# The Effects of Cell Density on Viability within PEGDA Hydrogel Microspheres

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**Statement of Purpose:** Cell microencapsulation can be used in tissue engineering as a scaffold or a physical barrier that provides immunoisolation. Microencapsulation shields cells from the host immune system when they are delivered into the body for cell therapies. In order to maximize the delivery of therapeutic product per volume of microencapsulated cells, we must optimize the seeding density of our hydrogels. In this study we microencapsulate mouse preosteoblast cells (MC3T3-E1) within poly(ethylene glycol) diacrylate (PEGDA) hydrogel microspheres at low, medium, and high seeding densities in order to assess the viability of these cells as a function of cell density.

**Methods:** Cells were microencapsulated as previously described.[1] Briefly, a prepolymer solution containing 0.1 g/ml 10 kDa PEGDA, 1.5% (v/v) triethanolamine/HEPES buffered saline (HBS, pH 7.4), 37mM 1-vinyl-2-pyrrolidinone, and 1.0 mM eosin Y was combined with a MC3T3-E1 cell suspension to achieve final concentrations of 2000, 10000, and 25000 cells/µL. The prepolymer solution was combined with mineral oil and agitated under white light to produce a vortex-induced emulsion and simultaneously photopolymerize the droplets. Cell-laden microspheres were then harvested and maintained in a humidified incubator at 37°C with 5% $CO_2$. Calcein AM/ethidium homodimer-1 live-dead assays were performed and microencapsulated cells were imaged using Zeiss Axiovert epifluorescent microscope. Images were analyzed with NIH ImageJ software.

**Results:** Our results showed that microspheres seeded with a higher density of cells have a greater viability than microspheres seeded at lower densities (Figures 1 and 2).

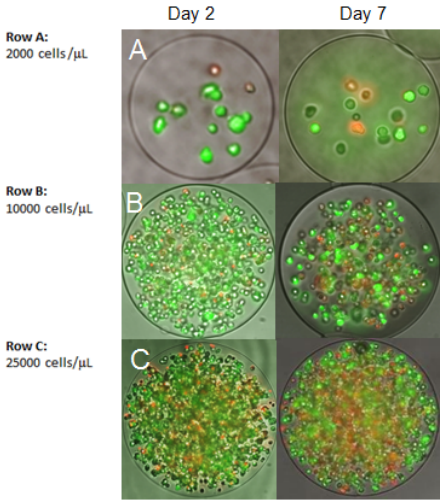


**Figure 1**. Fluorescent and phase contrast merge of microspheres at low, medium and high seeding densities at Days 2 and 7 (10x magnification). Live cells fluoresce green, dead cells fluoresce red.

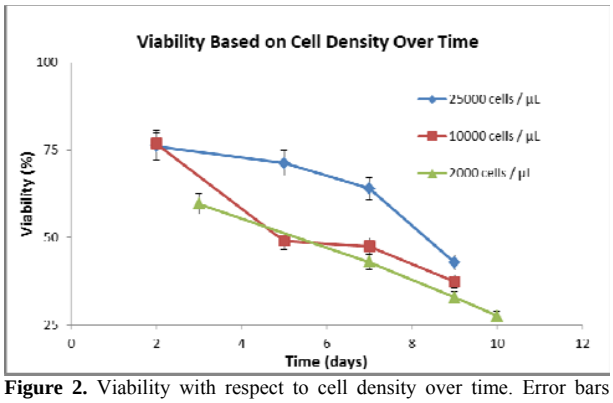


**Figure 2.** Viability with respect to cell density over time. Error bars show standard error.

**Conclusions:** Although microspheres seeded with a lower cell density should have less competition for oxygen and nutrients, and therefore higher cell viability than microspheres seeded at higher cell densities, our results were counterintuitive. Previous studies have demonstrated that in low density cell cultures, osteoblasts undergo apoptosis when serum is absent from the media. This effect is counteracted in high density cell cultures, where neighboring cells produce enough stimulatory growth factors to replace the function of serum. Commercially available fetal bovine serum contains over 90 identified proteins of varying molecular weights.[2] Although our media contained serum, the microspheres in our study were formed with 10% 10 kDa PEGDA, which has been estimated to have a mesh size of 280 Å.[3] It is possible that the mesh size of these hydrogel microspheres is not large enough to permit the free entry for all serum proteins, which can prevent apoptosis at lower densities. We are continuing to investigate the effects of larger molecular weight PEGDA with cell viability to confirm this.

## References

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3. Liao et al., 2008. "Influence of hydrogel mechanical properties and mesh size on vocal fold fibroblast extracellular matrix production and phenotype". Acta Biomaterialia. 4(5):1161-1171.