MODIFICATION OF MICRO-SPHERES' MICROSTRUCTURE FOR APPLICATION AS CELL CARRIERS IN MODULAR TISSUE ENGINEERING

BARTOSZ MIELAN*, MAŁGORZATA KROK-BORKOWICZ, KINGA PIELICHOWSKA, ELŻBIETA PAMUŁA

DEPARTMENT OF BIOMATERIALS AND COMPOSITES,
FACULTY OF MATERIALS SCIENCE AND CERAMICS,
AGH UNIVERSITY OF SCIENCE AND TECHNOLOGY, POLAND
*E-MAIL: BARMIE@AGH.EDU.PL

[ENGINEERING OF BIOMATERIALS 143 (2017) 72]

Introduction

Microspheres (MS) made of resorbable polymer have been proposed as a convenient cell growth support. They may be assembled to form cell constructs or suspended in hydrogels allowing injection into injury location. High relative surface area of MS provides more efficient cell culture environment than traditional 2D culture on flat substrates (multiwall plates, Petri dishes). In addition, MS structure, topography and surface chemistry can be modified to promote cell adhesion and proliferation [1].
The aim of this study was to modify MS properties by varying manufacturing conditions of oil-in-water emulsification to better control structural and microstructural properties of MS and their biological performance.

Materials and Methods

MS1 were prepared by oil-in-water emulsification method by pouring dissolved in dichloromethane (20% wt/vol) poly(L-lactide-*co*-glycolide) (PLGA 85:15, M_n = 100 kDa, M_w = 210 kDa) oil phase into water phase supplemented with 1.5% PVA. MS2 were produced in the same manner but water phase was additionally supplemented with 0.5% NaCl. For MS3 manufacturing oil phase was modified with addition of 20% PEG (M_n = 400 Da). After 24 h MS were vacuum filtered, rinsed with distilled water, dried at 37°C, sieved and fraction >100 µm was collected. MS were analysed with optical microscopes (Axiovert 40, Zeiss and VHX-900F, Keyence), scanning electron microscope (Nano Nova SEM 200) and DSC (DSC1 from Mettler Toledo). DSC measurements were performed in the temperature range of -90÷200°C at heating rate 10°C/min in nitrogen atmosphere, sample mass was ca. 6 mg. MG-63 osteoblast-like cells were cultured in TCPS 48-well Nunclon plates containing 200 µl 10% MS suspension in 70% ethanol for 1, 3 and 7 days at 37°C under 5% CO_2. Cell viability was assessed by Alamar Blue assay (Sigma Aldrich), live/dead staining (calcein AM/propidium iodide, Sigma-Aldrich) with fluorescence and optical microscopy (Axiovert, Zeiss, Keyence VHX-900F).

Results and Discussion

Modification of water phase (MS2) and oil phase (MS3) resulted in differences in appearance, transparency and microstructure of the MS. Addition of NaCl to water phase caused high transparency and low porosity of MS2 (FIG. 1A). When oil phase was modified with PEG400 opposite effect was observed: MS3 were opaque and porous. Microstructure of MS differed between samples and depended on the used modification approaches but in general the surface of MS was smooth and small pores (few µm in diameter) were found. DSC results (FIG. 2) showed that MS differed in crystallinity: MS1 had melting enthalpy ΔH_m = -2.47 J/g, MS2 ΔH_m = -0.92 J/g while MS3 ΔH_m = -4.82 J/g.
Thus modification of water phase with NaCl, which increased its ionic strength, resulted in more amorphous PLGA forming MS. Addition of PEG to PLGA solution (modification of oil phase) resulted in increase in crystallinity, probably due to the fact that PLGA and PEG do not form a physical mixture but undergo phase separation as shown in our previous study [2]. The findings regarding crystallinity are important from the point of view of using MS in cell culture, because it is known that crystallinity influences degradation kinetics of PLGA.

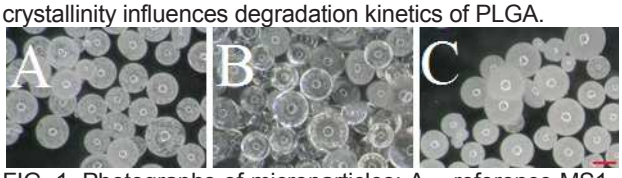

FIG. 1. Photographs of microparticles: A – reference MS1, B – MS2 – water phase modified with NaCl, C – MS3 – oil phase modified with PEG400. Scale bar 100 µm.

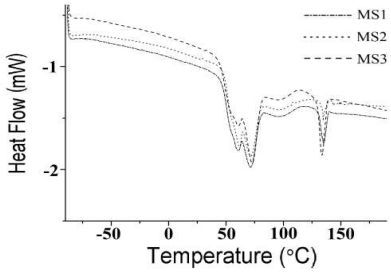

FIG. 2. DSC results of MS: reference MS1, MS2 – water phase modified with NaCl, MS3 – oil phase modified with PEG400.

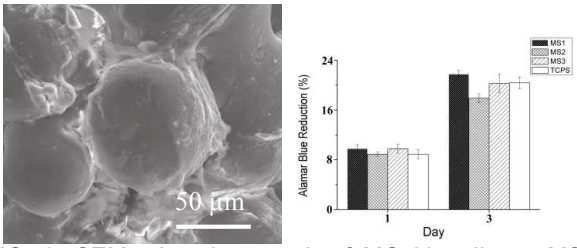

FIG. 3. SEM microphotograph of MG-63 cells on MS3 after 7 days of culture and Alamar Blue results.

In vitro tests showed good cytocompatibility of all MS. After 7 days cells grew on majority of microspheres and created cell-MS agglomerates (FIG. 3). Cell adhesion and proliferation on days 1 and 3 were similar to reference material (TCPS).

Conclusions

Method of emulsification is effective in manufacturing PLGA microparticles in diameter of >100 µm, of controlled crystallinity and morphology and allows for easy modification of these parameters by addition of NaCl and PEG, to water and oil phases, respectively. *In vitro* tests showed that MS support cell growth and formation of MPs-cell agglomerates which indicated their good cytocompatibility.

Acknowledgements

This study was financed from National Science Center, Poland (UMO-2016/21/D/ST8/0185).

References

[1] Hossain KMZ., Patel U., Ahmed I.: Development of microspheres for biomedical applications: a review. Prog Biomater 4:1-19. 2015.
[2] Krok M., Pamuła E.: Poly(L-lactide-*co*-glycolide) microporous membranes for medical applications produced with the use of polyethylene glycol as a pore former. J Appl Polym Sci 125:187-199. 2012.