**Primary Hepatocyte Culture on Polymeric Microsphere Scaffold with Human Hepatocyte Growth Factor Release**

Xin Hao ZHU[a], Chi-Hwa WANG[a], Yen Wah TONG[a,b]
[a]Department of Chemical & Biomolecular Engineering, National University of Singapore
[b]Division of Bioengineering, National University of Singapore
10 Kent Ridge Crescent, Singapore 119260

**Statement of Purpose:** A polymer scaffold with suitable three-dimensional structure to support cell growth as well as to provide suitable signal molecules, such as growth factor and cytokine, to regulate cell behavior, like migration, proliferation and differentiation would be the key to the ultimate success of tissue engineering. Our previous works have demonstrated that polymer microspheres are promising scaffolds to guide liver cell growth and the easily modified surface make the scaffolds more conductive and versatile for cell growth [1]. In order to further prove the viability of microsphere as a tissue engineering scaffold for growth factor delivery purpose, we prepared a PHBV/PLGA composite microsphere which can controlled deliver human hepatocyte growth factor (huHGF) up to three months with stable bioactivity.

**Methods:** A water-in-oil-in-water (w/o/w) double emulsion technique was used to fabricate the PHBV/PLGA (1:1, w/w) microspheres as well as to encapsulate huHGF. The microspheres were characterized by SEM and *in vitro* release. The bioactivity of released huHGF was tested using Hep3B cells since the growth of this cell line can be inhibited in the presence of bioactive huHGF [2].

**Results/Discussion:**

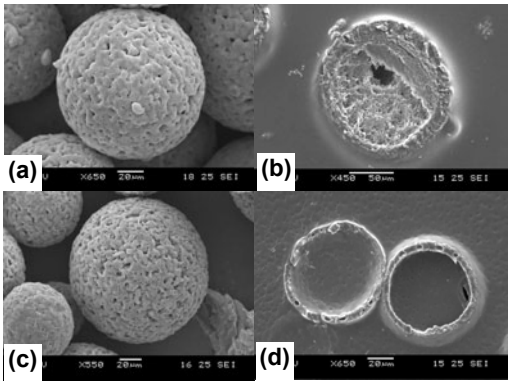


Figure 1 (a) and (b) SEM images of PHBV/PLGA microspheres; (c) and (d) partially dissolved microspheres with acetone

As shown in Figure 1, PHBV/PLGA microspheres show a core-shell structure, a porous core surrounded by a relative dense shell, which was due to the phase separation of the two polymers to get the most stable thermodynamic configuration [3]. The microspheres were suspended in acetone for 3 hrs followed by wash with DI water. It is interesting to see that the core of the composite microspheres was totally dissolved resulted in a hollow structure [Figure 1 (c) and (d)]. Since PHBV does not dissolve in acetone, the results suggest that the core is occupied by PLGA, while PHBV is located at the shell.

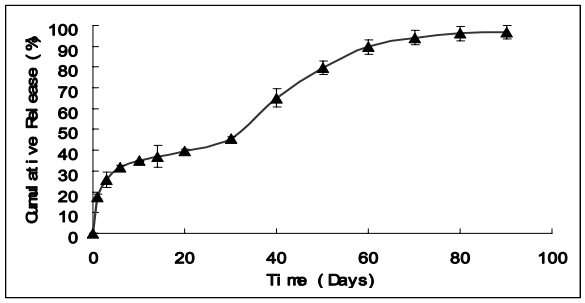


Figure 2 Cumulative release profile of huHGF from PHBV/PLGA composite microspheres with a 0.002% drug loading.

The release profile of huHGF up to three months was shown in Figure 2. The fast initial release was attributed to the porous surface which allowed the diffusion of proteins easily and the second accelerated release phase was mainly due the degradation of the core material. The hydrophobic PHBV shell could slow down the degradation as well as the release.

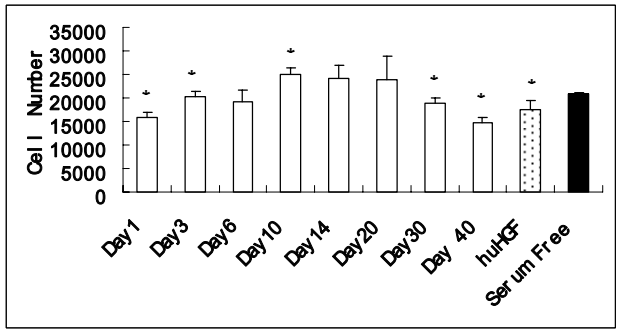


Figure 3 Bioactivity of huHGF assessed after 24 hr of incubating released huHGF with Hep3B cells by measuring cell proliferation. (□) released huHGF, (⊡)huHGF in serum free medium, 50 ng/ml, (■) serum free medium.

HuHGF is believed to inhibit the growth of a lot of cancer derived cells including Hep3B. The arrested growth of Hep3B cells as shown in Figure 3 indicated a sustained bioactivity of the growth factor.

**Conclusions:** A PHBV/PLGA composite microsphere with core-shell structure was successfully prepared by using solvent evaporation method. Long term release of huHGF from the microsphere was achieved with stable bioactivity. This composite microsphere is currently being applied as the tissue engineering scaffold for the in vitro culture of primary hepatocytes.

**References:**

[1] Zhu XH. J Biomed Mater Res: Appl Biomater. 2006 (online)
[2] Shiota G. Proc. Natl Acad. Sci. USA. 1992; 89:373-377.
[3] Pekarek KJ. Adv Mater. 1994; 6:684-687.