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Scaffolds with Magnetic Nanoparticles for Tissue Stimulation

Abstract: Magnetic nanoparticles (MNPs) have been used in several medical applications, including targeted hyperthermia, resonance tomography, diagnostic sensors, and localized drug delivery. Further applications of magnetic field manipulation through MNPs in tissue engineering have been described. The current study aims to develop tissue-engineered polymeric scaffolds with incorporated MNPs for applications that require stimulation of the tissues such as nerves, muscles, or heart. Electrospun scaffolds were obtained using 14%w/v poly-caprolactone (PCL) in 2,2,2-Trifluoroethanol (TFE) at concentrations of 5% & 7.5%w/v of dispersed MNPs (iron oxide, Fe_3O_4, or cobalt iron oxide, $CoFe_2O_4$). Scaffolds were analyzed using scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy, uniaxial tensile testing, and cell seeding for biocompatibility. Human bone marrow mesenchymal stem cells (bmMSCs) were seeded on the scaffolds. Biocompatibility was assessed by metabolic activity with Resazurin reduction assay on day 1, 3, 7, 10. Cell-cell and cell-scaffold interactions were analyzed by SEM. Electrospun scaffolds containing MNPs showed a decrease in fiber diameter as compared to scaffolds of pure PCL. The maximum force increases with the inclusion of MNPs, with higher values revealed for iron oxide. The metabolic activity decreased with MNPs, especially for cobalt iron oxide at a higher concentration. On the other hand, the cells developed good cell-scaffold and cell-cell interactions, making the proposed scaffolds good prospects for potential use in tissue stimulation.

Keywords: magnetic scaffolds, biocompatibility, tissue stimulation, mechanical testing, electrospinning.

https://doi.org/10.1515/cdbme-2021-2117

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1 Introduction

The morphology and components of scaffolds for tissue engineering play an essential role in cell adhesion and proliferation. Fibrous scaffolds possessing nano- to micro-sized fibers enhance cell-scaffold contact [1]. However, it is known that tissues such as nerve, muscle, heart, and bone require stimuli for their proper regeneration. In this regard, the standard method is to include growth factors like morpho-genetic proteins, though their short half-life limits its use. Thus, the inclusion of magnetic nanoparticles (MNPs) could provide a pathway for stimulation of these tissues. It has been found that the incorporated MNPs generate magnetic microenvironments that stimulate numerous sensitive receptors on the cell surface. The stimulus can increase the calcium content, enhance cell activity, and promote osteogenic or inductive scaffolds' response in the host tissue [2]. It is important to highlight that MNPs have proven their efficacy in medical applications such as targeted hyperthermia, resonance tomography, sensors for diagnostic among others showing no bio-compatibility issues. Moreover, magnetic structures like Fe_3O_4 are approved in iron preparations (carbohydrate-coated super-paramagnetic iron oxide nanoparticles, 510 mg) to treat anemia in chronic kidney disease by the FDA [3–5].

Different techniques have been used to fabricate scaffolds with MNPs. Scaffolds obtained by electrospinning have the advantage of nanofibers with a large surface volume to diameter ratio [6,7]. Thus, in this work we aimed to compare morphological and mechanical properties and biocompatibility of electrospun polycaprolactone (PCL)

scaffolds with the inclusion of iron oxide and cobalt iron oxide MNPs.

2 Materials and Methods

2.1 Materials

PCL (Mn=80,000), iron oxide (magnetite) and cobalt iron oxide nanoparticles in powder form were purchased from Sigma-Aldrich. 2,2,2-Trifluoroethanol (TFE) was purchased from abcr GmbH. Unless otherwise stated, all other chemicals were purchased from Sigma-Aldrich.

2.2 Electrospinning and characterization

2.2.1 Solution preparation and electrospinning

PCL was dissolved in TFE at a concentration of 140 mg/ml and stirred overnight at room temperature. Magnetic iron oxide (IONP) or cobalt iron oxide (CoNP) nanoparticles at two different concentrations 5% and 7.5% (w/v), were then included in the PCL solution. The MNPs were dispersed in the PCL solution using an ultrasonic bath for 4h in an ice bath to achieve a homogenous solution. After 4h, the solutions were transferred to 10 ml syringes (Omnifix, B.Braun, Germany) for electrospinning.

Electrospinning was performed with an in-house constructed device in horizontal orientation using a flow rate of 1 ml/h, an aluminum rotating collector with tangential velocity 35.3 m/s, an applied voltage of 17-22 kV to reach a stable process, a distance between needle and collector of 22 cm, and a 21G needle (Sterican 21G, B.Braun, Germany); mats were spun for 4h to obtain scaffolds with a thickness of ~ 80 µm. An electrospun mat of 14% PCL was used as the reference sample. Samples are labeled with their concentration and type of MNP (e.g., 7.5% IONP).

2.2.2 Scanning electronic microscopy (SEM) and energy dispersive x-ray (EDX)

SEM was used to analyze the surface morphology of the electrospun scaffolds. Small samples were cut and sputter coated with Au/Pd for 45s before imaging with the SEM (S3400N, Hitachi, Japan). Images were taken with an acceleration voltage of 20 kV and a working distance of 10 mm. The fiber diameter was measured from SEM images with ImageJ software (National Institutes of Health, Bethesda, MD, USA USA). Measurements were taken of 50 fibers per image on three images per sample. The chemical composition of the same samples was analyzed with the EDX (EDAX, Genesis APEX, Japan) at the same acceleration voltage.

2.2.3 Mechanical testing

In rectangular samples, mechanical properties were evaluated with a test section geometry of width = length = 1 cm cut with razor blades from the electrospun mats. The samples were pre-wetted in phosphate buffer saline (PBS) and mounted with sandpaper at each edge on the pneumatic grips of the testing device. Uniaxial tensile testing was performed using an Instron 5967 (Instron, Norwood, MA, USA) device with a controlled heating bath (Bioplus, Instron, USA) and a load cell of 100 N (Instron, USA). All experiments were done in 37°C PBS with a 10 mm/min strain rate until failure. The thickness of the samples was assumed to be 80 µm based on SEM measurements. The data from the test was post-processed in MATLAB to obtain ultimate force and strain. Experiments were repeated six times, and median values were presented.

2.2.4 Cell seeding and biocompatibility

To evaluate the electrospun scaffold's biocompatibility human bone marrow mesenchymal stem cells (bmMSCs) provided by Dr. Yvonne Roger from Clinic for Orthopedy at Hannover Medical School were employed. bmMSCs (4-6 passages) were seeded onto circular 1.13 cm^2 scaffolds (pre-wet in supplemented medium overnight) at a density of 25,000 cells/cm^2, PCL scaffolds without MNPs served as control. The seeded scaffolds were cultivated in 12 well plates (TPP, Switzerland) in supplemented growth medium made of Dulbecco's modified Eagle's medium (DMEM) (Bio&Sell, Germany) with 2.3 % (v/v) estable glutamine, 2.3% (v/v) HEPES, 0.002 % (v/v) FGF-2, 11.6 % (v/v) fetal bovine serum (Bio&Sell, Germany), and 1.2 % (v/v) Penicillin/Streptomycin; medium was replaced every 3 days.

On day 1, 3, 7, and 10, the metabolic activity of bmMSCs on the scaffolds was assessed using a resazurin reduction assay. A working solution of 44 µM was prepared in pre-warmed culture medium directly before experiments, and 1 ml was added to the samples. After incubation for 2 h, 100 µl of the reduced resazurin solution was transferred to a 96-well TPP cell culture plate and fluorescence was analyzed at 570 nm (excitation) and 600 nm (emission) with a microplate reader (Tecan Genios; Tecan, Austria). The obtained data were analyzed and presented as Relative Fluorescent Units (RFU)

per well. The RFU were calculated as F_{sample}—$F_{sample\ blank}$, where F_{sample} are the fluorescence values of reduced resazurin for cell-seeded scaffolds and $F_{sample\ blank}$ are the values of resazurin solution incubated with unseeded scaffolds.

The morphology of adherent cells on the scaffolds and their interaction with the fibers was visualized using SEM. Scaffolds were prepared by rinsing with 0.1 M cacodylate buffer twice, followed by fixation in 2.5% glutaraldehyde prepared in 0.1 M cacodylate buffer (pH 7.4) at 4°C for one week. Subsequently, the samples were washed in bi-distilled water and dehydrated in ethanol (20, 35, 50, 70 and 99 % (v/v)). Scaffolds were air-dried overnight, followed by sputter coating and imaging as described above.

3 Results and Discussion

3.1 Morphological and chemical characterization

Measurements showed that all the scaffolds with MNPs had similar fiber diameters, which were around two times thinner as compared to 14% PCL scaffolds. This could result from the increase in conductivity of the polymeric solution with incorporated MNPs. The fiber diameter was 1.18 ± 0.56 µm for PCL, 0.67 ± 0.22 µm for 5% IONP, 0.59 ± 0.18 µm for 7.5% IONP, 0.55 ± 0.14 µm for 5% CoNP and 0.68 ± 0.19 µm for 7.5% CoNP. The surface morphology is presented in Figure 1.

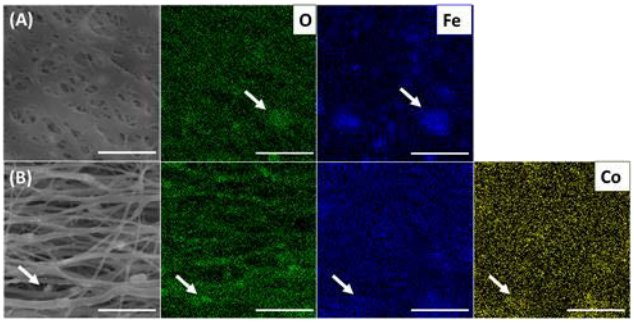

Figure 1: SEM from 7.5% IONP (A) and 7.5% CoNP (B), with the corresponding EDX maps (oxygen (O, green), iron (Fe, blue) and cobalt (Co, yellow)). Scale bar: 10µm

The EDX mapping of elemental composition identified oxygen along the scaffold's fibers as expected for the chemical formula of PCL ($(C_6H_{10}O_2)_n$). In addition, in IONP scaffolds, iron and oxygen were found in higher contrast in the particle agglomerates, as indicated by white arrows in Figure 1. The same was observed for cobalt in the samples with CoNP. The presence of iron and cobalt were mapped in all the area, indicating that the particles are also included inside the electrospun fibers. This agrees with similar research by Guadagno et al. for PCL-IONP electrospun membranes [8].

The mapping is presented only for the samples with higher MNPs concentrations since there was no appreciable difference between the analyzed concentrations. Further work should be performed with transmission scanning microscopy to corroborate the EDX results showing inclusion of MNPs inside the fibers or other techniques as Raman spectroscopy.

3.2 Mechanical properties

Methods for mechanical analysis have been a point of debate due to the non-uniform and heterogeneous cross-sections of electrospun fiber mats. The force strain data is presented to provide a complete picture of the scaffold behavior throughout the loading process. Scaffolds containing MNPs showed different mechanical behavior as compared to scaffolds of PCL alone. Scaffolds with 5% and 7.5% IONP had a maximum force of 5.09 N and 6.02 N and an ultimate strain of 215% and 217%, respectively. Scaffolds containing 5% and 7.5% CoNP showed a maximum force of 1.62 N and 1.45 N and an ultimate strain of 397% for both. The PCL scaffolds had a maximum force of 1.23 N and an ultimate strain of 1051%.

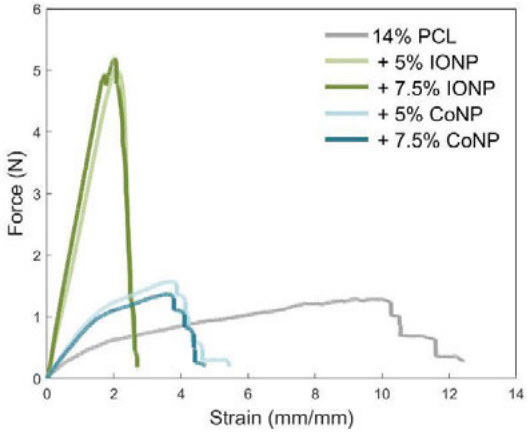

Figure 2: Force-strain diagram of samples compared to pure PCL.

This could mean that the inclusion of MNPs changes the elastic strain range with increase in the relative stiffness of the scaffolds. One cause for this is differences in fiber alignment: PCL scaffolds showed more distinct alignment than CoNP scaffolds, which in turn were more aligned than IONP scaffolds. Delp and Becker et al. [9] recently showed that electrospun scaffolds of aligned fibers go through a plastic "straightening" period before failure. CoNP scaffolds in Figure 1B show certain alignment, which agrees with the plastic deformation shown in Figure 2. IONP scaffolds did not show any alignment (Figure 1A), and fibers were not distinct, which could explain no plastic deformation (Figure 2).

3.3 Cell seeding and biocompatibility

Metabolic results show more activity of the cells seeded on the PCL scaffolds. However, fluorescence values were lower by only 20% for IONP scaffolds, standing out as a better alternative to CoNP (30% decrease) over the 7 days. The increase in the concentration of MNPs results in lower metabolic activity due to possible cytotoxic effects that the released particles and the agglomerates on the surface may have.

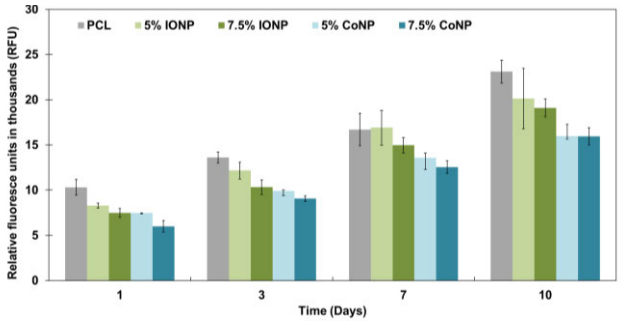

Figure 3: Metabolic activity of the bmMSCs measured using resazurin reduction assay for 10 days in culture.

Despite the decrease in metabolic activity for CoNP, the experiments should be repeated to obtain more statistically significant results. Moreover, osteogenic and neurogenic differentiation of bmMSCs should be conducted, since this scaffold type has been used for stem cell differentiation into neural cells without adding differentiation factors [1,10].

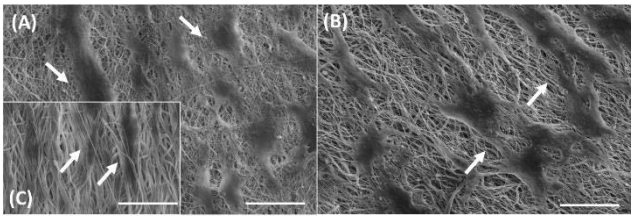

Figure 4: SEM of bmMSCs seeded on the scaffolds on day 7 with 7.5% IONP (A), 7.5% CoNP (B), PCL (C). White arrows indicate cell contact. Scale bar 50µm.

Interaction between cell-scaffold and cell-cell is presented in Figure 4. The bmMSCs had an aligned morphology related to the fiber orientation. Even with the small reduction in metabolic activity, the scaffolds with MNPs have visually larger cells-scaffold adhesion area and cell-cell contact (white arrows) in comparison with PCL.

4 Conclusions

PCL scaffolds containing different amounts of MNPs were successfully obtained using electrospinning. The fiber diameter decreased with the MNPs, and the maximum force increased. The scaffolds showed well in vitro performance with bmMSCs. The cell-cell and cell-scaffold interactions improved with addition of MNPs in comparison with PCL. The morphological, mechanical and biological results suggest a promising application of this type of scaffolds in magnetic field for tissue stimulation and differentiation of bmMSCs.

Author Statement

The authors acknowledge Dr. Yvonne Roger, Orthopedic Clinic of Hannover Medical School, for the bmMSCs, Dr. Sara Knigge, HTTG in NIFE, for support in mechanical testing and Prof. Chichkov, Institute of Quantum Optics at LUH, for support with a multi-well plate reader.

Research funding: The authors acknowledge the funding of German Academic Exchange Service (DAAD, project number 91725466), Caroline Herschel Program Leibniz University Hannover and Fulbright Germany U.S. Student Research Grant.

Conflict of interest: Authors declare no conflict of interest.

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