Implantable hydrogel beads entrapping PLGA-paclitaxel microspheres: Exploring the effects of near-zero-order drug release for intracranial chemotherapy

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INTRODUCTION

Local intracranial chemotherapy using drug-loaded microspheres has shown considerable potential in many pre-clinical studies [1,2]. However, its translation into clinical trials has been hindered by two major disadvantages: implantability and excessively high initial drug release. Intra-tumoral injection of microspheres (suspended in saline) reduces the efficiency of implantation due to the expelling of microspheres from the target site because of high interstitial pressure in the tumor. Also, owing to the concentration of drug near the surface, the microspheres exhibit a high initial drug release that can cause neurotoxicity. In an effort to overcome these limitations, we have developed alginate hydrogel beads that entrap PLGA-paclitaxel microspheres.

Thus in this study, the main objectives were to design, develop and evaluate a novel composite implant that couples the sustained release properties of PLGA-paclitaxel microspheres with the intracranial implantability advantages of alginate matrix for post-surgical chemotherapy to treat malignant glioma. PLGA-paclitaxel microspheres were fabricated using Electrohydrodynamic Atomization (EHDA) since this process was reported to be very efficient in controlling the microsphere size, morphology and monodispersity (3). An electrospray technique in dripping mode was utilized to entrap the microspheres in alginate hydrogel beads owing to its ease of operation and reported monodispersity (4). Furthermore, the effect of various parameters such as gelation time, gelation bath concentration and microsphere loading on in vitro drug release was studied followed by cell culture studies (evaluation of cytotoxicity against C6 glioma cells). Studies of drug release sustainability of the hydrogel beads, apoptosis against C6 glioma cell in vitro, and in vivo anti tumor efficacy in a subcutaneous tumor model are ongoing. These studies will be followed by in vivo intracranial studies such as drug bio-distribution and treatment efficacy (tumor volume and survival analysis) and are warranted.

EXPERIMENTAL METHODS

Fabrication of PLGA-Paclitaxel Microspheres by EHDA Process

Fabrication of PLGA-paclitaxel microspheres was performed using EHDA as reported earlier (3).

Fabrication of Alginate Beads Entrapping PLGA-Paclitaxel Microspheres by Electrospray

The electrospray process as depicted in Fig. 1 was employed to entrap PLGA-paclitaxel microspheres in alginate beads. The setup constituted a high voltage generator, syringe pump with syringe and a metal tip (tapered conical section made of copper sheet) and a grounded stage. The metal tip was connected to the syringe and used instead of a needle to avoid clogging by the microspheres. A petri dish filled with $CaCl_2$ solution was placed on the grounded stage. PLGA-paclitaxel microspheres were suspended in deionized water (with 0.1% (w/v) of Tween 80) to form a uniform dispersion. Sodium alginate was added to the dispersion at 1% (w/v) and then completely dissolved to result in a viscous suspension. The suspension was then pumped at 1 ml/min using the syringe pump with the metal tip connected to a high voltage generator at 6 kV. Due to the high voltage at the metal tip, the suspension broke into monodisperse droplets and was dripped directly into $CaCl_2$ solution. The alginate in the droplets instantaneously underwent gelation in $CaCl_2$ solution resulting in calcium crosslinked hydrogel beads entrapping microspheres in the matrix. In addition, hydrogel beads were also fabricated by manually dripping without the use of the electrospray and were used to compare with the beads fabricated by the electrospray.

The following preparative variables were evaluated in an effort to understand their effect on drug release profiles: Gelation time (1, 5 and 15 min), $CaCl_2$ concentration (0.5, 1, and 2% w/v) and microsphere loading (50, 80, and 90% w/w). Following gelation, the beads were washed with deionized water (to remove non-crosslinked Ca^{2+} ions) and freeze-dried for at least 7 days to remove water and residual DCM. The beads were sterilized by exposing to UV light for 12 hours before using for cell culture and animal studies. Table 1 shows the different formulations fabricated and evaluated.

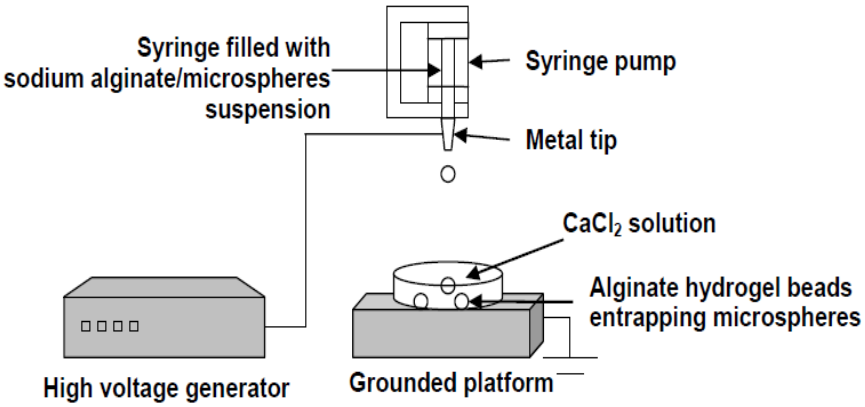


Figure 1: Schematic representation of microsphere entrapment in hydrogel beads using electrospray.

Table 1. Preparative Variables for Hydrogel Bead Fabrication and Respective Formulations

Preparative Variables	**Formulations**								
	H50	M50	L50	H80	M80	L80	H90	M90	L90
Microsphere Loading (% w/v)	50	50	50	80	80	80	90	90	90
Gelation Time (min)	15	5	1	15	5	1	15	5	1
$CaCl_2$ Concentration (% w/v)	2.0	1.0	0.5	2.0	1.0	0.5	2.0	1.0	0.5

RESULTS AND DISCUSSION

Physicochemical Characterization of Hydrogel Beads

Morphology, Size Distribution, Uniformity of Microsphere Entrapment and Loading Efficiency

The beads were prepared using two types of dripping methods, manual dripping (MD) and electrospray dripping (ED), after which they were characterized and compared. Manual dripping yielded polydisperse non-spherical beads with a non-uniform distribution of the microspheres within them (data not shown). The ED method yielded highly monodisperse spherical beads with a uniform microsphere distribution within them as revealed by the SEM images in Figure 2. Also, from Table 2 we can observe that the hydrogel beads possess acceptable monodispersity. However, the ED method yielded hydrogel beads with better uniformity in microsphere distribution compared to the MD method; hence, for further studies beads fabricated using the ED method were used.

Table 2. Physicochemical Characterization of Hydrogel Beads Fabricated by Electrospray

Formulations	**Average Bead Diameter (mm)**	**Loading Efficiency (%)**
50% microsphere loaded beads	1.61 ± 0.041	144.2 ± 11.07
80% microsphere loaded beads	1.65 ± 0.057	73.5 ± 11.02
90% microsphere loaded beads	1.68 ± 0.046	52.1 ± 3.77

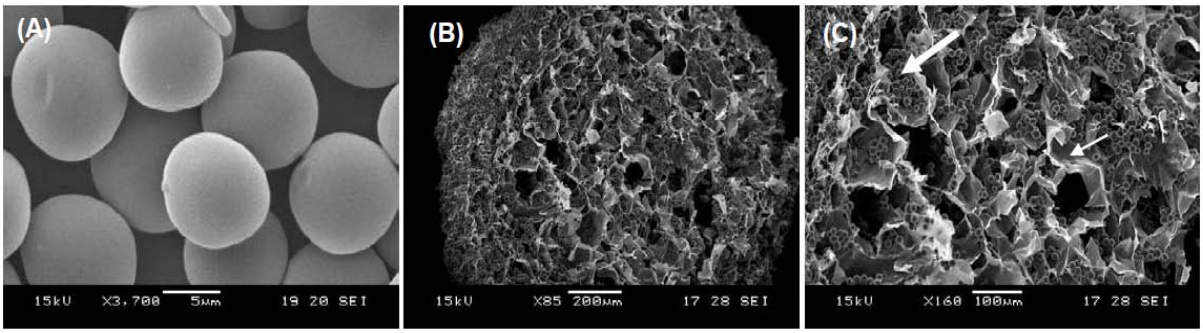


Figure 2: SEM images of hydrogel beads entrapping PLGA-paclitaxel microspheres. (A) PLGA-paclitaxel microspheres; (B) & (C) Alginate hydrogel beads entrapping PLGA-paclitaxel microspheres. Big arrow shows microspheres, while small arrow shows alginate matrix.

Thermal Analysis of Hydrogel Beads

Differential scanning calorimetery (DSC) was performed in order characterize the solid-state properties of paclitaxel encapsulated in the alginate gel beads by thermal analysis on the melting of crystalline regions and phase transitions. Polymer-drug crystallinity of PLGA microspheres in alginate is a key concern related to rate-controlled delivery and drug delivery efficiency. It has been established that molecular dispersions give better release profiles as compared with particulate dispersions of the same drug [5]. The characteristic DSC thermographs were obtained for the individual components of the bead. The thermograms for pure calcium alginate, PLGA 50:50 and pure crystalline anhydrous paclitaxel were analyzed (data not shown) and then compared with the thermograms of the hydrogel beads.

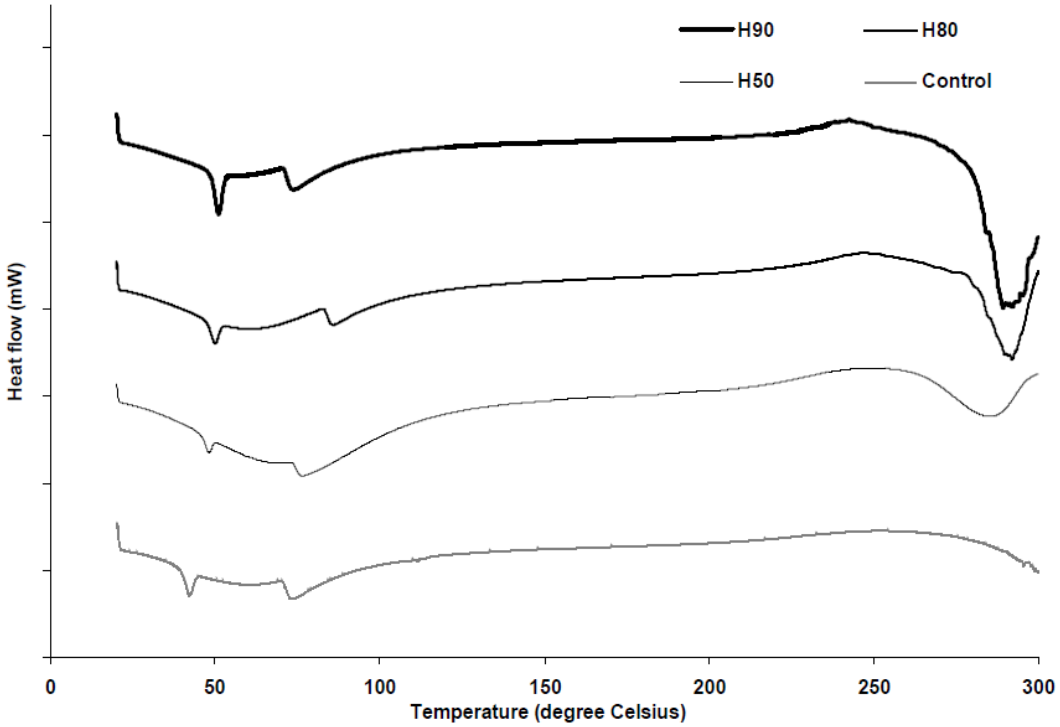


Figure 3: Thermograms of H90, H80, H50 and control beads (no paclitaxel).

According to the DSC thermograms in Fig. 3 the endotherms for the pure and anhydrous paclitaxel were missing in all the formulations. This indicated that the drug was in an amorphous phase in the polymer matrix after the EHDA and electrospray dripping processes. Decreasing the proportion of microspheres to alginate resulted in thermograms that resembled those of the pure calcium alginate. Furthermore, an increase in glass transition temperature was noted as increasing proportions of drug-loaded microspheres were used in each formulation. As the percentage of microsphere loading increased, the increase in T_g may be explained by the additional endothermic overlap, representing the energy required to overcome the microstructure of the polymer which had developed during the EHDA process [6]. These results suggested that paclitaxel was in an amorphous state that was ideal for drug diffusion.

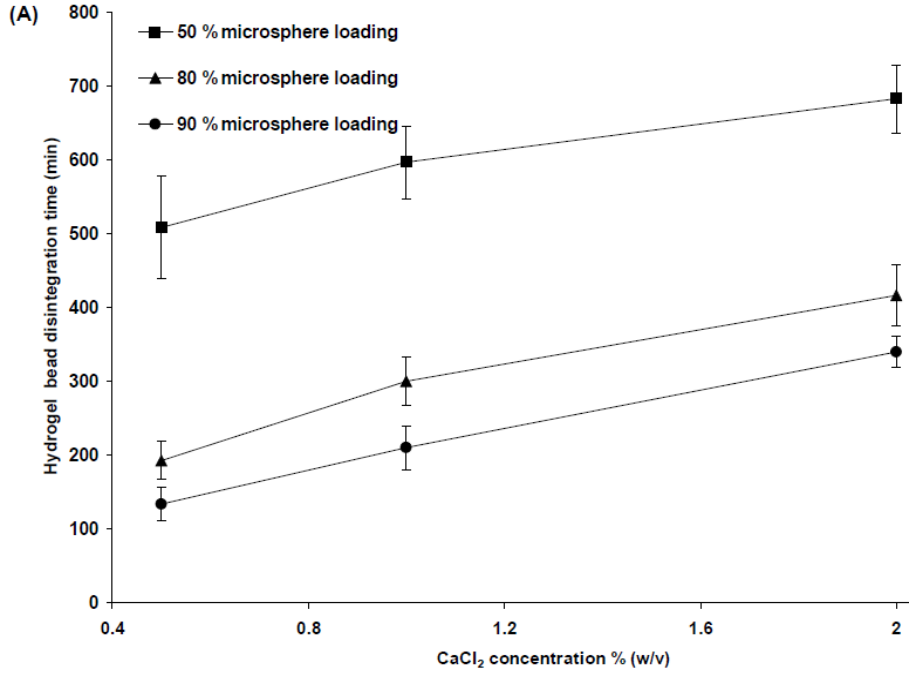
In vitro Characterization of Hydrogel Beads

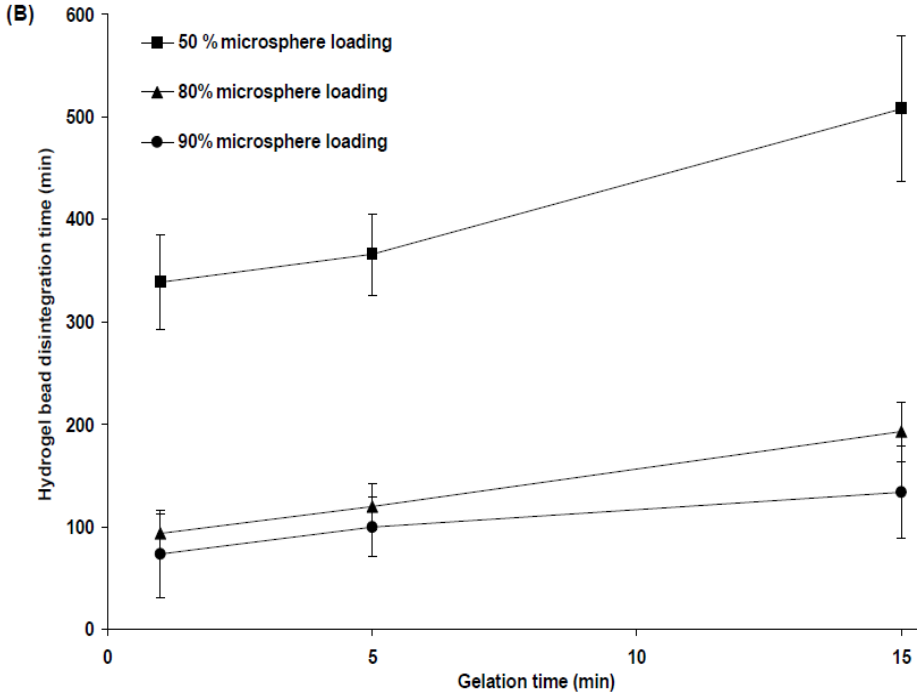
Hydrogel Bead Disintegration Study

The disintegration time of hydrogel beads containing the entrapped microspheres is affected by gelation time, calcium chloride concentration in the gelling bath and microsphere loading per bead. Experiments were performed by varying the three parameters to evaluate the disintegration time.

When subjected to monovalent ions in the buffer solution, the beads were disintegrated by the substitution of mainly sodium ions with crosslinked calcium ions. Longer exposure of beads to the counter ion solution (Fig. 4A) and exposure of beads to a more concentrated counter ion solution (Fig. 4B) increased the extent of cross-linking resulting in stronger beads. This increased the disintegration time under the simulated conditions. However, in Fig. 4C, as the microsphere loading increased, both the effects of calcium concentration and gelation time on disintegration time diminished. Reduced slopes were observed as the microsphere loading

increased from 50 to 90%; this was due to the decreasing amount of alginate content and lower extent of calcium crosslinking. These data suggested that by controlling the three parameters, disintegration time of the beads can be controlled.





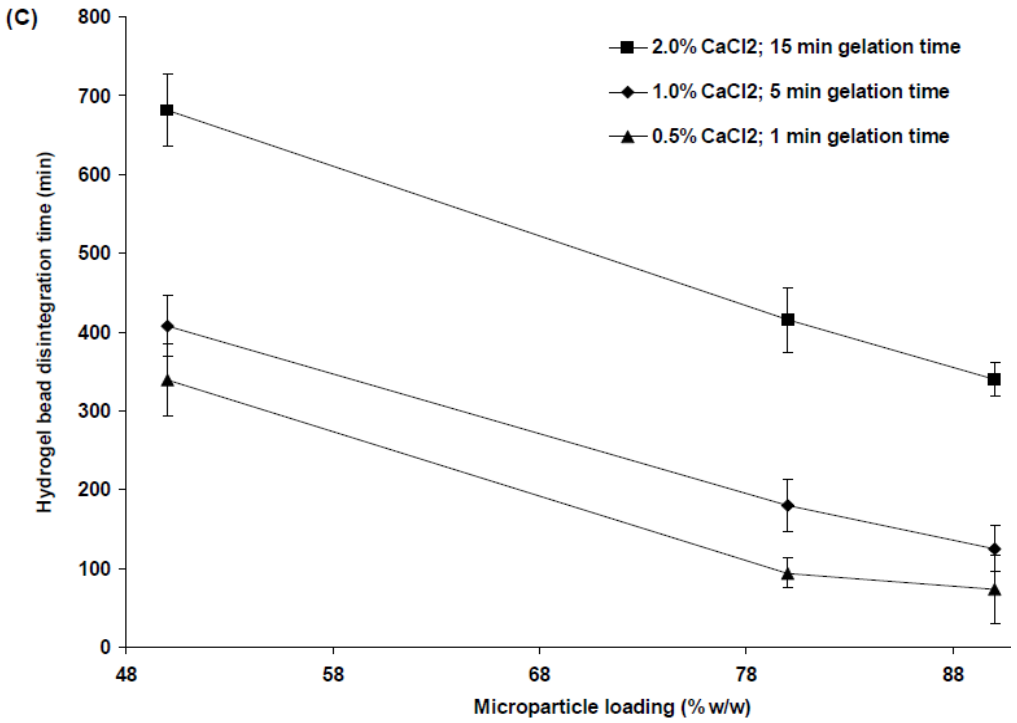
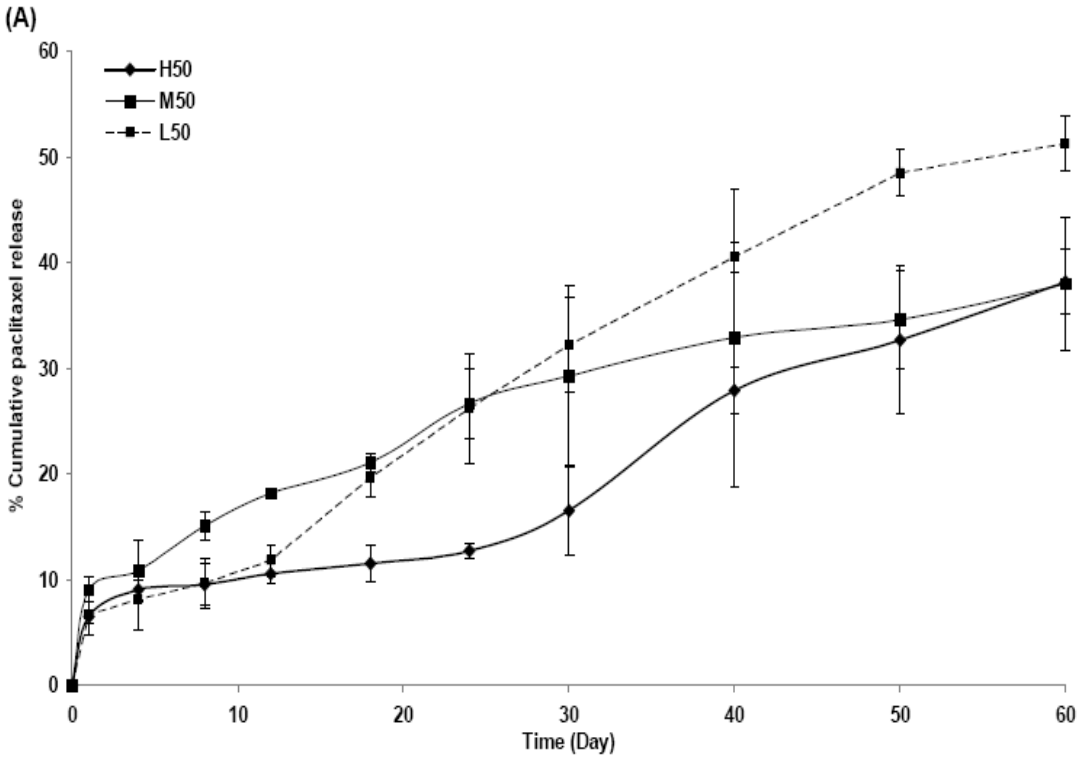


Figure 4: (A) Effect of gelation bath concentration on bead disintegration time (at 15 minute gelation time); (B) Effect of gelation time on bead disintegration time (at 2% w/v gelation bath concentration); (C) Effect of microsphere loading on bead disintegration time.

In vitro Paclitaxel Release from Hydrogel Beads

The nine formulations stated in Table 1 were used in this study to evaluate paclitaxel release under simulated physiological conditions in vitro. Figure 5 depicts the release profiles of the formulations.



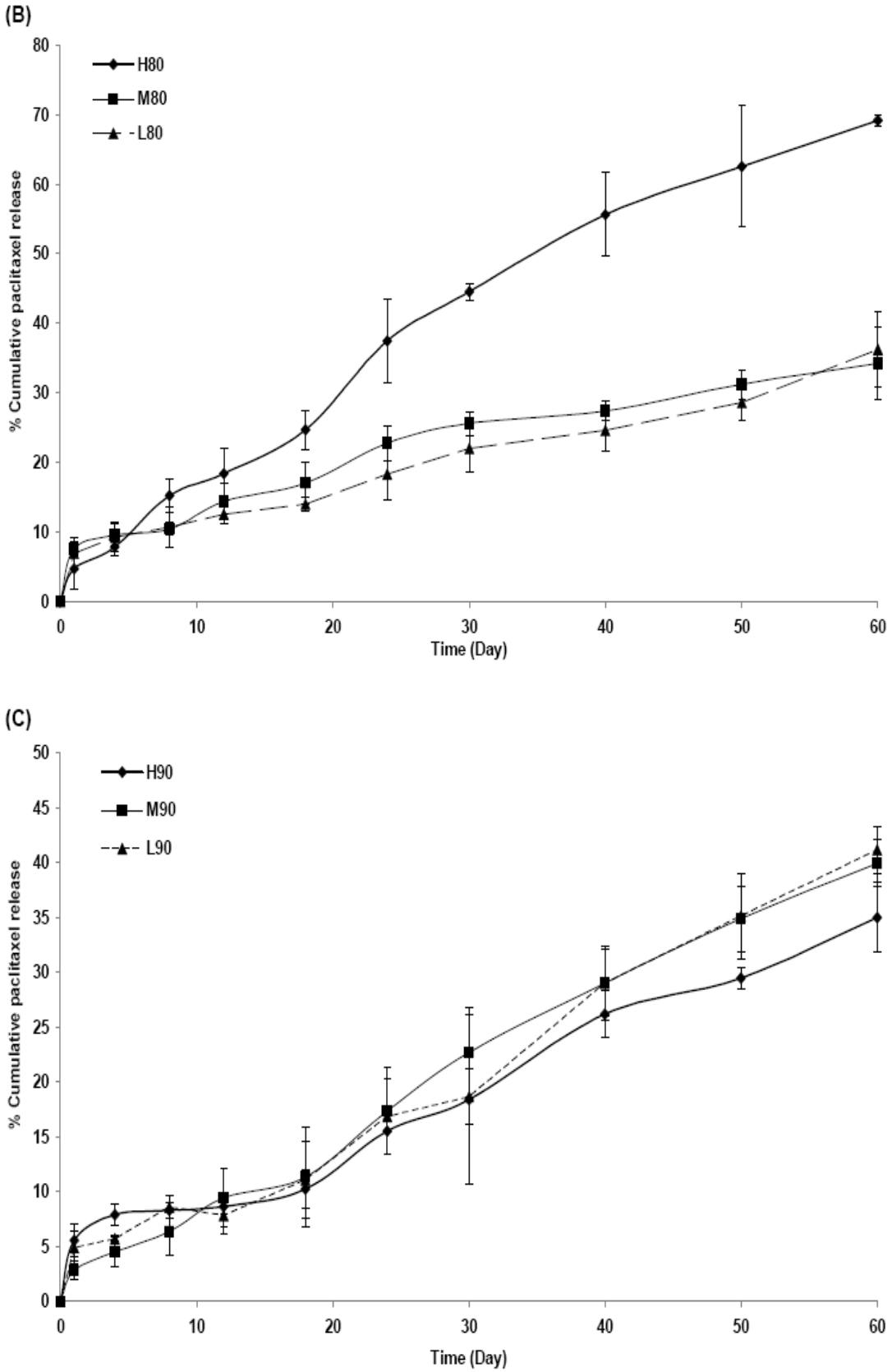


Figure 5: In vitro paclitaxel release from (A) 50 % microsphere loaded beads; (B) 80 % microsphere loaded beads; (C) 90 % microsphere loaded beads under specified conditions in Table 1.

From Figure 5 it can be noted that as the microsphere loading increases, a decrease in the drug initial burst results. The reason could be that at lower microsphere loading the microspheres were less compactly distributed and had good contact with the release buffer. Whereas in case of the higher microsphere loaded beads, due to densely distributed microspheres the initial burst was minimized. The later part of the release profile for all the formulations was near zero order. Although 50% microsphere loaded beads exhibited the highest initial burst, the release rate later was relatively slow. This could be due to the barrier of non-disintegrated alginate matrix through which the drug has to diffuse out. Whereas the 90% microsphere loaded beads exhibited a slow release rate probably because the higher compaction resulted in low penetration of buffer into the bead. Of the three formulations, the 80% microsphere loaded beads exhibited the highest release rate (especially H80) with near zero-order kinetics; this may be because this formulation has the optimum loading (optimum microsphere compaction and degree of crosslinking) that results in low initial burst and higher release rate with zero-order kinetics. Hence, the H80 and M80 formulations were screened for the subsequent in vitro cell culture and in vivo animal studies. Also, H80 and M80 having different release rates would be useful to neurosurgeons for applying them for patient-specific glioma chemotherapy.

In vitro Cytotoxicity Against C6 Glioma Cells

The H80 and M80 beads were evaluated against C6 glioma cells in vitro for their cytotoxic potency using IC_{50} values. IC_{50} refers to the concentration of the drug required to inhibit 50% of cell growth in vitro against the control sample. The C6 glioma cells were treated with control (no drug), H80 and M80 beads at different paclitaxel concentrations for three days. Figure 6 shows the decrease in cell viability after three days of incubation with varying paclitaxel concentrations. The M80 has an IC_{50} value of 16 µg/ml while the H80 has 37 µg/ml. This difference could be explained by the in vitro release profiles. After 3 days, the M80 exhibits a higher release (around double) of drug compared to the H80 and thus is more potent. However the IC_{50} values of both M80 and H80 correspond to that of Taxol®, which is reported to be about 30 µg/ml for a three-day incubation period [3]. In fact, the M80 outperformed Taxol® while the H80 had a similar IC_{50} value.

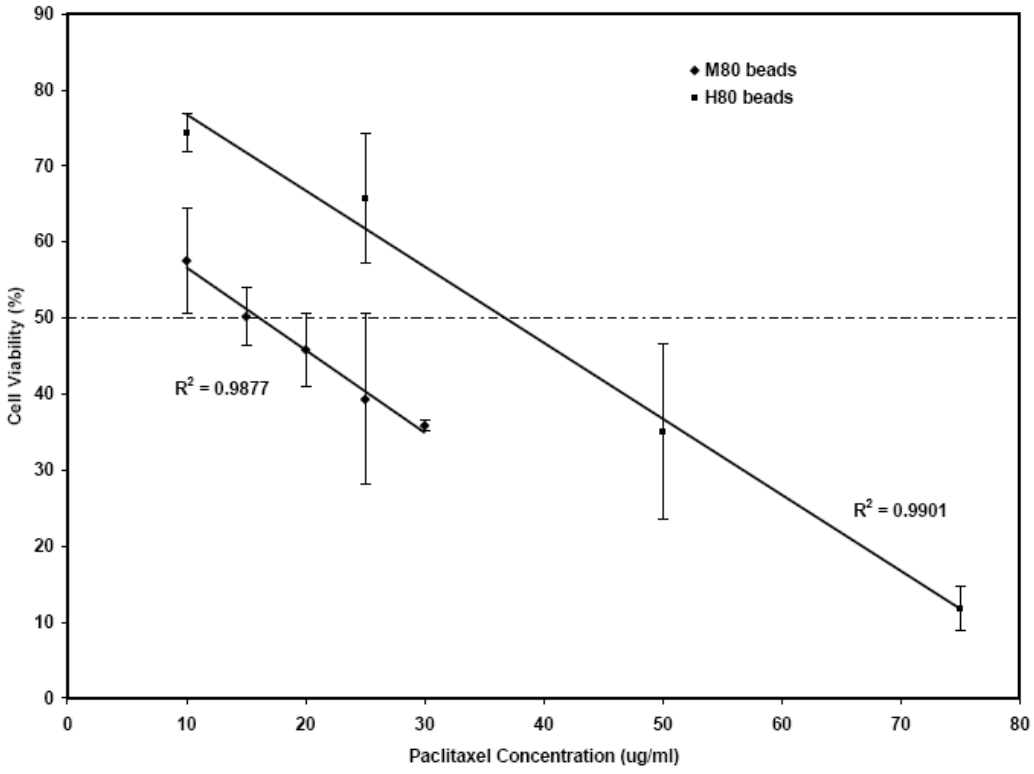


Figure 6: IC_{50} values for H80 and M80 beads used to treat C6 glioma cells in vitro for 72 hours.

These values indicate the cytotoxic potency of the H80 and M80 beads against C6 glioma cells in vitro. Studies of the drug release sustainability of the hydrogel beads, apoptosis against C6 glioma cell in vitro, and in vivo anti tumor efficacy in a subcutaneous tumor model are ongoing. These studies will be followed by in vivo

intracranial studies such as drug bio-distribution and treatment efficacy (tumor volume and survival analysis) and are warranted.

CONCLUSIONS

The entrapment of microspheres was done using two types of dripping methods, manual dripping (MD) and electrospray dripping (ED). Manual dripping yielded polydisperse non-spherical beads with a non-uniform distribution of the microspheres within them. In the ED method highly monodisperse spherical beads with a uniform microsphere distribution within them were obtained. Hence, for further studies, beads fabricated using the ED method were used. The hydrogel bead disintegration study revealed that with higher gelling bath concentration and gelation time the beads disintegrate slowly, whereas with an increase in microsphere loading the disintegration time decreased. *In vitro* release of paclitaxel from nine formulations revealed that 80% microsphere loaded beads provided the optimum release profile with low initial burst with a higher release rate and near zero-order kinetics. The study of the IC_{50} value for the H80 and M80 in comparison with Taxol® against C6 glioma cells showed that in vitro, the beads were more potent. In vivo intracranial studies focusing on paclitaxel bio-distribution in the brain, survivability analysis and tumor volume through bio-imaging are ongoing and will give more insight into the performance of the hydrogel beads.

REFERENCES

[1] Naraharisetti PK, Ong BYS, Xie JW, Lee TKY, Wang CH, Sahinidis NV. In vivo performance of implantable biodegradable preparations delivering paclitaxel and etanidazole for the treatment of glioma. Biomaterials 2007;28(5):886-894.

[2] Li KW, Dang W, Tyler BM, Troiano G, Tihan T, Brem H, et al. Polilactofate microspheres for paclitaxel delivery to central nervous system malignancies. Clin Cancer Res 2003;19:3441-3447.

[3] Xie J, Marijnissen JCM, and Wang CH. Microparticles developed by electrohydrodynamic atomization for the local delivery of anticancer drug to treat C6 glioma in vitro. Biomaterials. 27:3321-3332 (2006).

[4] Xie J, and Wang CH. Electrospray in the dripping mode for cell microencapsulation. J Colloid Interf Sci. 312:247-255 (2007).

[5] Freiberg S, Zhu XX. Polymer microspheres for controlled drug release. Intl. J. Pharm., 282:1-18, 2004.

[6] Dubernet C. Thermoanalysis of microspheres. Thermochimica Acta, 248: 259-269, 1995.