were maintained in EMEM (ATCC) with 10% (v/v) FBS (Lonza) and 1% penicillin/streptomycin/amfotericin B (Lonza). The cells were cultivated in the incubator (37 °C/5% CO_2). The medium was changed 3 times a week. The second passage culture was used for the experiments.

2.5. Sample preparation, cell seeding

The discs of a diameter of 15 mm and thickness of 5 mm were cut from prepared layers. Discs were sterilized (70% ethanol, 30 min) and washed in PBS (pH 7.4) prior to the cell seeding.



	ME26	ME55	ME71
Production time [min]	30	60	120
Speed of extruder screw [rpm]	15	7.5	3.7
Average pore diameter [μm]	87.9±293.4	48.1±141.7	35.5±90.2
Nanofibers volume fraction [%]	0.5	1	2
Nanofibers numerical fraction [%]	26	55	71

Fig. 2. The upper part – scaffold contour for all three tested materials observed by a projector, the scale bars are 10 mm long. The middle part-scaffold morphology observed by SEM scale bar is 20 μm. The lower part describes the production and structural variables for all three created samples.

MG63 cells were seeded ($1^{*}10^{5}$ cells per sample) on scaffolds placed in 24-well tissue culture plates.

2.6. MTT assay for the cell proliferation

Cell proliferation was monitored after 1, 7, 14 and 21 days by MTT assay. A 250 μl of MTT solution (2 mg/ml in PBS pH 7.4) was added to 750 μl of a medium (EMEM) and incubated with a sample for 3 h at 37 °C/5% CO_2. Formazane crystals were solubilized with isopropyl alcohol. Absorbance of the formazane solution was measured at 570 nm (ref. wavelength at 650 nm).

2.7. Microscopy analysis (SEM and fluorescence)

After day 1 and 21 after the cell seeding, the cell-cultured scaffolds were processed for microscopy analysis. The scaffolds were fixed by 2.5% glutaraldehyde and dehydrated with upgrading concentrations of ethanol (60–100%). The samples were analyzed by SEM (TescanVega 3SB) and image analysis software (NIS Elements, Nikon). For fluorescence microscopy (FM) the cells were fixed in ice – cold methanol for 15 min at 4 °C, washed in PBS and stained with propidium iodide for 15 min in the dark. Then the samples were washed in PBS and analyzed by means of a fluorescence microscope (NICON Eclipse). Splitting of the samples into halves allowed us to observe the cell behavior inside the materials.

3. Results and discussion

Each sample had the same area density of 250 g^{-2}. When the density of PCL is 1.145 g cm^{-3} and the thickness of all three materials was 5 mm with the same 95% porosity. Morphology characteristics of the materials (fibers and pores diameters) were studied by SEM. The produced fibrous structures (Fig. 2) were analyzed by the image analysis software. Overall, the average electrospun fiber diameter was 701 ± 227 nm and the meltblown fibers diameter was 7.1 ± 4.9 μm in all samples. The volume fraction of nanofibers in all three materials was compared with micro fibers, these fractions were 0,5%, 1% and 2%. The numerical ratios of nanofibers and microfibers were 26%, 55% and 71%. The contour projection of samples ME55 and ME71 (Fig. 2) showed very good shape integrity of these two samples in comparison to the sample ME26, which was disintegrated to layers of irregular thickness. The sample ME26 with the numerical ratio of the nanofibers of 26% was thus unsuitable in terms of mechanical properties (Fig. 3).

3.1. In-vitro tests

The images from FM showed a similar degree of adhered cells on the first day of testing. From the 7th to 21st testing day the sample with the highest ratio of nanofibers (ME71) showed the highest rate of cell proliferation. Generally, microscopy shows that

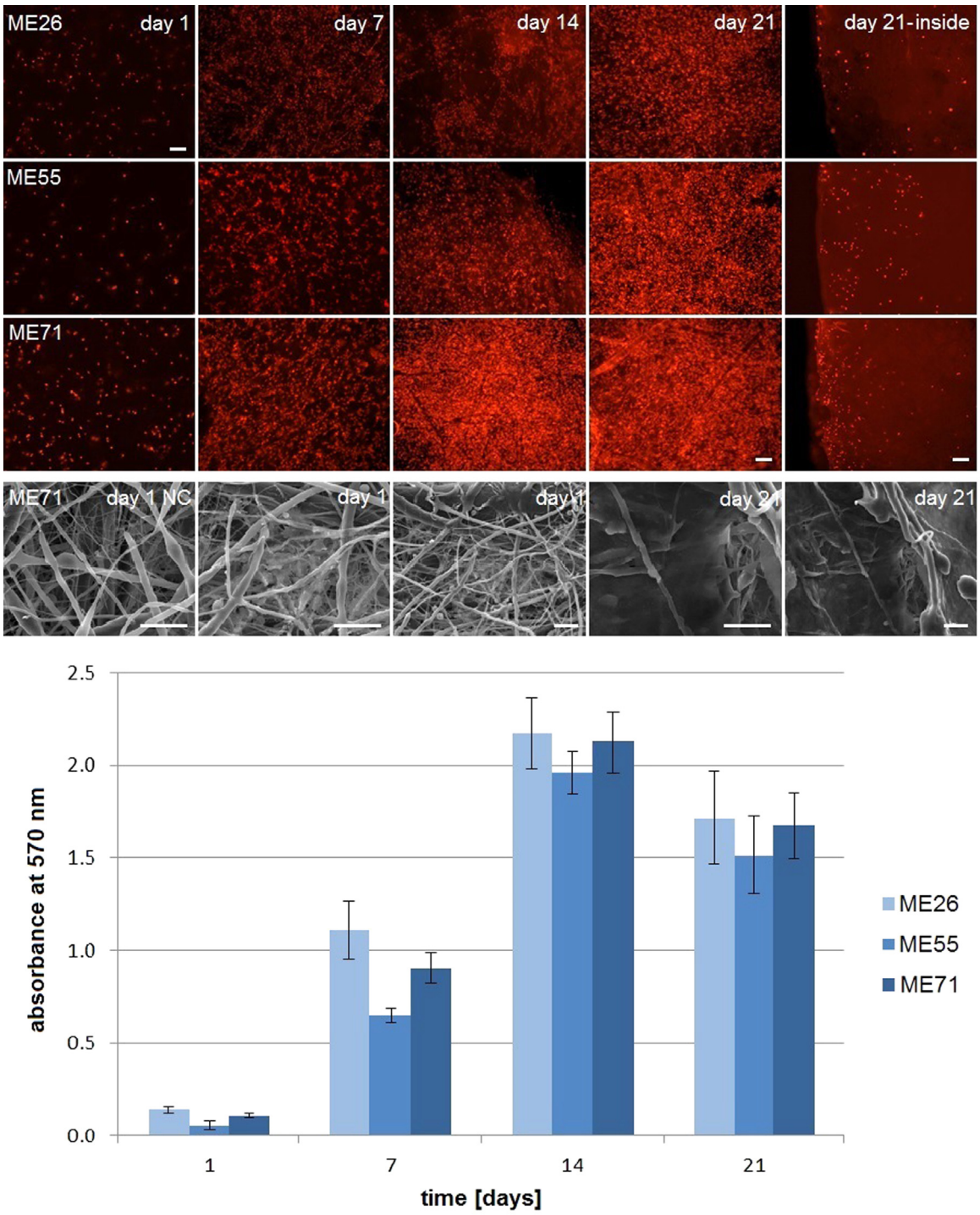


Fig. 3. FM images (upper part) of MG-63 cells on the materials (external view) on days 1, 7, 14 and 21. Images from a FM composed of 100 images was captured by a motorized microscope stage with a changing focus in the z axis of the 1 μm distance, the scale bar was 100 μm. FM images of MG-63 cells on the inner surface of the tested materials on day 21 (day 21 included) are presented here as basic views. Cell proliferation on the scaffolds was determined by MTT assay (lower part).

together with the increasing ratio of nanofibers in the composite the cell proliferation and confluence colonization of material increase as well. Proliferation into the inner structure was observed mainly from the edges into the center of the materials, and was similar in extent for all tested samples. The results from MTT assay showed contradictory results compared to the microscopy of the ME26 sample, where this material showed the highest cell viability. However, this was caused by shape disintegration. The samples made from this material were disintegrated to individual layers with different thickness. In comparison with other samples, these layers provided a higher surface area for cell proliferation. Therefore, the results from MTT assay for material ME26 should be taken as relevant.

4. Conclusion

Various materials with controllable ratio of micro and nanofibers and their manufacturing technology have been developed. In this article we have demonstrated the beneficial effect of the

increasing ratio of nanofibers in the composite on the cell proliferation. The critical limit of the minimal ratio of nanofibers in the composite has been determined. The composite with lower nanofiber content disintegrates. On the basis of these results from *in-vitro* testing, we propose the ME71 composite as most suitable for bone tissue engineering. Further studies will include *in-vivo* tests and tests of manufacture reproducibility.

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