MODIFICATION OF MICROPARTICLES' MICROSTRUCTURE WITH CARBON DIOXIDE FOR APPLICATION AS CELL CARRIERS IN MODULAR TISSUE ENGINEERING

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Introduction

Microparticles (MP) made of resorbable polymers are considered as convenient tools to culture cells. MP may be assembled to cell constructs or suspended in hydrogels suitable for administration into the tissues by injection. High surface area of MP provides more advanced cell culture conditions than traditional culture on flat substrates. Moreover, MP with proper porosity were found to promote cell adhesion [1].
In this study we aimed to modify microstructure of MP by changing composition of both water and oil phases in the process of emulsification. Particularly we were interested in chemicals that undergo decomposition and release carbon dioxide (CO_2). Our hypothesis was that CO_2 will create pores in the MP. Moreover, we intended to find out how these modifications influence cytocompatibility of MP.

Materials and Methods

MP were prepared by oil-in-water emulsification method by pouring solution of poly(L-lactide-*co*-glycolide) (PLGA 85:15, M_n = 100 kDa, M_w = 210 kDa) in dichloromethane (DCM) to *aq.* solution of polyvinyl alcohol (PVA, Mowiol 4-88, Sigma-Aldrich, avg. M_w 31 kDa) during stirring with magnetic stirrer (250 rpm). Some of oil phases and all of water phases were modified with citric acid or Na_2CO_3. After 24 h MP were vacuum filtrated, rinsed with distilled water and left at 37°C to dry. After that MP were sieved with laboratory sieves (mesh diameter 100 µm) to receive fraction with diameters above 100 µm. Obtained MP were analysed with optical microscope (Axiovert 40, Zeiss, Keyence VHX-900F) to characterise their diameter. Additionally, to observe surface, porosity and roughness scanning electron microscopy (Nano Nova SEM 200) was used. For preliminary assessment of MP cytocompatibility MG-63 osteoblast-like cells were cultured in TCPS 48-well Nunclon plates for 1, 3 and 7 days at 37°C under 5% CO_2 in contact with 2 mg MP per well. Cell viability was measured by Alamar Blue assay (Sigma Aldrich). Live/dead (calcein AM/propidium iodide Sigma-Aldrich) and haematoxylin/eosin staining were used and the samples were observed with fluorescence and optical microscopes (Axiovert, Zeiss and Keyence VHX-900F), respectively.

Results and Discussion

Addition of citric acid and Na_2CO_3 to both phases resulted in differences of MS microstructure. MS obtained when water phase was modified were transparent (FIG. 1 a, b). When both phases were modified and Na_2CO_3-citric acid reaction took place, released CO_2 was encapsulated inside MP creating closed porosity, but also modified roughness of the MP surface. As a result MP became opaque (FIG. 1 c, d).

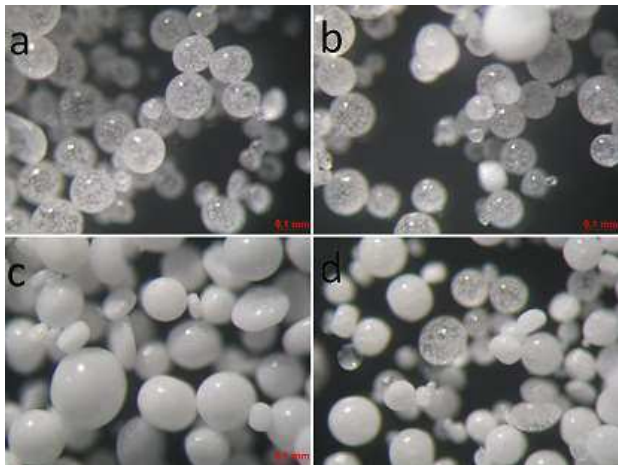

FIG. 1. Photographs of MP obtained from pure PLGA/DCM solution (a, b) poured to 1.5% PVA solution modified with citric acid (a) and Na_2CO_3 (b) and PLGA/DCM with Na_2CO_3 to *aq.* PVA with citric acid (c) and PLGA/DCM with citric acid to *aq.* PVA with Na_2CO_3 (d).

In vitro tests showed good cytocompatibility of microparticles (FIG. 2). After 7 days higher coverage with cells was observed for MP where CO_2 was produced in reaction of citric acid and Na_2CO_3 (FIG. 2 c, d) and those samples showed higher cell viability.

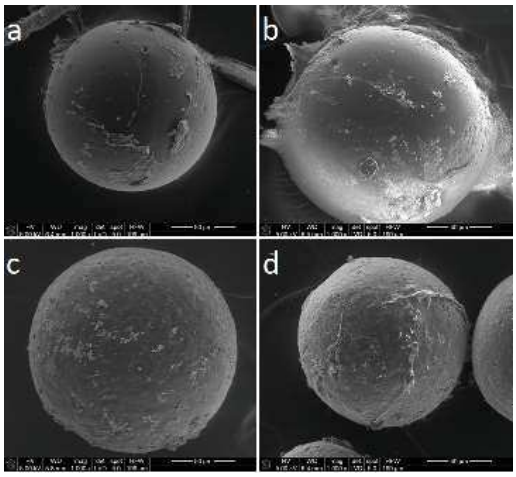

FIG. 2. SEM microphotographs of MG63 cells cultured for 7 days on MS obtained from pure PLGA/DCM solution (a, b) poured to 1.5% PVA solution modified with citric acid (a) and Na_2CO_3 (b) and PLGA/DCM with Na_2CO_3 to *aq.* PVA with citric acid (c) and PLGA/DCM with citric acid to *aq.* PVA with Na_2CO_3 (d).

Conclusions

Method of emulsification allowed to obtain MP differing in diameter, porosity and morphology. Such parameters can be easily modified by addition of other chemicals to both water and oil phases. *In vitro* tests showed good adhesion and growth of cells on MP, particularly those with modified microstructure.

Acknowledgments

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References

[1] Hossain KMZ., Patel U., Ahmed I.: Development of microspheres for biomedical applications : a review. Prog Biomater 4:1–19, 2015.