3D MICRO-NANO FIBROUS SCAFFOLD PREPARED BY MELTBLOWN IN COMBINATION WITH ELECTROSPINNING FOR THE BONE TISSUE ENGENEERING

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Abstract

This study describes the development of a novel composite material for tissue engineering consisting of microfibers produced by meltblown technology ensuring optimal three dimensional porous structure and sufficient mechanical properties, electrospun nanofibers for good cell adhesion and particles of hydroxyapatite enhancing increasing the biological activity and mineralization of regenerated bone tissues. The production is continuous and all technologies are composed into one step. This newly formed composite set the excellent biodegradable properties tested by enzymatically catalyzed hydrolysis The preliminary in-vitro tests showed the high potential of developed composite materials in TE - good porosity of the tested materials allows osteoblasts to proliferate into the sample inner structure with the significant contribution of nanofibers content to the cell proliferation rate.

Keywords: Meltblown, electrospinning, nanofibres, scaffolds, tissue engineering, polycaprolactone

1. INTRODUCTION

Meltblown is a nonwoven technology, which produce fine fibers (1-5μm) typically from thermoplastic fiber forming polymers by extrusion through a die containing small orifices. Then the fibers are rapidly attenuated by converging streams of hot air and subsequently blown by high-velocity air onto a collector [1]. The technology is very usable one and their products are used in many technical, hygiene and medical applications (filtration media, sorption pads, diapers, breathing masks, disposable cloth..). The basic polymers used for meltblown nonwoven production (PP, PE, PS, PET, PLA..) are not biodegradable and therefore are not used as scaffolds for tissue engineering (except PLA). Nevertheless structurally, meltblown materials are destined to be the bone or knee cartilage scaffold for their small diameter of fibers, random orientation of fibers, threedimensional structure, high porosity, optimal pore size etc [2]. Electrospinning is generally well known technology for polymer nanofibrous materials production with respect to application in biomedical applications [3]. There is many publications confirming their appropriateness as scaffolds for tissue engineering [4]. The combination of electrospun materials and particles as hydroxyapatite either inside fibers or in between fibers [5] was observed and proposed.

This study aims to fabricate a novel composite material for bone tissue engineering consisting of microfibers produced by meltblown technology ensuring optimal threedimensional porous structure and sufficient mechanical properties, electrospun nanofibers for good cell adhesion [6, 7] and particles enhancing the biological activity and mineralization of regenerated bone tissue. The production is continuous and all technologies are composed into one step. There is not necessary any additional bonding, shaping etc. The succesfull preliminary in-vitro tests of the scaffolds prepared by the method are described here. The results showed that the composite materials significantly promoted proliferation, viability and cell adhesion compared to simple meltblown materials

2. EXPERIMENTAL APPROACH

Materials: Poly-ε-caprolactone (PCL; Mw 45,000; Sigma Aldrich), chloroform (Penta), ethanol (Penta), hydroxyapatite (HA; calcium hydroxyphosphate; Mw 502.31; powder; Sigma Aldrich) were used for the composite materials production.

*Scaffold fabrication***:** Solution of 16% by weight PCL (Poly-ε-caprolactone, Mw 45000) in chloroform/ethanol (9:1 by weight) was prepared for electrospinning. The production equipment set-up consisting of meltblown (laboratory equipment J&M Laboratories, USA), electrospinning (needle-less roller electrospinning) and sputtering devices based on vibration principle. The meltblown extruder screw rotated 40 rpm (100g of polymer per 1 hour). The spinning of electrospin roller was 50 rpm, the roller charging was 35kV positive and collector charging 20kV negative. The study compare four different materials: i) meltblown material (M); ii) meltblown material with sputtered particles (MS); iii) meltblown material combining with electrospun fibers (ME); iv) meltblown material combining with electrospun fibers and sputterd particles (MES).

Characterization: Dry scaffolds were characterized in term of morphology. Samples were sputter-coated by gold and then observed by a scanning electron microscopy (SEM, Tescan Vega 3SB Easy Probe). The biocompatibility of the material cell proliferation and ability migrate into the structure of scaffold was tested *in vitro* using MG-63 osteoblasts.

In-vitro testing: In-vitro culture of MG-63 osteoblasts: human osteoblasts (MG63) were maintained in EMEM (ATCC) with 10% (v/v) FBS (Lonza) and 1% penicilin/streptomycin/amfotericin B (Lonza). Cells were maintained in a incubator (37 °C/5% CO_2). Medium was changed 3 times a week. The 2nd passage culture was used for experiments.

Sample preparation, cell seeding: from prepared layers were cutted discs (diameter 15mm, thickness 5mm) which were sterilized (70% Et-OH, 30min) and washed in PBS (pH7.4) prior the cell seeding. MG63 cells were seeded ($1*10^5$ cells per sample) on scaffolds placed in 24-well Tissue culture plates.

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

Microscopy analysis (SEM and fluorescence): After 1, 3, 7 and 14 days of cell seeding, the cell-cultured scaffolds were processed for microscopy analysis. SEM: the scaffolds were fixed by 2.5% glutaraldehyde and dehydrated with upgrading concentrations of Et-OH (60%, 70%, 80%, 90% and 100%). Samples were analysed by scanning electron microscope (Tescan, VEGA3 SB easy probe). Fluorescence microscopy: The cells were fixed in frozen methanol for 15 minutes, washed in PBS and stained with propidium iodide for 15 minutes in the dark. Then the layers were washed in PBS and analyzed using fluorescence microscope (NICON Eclipse Ti-E)

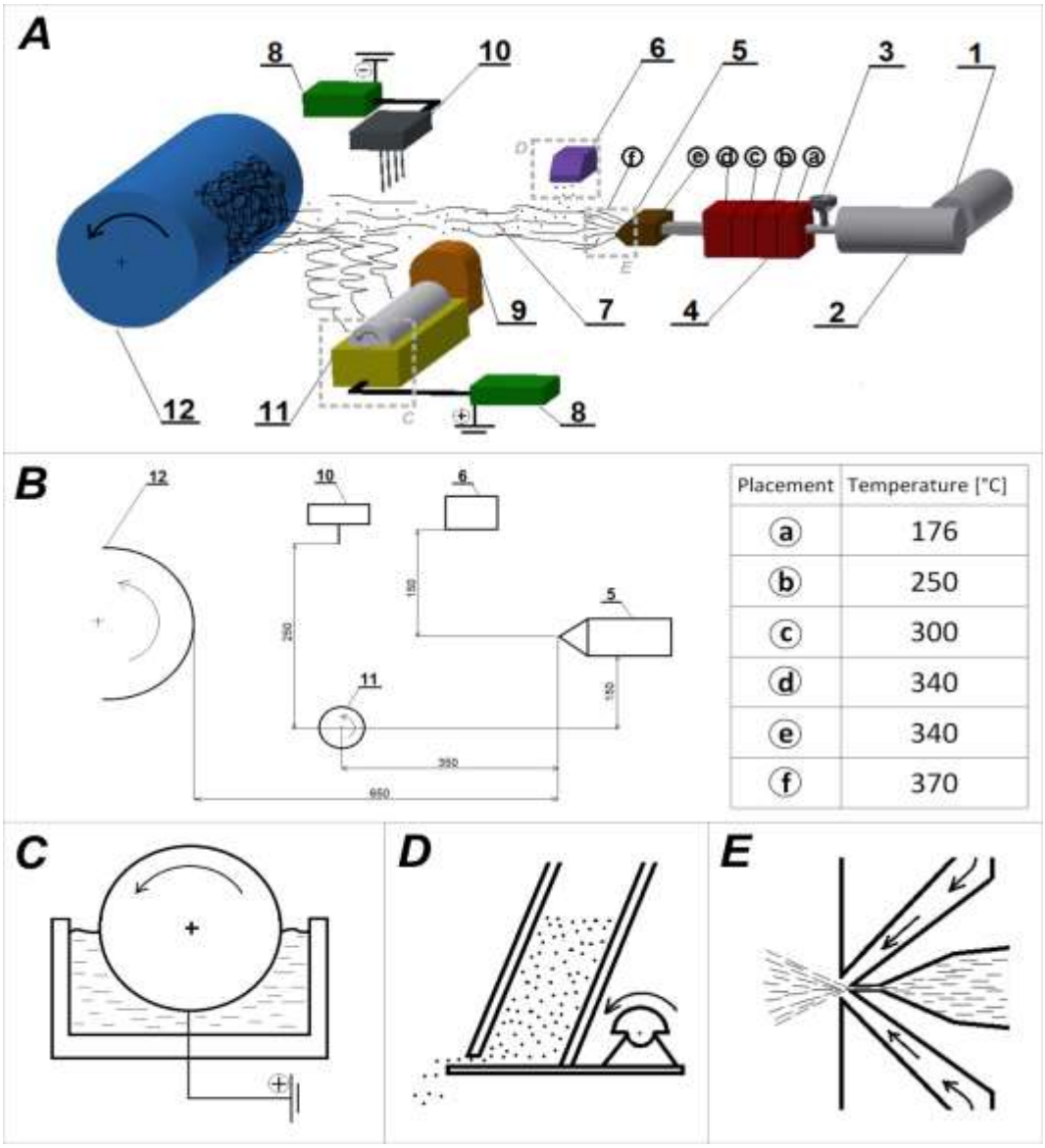


Fig.1. Scheme of the combination of meltblown and electrospinning with integration of particles in-situ into the producing fibrous material: scheme of all set-up (A); proportion description in millimeters and optimal temperature set-up (B); details of needle-less electrospinning spinning electrode (C), sputtering device (D) and meltblown die (E) set-ups.

3. RESULTS AND DUSCUSION

The result materials without HA particles (M and ME) had surface density 250 gm^{-2}. When density of PCL is 1.145 gcm^{-3} and the thickness of materials is 5 mm then porosity is about 95%. The weight percentage of HA in the materials was 10 %, thus the final surface density of materials with HA particles (MS and MES) was 275 gm^{-2}. Morphology characteristics were studied by SEM visualization of the produced fibrous structures, see Fig.2 and image analysis. The average electrospun fiber diameter was 732±292 nm. Average meltblown fiber diameter was 6,5±4,4μm.

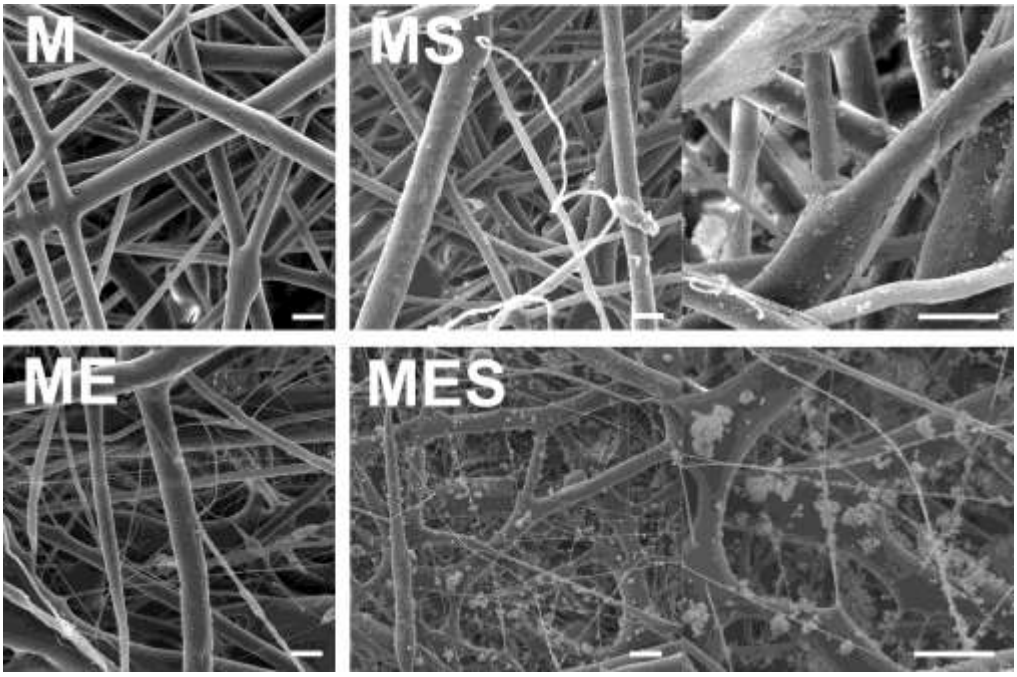
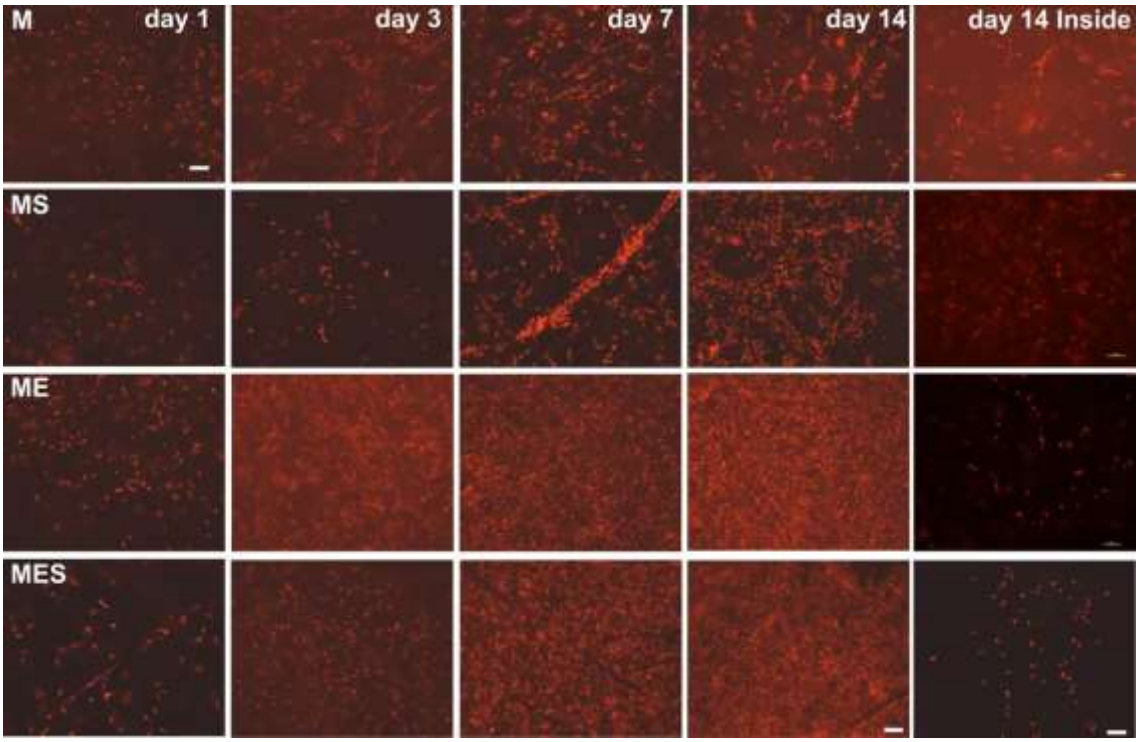


Fig. 2: Scaffold morphology observed by SEM for all four tested materials, scale bar is 20 μm.

The pictures from microscopy and MTT assay data (fig.3) reveal similar rates of adhered MG-63 osteoblasts in all kinds of tested materials. However from the 7th day after cell seeding the significant differences in the cell proliferation rates had been observed. Osteoblasts on samples containing electrospun fibers (ME, MES) showed significant increase in proliferation. Also sputterd particles (MS, MES) seems to have a positive effect on the proliferation rate compared to materials without sputterd particles (M, ME). Fig.3. confirms osteoblasts proliferation into the inner structure of the materials with the significantly higher rate in materials containing nanofibers (ME, MES). Good porosity of the tested materials allows osteoblasts to proliferate into the sample inner structure with the significant contribution of nanofibers content to the cell proliferation rate.



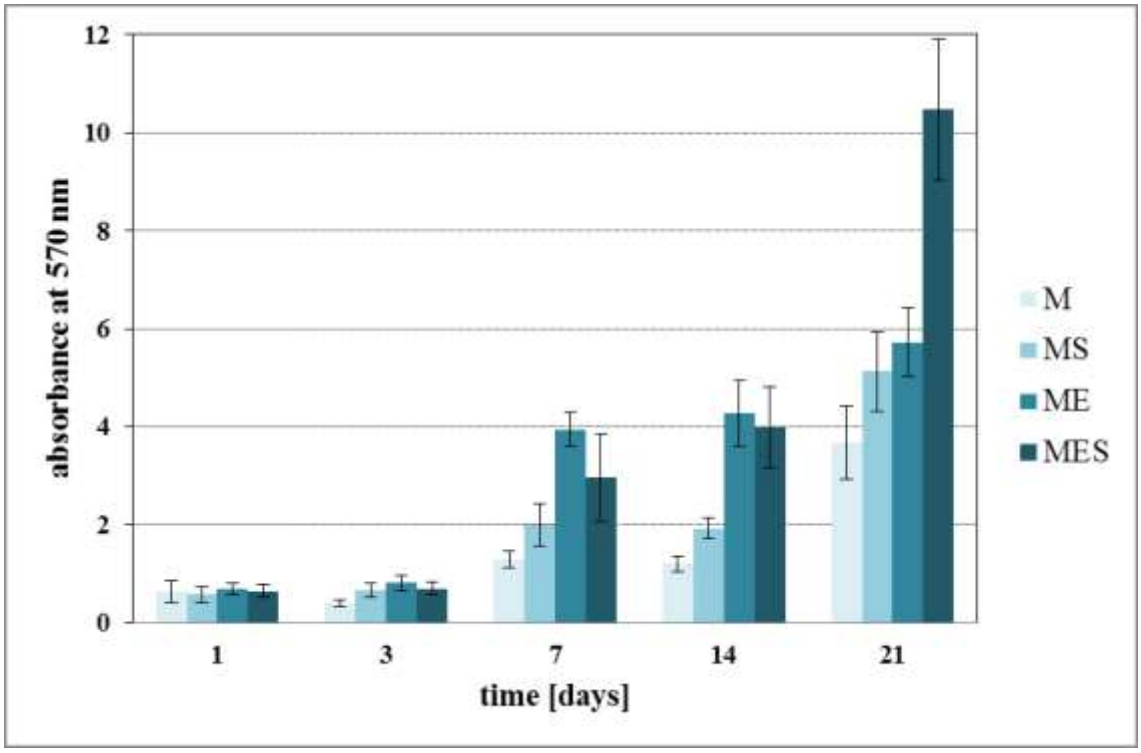


Fig. 3 Fluorescent microscopy images (upper part) of MG-63 cells on the materials (external view) in days 1, 3, 7 and 14 Images from fluorescence microscope composed of 100 images captured by motorized microscope stage with a changing focus in the z axis of the 1 micrometer distance. Fluorescence microscopy images of MG-63 cells on the inner surface of tested materials in day 14 (day 14 inside) presented here as basic views. Cell proliferation on the scaffolds was determined by MTT assay (bottom).

4. CONCLUSIONS

A novel scaffold produced by combining meltblown and electrospinning technology with in-situ particle integration in-between fibers was developed. The scaffold has sufficient surface properties and porous structure. This scaffold is beneficial for cell growth, adhesion and proliferation and may be served as bone substitutes in tissue engineering application. Further studies are focused on its implantation into animal models for the investigation of its behavior in vivo.

5. FUTURE WORKS

Prepare and test materials with diverse micro/nano fibre content. *In-vivo* tests of scaffolds with the most promising *in-vitro* tests results (ME, MES).

ACKNOWLEDGEMENTS

This material was supported by the project "Nanofiber materials for tissue engineering", reg. No. CZ.1.05 / 3.1.00 / 14.0308, which is co-financed by the European Social Fund and the state budget of the Czech Republic.

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