Preparation of Polyvinyl Alcohol (PVA) microspheres using freeze-thaw cycling methods

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Statement of Purpose:

Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer, with excellent film forming, emulsifying, and adhesive properties. Preparation of PVA microsphere without inclusion of toxic solvents and/or chemical cross-linkers is a challenging. The purpose of this study was to determine the optimal conditions that can be used to prepare sufficient amount of PVA microspheres though physical freeze-thaw cycling approach.

Methods:

Experiment design: Briefly, the microsphere was prepared through a water/oil (w/o) emulsification process. The freeze-thaw cycling was performed for physically crosslinking. Five crucial factors were tested to determine the efficiency of microsphere formation: stirring speed (x), number of freeze-thaw cycling (p), PVA concentration wt/vol (y), PVA/vegetable oil ratio (z), and cycling at room temp (n). Freeze-thaw cycling was performed at -20ºC for physical cross-linking. With the orthogonal experiment design (Table 1), the number of experiments was reduced to 16. 10% of Sodium dodecyl sulfate (SDS) (0.1g/mL) was dissolved into PVA solution as emulsifier.

Particle morphology and size-distribution: The particles were visualized with a light microscope (Zeiss) at 5×, 10×, 20×, and 40×. Particle-size distribution was analyzed and quantified based upon 5× images. Selected microspheres were loaded onto alumina film and 3-D Ca-P ceramics scaffold. The microstructure of microspheres was analyzed by scanning electron microscopy (Hitachi).

Cytotoxicity: The PVA particles were extracted into an aqueous phase in a 3:1 water/sample ratio. Mouse MC3T3 preosteoblast cells were seeded onto a 24-well plat in a density of 2,000 cells/well in the presence of PVA (aq) particles. Cells were cultured in the αMEM medium (invitrogen) for 1 week at 37ºC. The medium was replaced every 3 days. Live/Dead cell kit (invitrogen) was used to determine cell viability after cell culture. PVA-untreated cells were included as a control.

Test No.	Level combination	Test factors				
		x /rpm	*p*	*y* / PVA%	*z*	*n*
1	$A_1B_1C_1D_1E_1$	250	3	5	1:20	5
2	$A_1B_2C_2D_2E_2$	250	4	8	1:15	6
3	$A_1B_3C_3D_3E_3$	250	5	11	1:10	7
4	$A_1B_4C_4D_4E_4$	250	6	13	1:5	8
5	$A_2B_1C_2D_3E_4$	500	3	8	1:10	8
6	$A_2B_2C_1D_4E_3$	500	4	5	1:10	7
7	$A_2B_3C_4D_1E_2$	500	5	8	1:20	6
8	$A_2B_4C_3D_2E_1$	500	6	13	1:15	5
9	$A_3B_1C_3D_4E_2$	750	3	11	1:5	6
10	$A_3B_2C_4D_3E_1$	750	4	13	1:10	5
11	$A_3B_3C_1D_2E_4$	750	5	5	1:15	8
12	$A_3B_4C_2D_1E_3$	750	6	8	1:20	7
13	$A_4B_1C_4D_2E_3$	1,000	3	13	1:15	7
14	$A_4B_2C_3D_1E_4$	1,000	4	11	1:20	8
15	$A_4B_3C_2D_4E_1$	1,000	5	8	1:5	5
16	$A_4B_4C_1D_3E_2$	1,000	6	5	1:10	6

Tables 1: Orthogonal experimental scheme. (x: rpm, p: freeze-thaw cycles,
y: PVA % (wt/vol), z: PVA-to-oil ratio, n: cycling at room temp).

Results:

Spherical particles were observed for samples No. 2, 5, 7, 9, and 1 (Fig 1)
No spherical particles were observed at the concentration of 13% PVA. Particle size decreased with increasing stirring speed (Fig 1,f).

With increasing particle size, the distribution percentage decreased (Fig 2)
The major proportion of particles ranged from 1.5 to 100 μm for all samples with spherical particles.

A reduced cell proliferation was found in PVA microsphere- treated MC3T3 cells (Fig 3). We found that PVA microsphere inhibited cell growth, as manifested by the reduced cell number (live and dead staining), compared to PVA untreated cells. This is probably due to the addition of SDS to the system as an emulsifier.

Discussion and Conclusions:

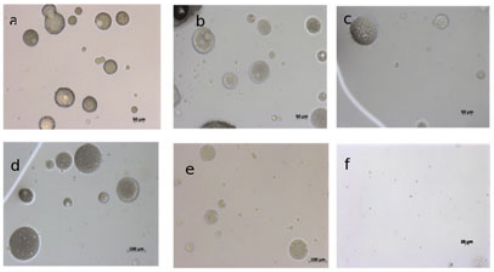

Fig 1: Particle morphology 10× for samples w/spherical particle morphology (a: #2, b: #5, c: #7, d: #9, e: # 1, f: #11)

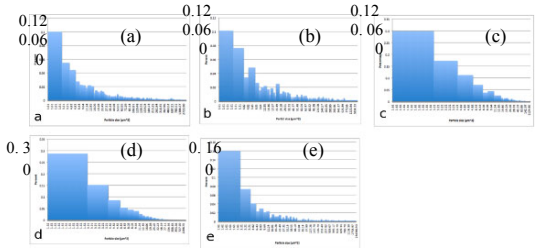

Fig 2: Particle-size distribution. (a: #1, b: #2, c: #5, d: #7, and e: #9). X-axis: particle size, 1.6 to 2700 (μm^2), Y-axis: size-distribution. With the size increasing, the distribution percentage of particles is decreased.

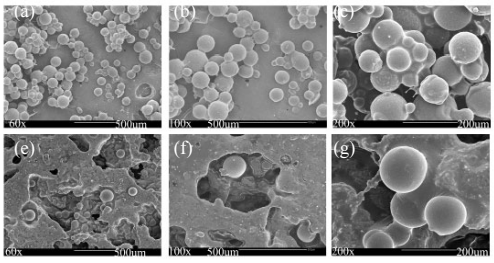

Fig 3: SEM Morphology of (a-c) microsphere spread on a film and (e-g) microsphere loaded into a 3D Ca-P scaffold

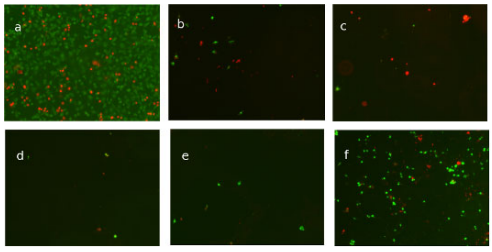

Fig 4: Live/Dead staining of MC3T3 cells co-cultured with 100uL of PVA microspheres suspended aqueous solution in well plate for 7d. (a: control, b: #9, c: #7, d: #5, e: #2, f: #1)

Our data demonstrated that (1) the increase of stirring speeds will lead to reduction of PVA microsphere size; (2) Microsphere could not be when the concentration of PVA is ~13%; (3) PVA microsphere- induced cytotoxicity is related to the incorporation of SDS during process, and (5) The particle-size distribution displayed an exponential decay pattern. As particle size increased, the proportion comprising the sample decreased. Based on these preliminary data, additional experimental approaches have been designed to minimize, or eliminate the use of SDS in PVA microsphere preparation and to further improve the purification and production of PVA microspheres.

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