Open Porous Microscaffolds for Cell Manufacturing and Tissue Engineering by Solid Lipid Templating

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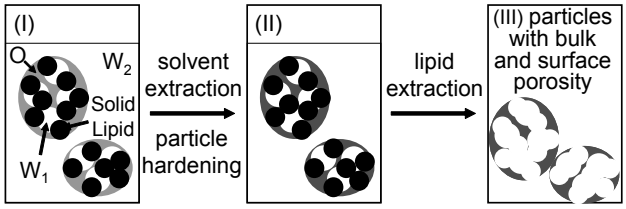
Statement of Purpose: Open porous polymeric microspheres (microscaffolds) have attracted interest as cell carriers for different applications in tissue engineering not only because they can be easily administered using a syringe but also because of the possibility of their application in suspension culture. Due to the small diameters their well interconnected pore network facilitates the transport of nutrients, oxygen and waste throughout the 3-D carrier. To be used in such application, the microscaffolds should be of the following design: diameter: up to 1 mm, high bulk and surface porosity with pores > 50 µm. Conventional fabrication techniques for microparticles often do not provide the controlled generation of interconnected macroporous networks necessary to ensure the migration of cells into the microscaffold. This study presents a novel fabrication technique for microscaffolds that combines an adjusted double emulsion technique with Solid Lipid Templating (SLT) [1] to yield particles of desired structure. Key processing parameters and their effects on microscaffold structure are described.

Methods: *Microscaffold fabrication (scheme 1)*: Solid lipid particles were fabricated using a melt dispersion technique, dried and sieved into the desired size range of 50 – 150 µm. Following a $W_1/O/W_2$ double emulsion method strategy, the inner aqueous phase containing HPMC was mixed with different amounts of solid lipid particles (W_1). PLGA (Resomer® RG 756, Boehringer, Ingelheim) was dissolved in a mixture of acetone and methylene chloride with or without the addition of a second pore forming lipid (O). The resulting emulsion (W_1/O) was poured into an aqueous solution of 0.1% HPMC as stabilizing agent (W_2) and emulsified (I) using an overhead propeller stirrer at varying speed for at least 4 hours to evaporate the polymer solvent mixture (II). Hardened polymeric microspheres were collected by filtration, dried overnight and immersed in n-hexane to leach out the remaining embedded lipids (III). *Microscaffold characterization*: Sieve analysis and laser diffractometry were used to determine microscaffolds size distribution. Particle morphology was analyzed by optical microscopy and scanning electron microscopy, intrusion tests were done using a n-propanol/water mixture.



Scheme 1. Microscaffold fabrication process

Results: A classic double emulsion technique for the fabrication of polymeric microparticles was modified by increasing the viscosity of aqueous phase W_1 in order to allow for the incorporation of dispersed solid lipid microspheres. The stable dispersion/double emulsion was processed to yield particles with incorporated solid lipid. After lipid extraction, microparticles with interconnected bulk porosity and open surface pores were obtained (Fig. 1). The effect of amount of dispersed solid lipid particles on microscaffold porosity was determined. Higher amounts of lipid particles yielded microscaffolds with higher porosity. Surface porosity was mainly dependend on the presence of a second lipid soluble in the polymer solvent.

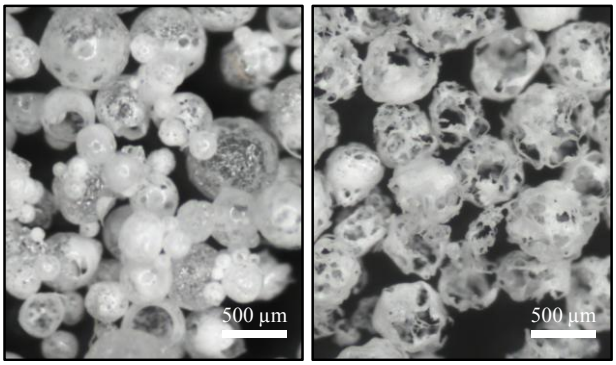


Figure 1. Light micrographs of microscaffolds fabricated without (left) and with (right) dispersed solid lipid particles.

Microscaffolds size distribution was determined by laser diffractometry. Effects of stirring speed and concentration of polymer solution on particle size distribution were evaluated. Mean diameters decreased with increasing the stirring speed from 600 rpm to 900 rpm and decreased with decreasing polymer concentration. An increase in the amount of lipid particles as well as the addition of dissolved lipid or higher concentrated polymer solutions resulted in larger mean diameters of the resulting microscaffolds.

Conclusions: Macroporous microscaffolds with open surface pores were successfully fabricated by solid lipid templating combined with a $W_1/O/W_2$ double emulsion technique. Processing parameters that control particle size distribution and surface porosity were identified and described. Microsaffold of desired structure were synthesized and results of early biocompatibility studies are presented.

References: Hacker M. et al. Biomaterials 28: 3497-3507 (2007).