The Study of Collagen-Chitosan Complex Film Containing VCR-microspheres

Hongli Chen[1], Han Chen[1], Lingrong Liu[1], Ping yuan1, Qiqing Zhang[1, 2]

[1] Institute of Biomedical Engineering, Chinese Academy of Medical Sciences, Tianjin Biomedical Material Key Laboratory, Tianjin 300192, China

[2] Research Center of Biomedical Engineering, Xiamen University, Research Center of Biomedical Engineering Technology of Xiamen City, The Key Laboratory of Biomedical Engineering of Fujian Province Fujian 361005, China

Introduction: Cancer chemotherapy is not always effective. Difficulties in drug delivery to the tumor site, drug toxicity to normal tissues, and drug stability in the body contribute to this problem. Interstitial chemotherapy could minimize the systemic toxicity and achieve the goal of target therapy. Sustained release implant films of VCR could decrease the initial burst release and prolong the release. The use of collagen and chitosan for the controlled release of therapeutic agents has been extensively studied. The production and characterization of implant films based on collagen- chitosan containing PLGA microspheres for the delivery of VCR is described. The objective of this study is to investigate the films and their release drug characters.

Experiments: Vincristine sulfate (purity>98%) was purchased from Shanghai Biopharmaceutical. Zinc carbonate chemica was purchased from Sigma. PLGA（MW50,000-800,000，50:50）was purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences. Chitosan（MW200,000，85%）was purchased from Qingdao Institute of Meteria Medica. Collagen type I was harvested from cattle tendon by using pepsin. All other solvents and chemicals were of analytical grade or HPLC grade.

A water-in-oil-in-oil double-emulsion /solvent evaporation method was used to prepare VCR-loaded PLGA microspheres, then microspheres were mixed with collagen and (or) chitosan swelling solution, and lyophilized.The films were cross-linked by 0.3% glutaraldehyde [2]. We fully characterized for encapsulation efficiency and release kinetics of VCR microspheres, in vitro degradation and release kinetics of the film. The rate of VCR release from the film submerged in PBS (pH6.8) and the content were measured by high-performance liquid chromatography (HPLC). We observed the physical & chemical properties of the film such as surface morphology, hygroscopicity, mechanical function, differential scanning calorimetry.All data were analyzed using ANOVA. Results are reported as mean±standard deviation.

Results and Discussion: The microparticles fabricated with PLGA have smooth surfaces, and average particle size was (27.97±0.90)μm.(Fig.1) $ZnCO_3$ was suspended in the polymer solution to raise the microclimate pH inside the microspheres[3,4]. The encapsulation efficiency of microspheres was (79.0±1.0)%. In the degradation experiment, figure 2 shows the degradation curve of collagen with 10%, 20% and 40% chitosan (w/w). The film without chitosan degraded 94% incubation in PBS (pH6.8) containing 200U/mg collagenase type I after 32h, while collagen with 40% chitosan degrade 23% only.

The initial release of microspheres is (27.3±1.2)%, but as for the film of collagan and collagen-chitosan(9:1,4:1,3:2 ,w/w) it is(20.3±1.2) %、(18.2±1.0) %，(16.6±1.6) % respectively. In vitro degradation experiment, the film containing chitosan degraded more slowly than that without chitosan. The chitosan-free collagen film is loosen after 2 weeks during the release period, and microspheres were fewer than ever. With more proportion of chitosan, the tensile strength of the film was strenghened, and the breaking elongating rate of the film was decreased. DSC thermogram of the collagan film suggested that more degradation product than that of the film with chitosan. The results suggest that chitosan could significantly affect physical & chemical properties of the film,especially the degradation rate and initial burst release of VCR. The film (collagen:chitosan 4:1) is properly.

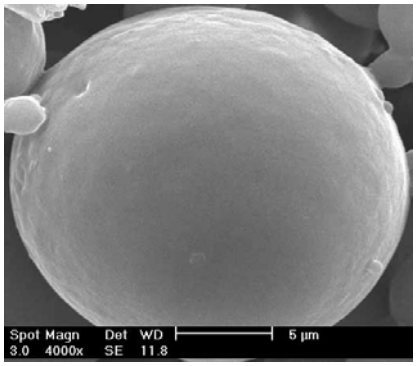

Figure 1. SEM images of PLGA microspheres

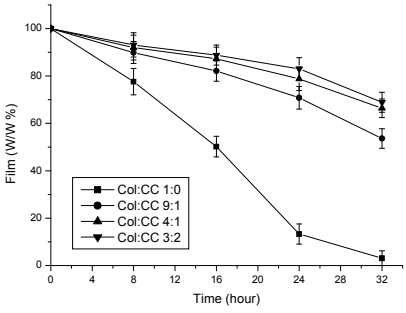

Figure 2. Degradation profile of films

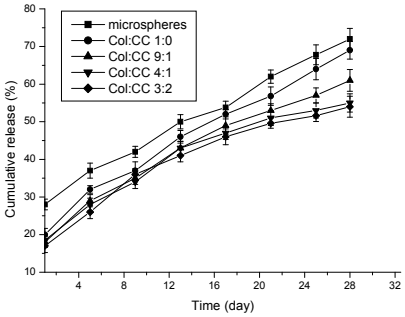

Figure 3. In vitro release curves of VCR microspheres and films

Conclusion: In this study, the improvements by adding chitosan have led to more effective chemotherapeutic agents for use against malignant tumors. The films that comprise collagen and chitosan could achieve the goal of constant release of drug and decrease the initial burst release. It has a prospect future.

Acknolegment: This work was supported by the Programs of Science and Technology Commission Foundation of Tianjin (No. 05YFGZGX03800) and the Doctoral Fund of Ministry of Education of China (No. 20060023050).

References: Brem, H. and P. Gabikian. Biodegradable polymer implants to treat brain tumors. [J] *J Control Release* 2001 , 74 (1-3): 63-67. Goissis G,M arcantonio EJR,M arcantonio RA , *et al.* Biocompatibility studies of anionic collagen membranes with different degree of GA cross-linking [J]. Biomaterials, 1999, 20 (1) : 27 - 34. C. Gidding , S. Kellie b, W. Kamps *et al*., Vincristine revisited [J] *Critical Reviews in Oncology:Hematology* , 1999, 29: 267–287. Mayer LD, Masin D, Nayar R, *et al.* Pharmacology of liposomal vincristine in mice bearing L1210 ascitic and B16/BL6 solid tumours. [J] *Br J Cancer,* 1995, 71:482-488.