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# Preparation and biocompatibility of chitosan microcarriers as biomaterial

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**Abstract**

Chitosan microcarriers (100–200 μm) were prepared by the methods of emulsification and ethanol coagulant. It has smooth surface and was stable in phosphate buffer solution (PBS) of at pH 7.2 in the treatment of temperature 120 °C and pressure 150 kPa. The chitosan microcarriers showed molecular affinity to the bovine serum proteins at pH 7.2. The adsorptive capacity of the microcarriers to the serum albumin was 6.8 mg protein/g chitosan bead. The chitosan microcarriers were found to have good biocompatibility and no cytotoxicity to both human and mouse fibroblasts in tissue cell culture. The fibroblasts well adhered on the smooth surface of the chitosan microcarriers and grew in high cell density. The results suggest a good potential of the chitosan microcarriers as a wound-healing biomaterial.


*Keywords:* Affinity; Microcarrier; Tissue cell culture; Adsorption

## 1. Introduction

Chitosan (β-1,4-linked-2-amino-2-deoxy-D-glucopyranose), made from deacetylated chitin (β-1,4-linked-2-acetamido-2-deoxy-D-glucose) is a polysaccharide of considerable promise in the field of biomedical research. Their biodegradability, biocompatibility, and nontoxicity allow widespread applications in wound healing [1]. The ability of chitosan and chitin to form gels, films, fibers, and sponges demonstrate their inherent versatility [2–4].

Chitin beads are potentially useful as a wound dressing material [1]. Chitin-based wound dressing materials promote and accelerate wound healing. Chitin may possess a tissue cell growth function and may action as a favorable scaffold for cell attachment and proliferation. This promotes rapid dermal regeneration allow accelerated wound healing [5]. The formation of chitin beads has been extensively investigated. Methods to prepare chitin beads include emulsification, suspension and homogenization, freeze-drying and hammer milling [6–11]. The water insolubility of chitin is a limitation for the preparation process of the beads, some special solvents or compounds, such as hexafluoroisopropanol, hexafluoroacetone, chloroalcohols, need to be added into the solvent in order to make chitin solutions [2,12].

Chitosan is soluble in the diluted acetic acid solution, and can be made into microcarriers. Chitosan microcarriers were found to have more utility in drug carrier and delivery systems [13]. The preparation of chitosan microcarriers involves the crushing and precipitation of chitosan solution droplets in an anionic coagulant such as alginate, tripolyphosphate [14]. The emulsion process had been employed for the preparation of chitosan beads with smooth surface and uniform size distribution [15]. The chitosan microcarriers with smooth-surface are thought to be good condition for cells adherence and growth on it. The characteristics and biocompatibility of the chitosan microcarriers are necessary to be described.

In this paper, a smooth-surface chitosan microcarriers were prepared by emulsion and cross-linking method. The characteristics, bio-affinity to bovine serum albumin, and

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cytotoxicity of the chitosan microcarriers were investigated. The cell growth status on the chitosan microcarriers in vitro was described.

## 2. Materials and methods

### 2.1. Materials

Chitosan (MW 480,000 Da, DD 90%) was made from crab-shell and obtained from Biochemical Medicine Plant of Qingdao (Qingdao, China). Bovine serum albumin (BSA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (Sigma Co., St. Louis, MO, USA).

### 2.2. Chitosan bead preparation

The preparation of chitosan microcarriers were followed a patented procedure [16]. Chitosan powder was dissolved in 1% acetic acid to give a 2.5% chitosan solution. The solution was filtered through glass wool. Filtered chitosan solution (50 ml), tween-80 (2.5 ml) and toluene (150 ml) were added into a 500 ml flask. The mixture solution were stirred 30 min with an electromagnetic bar to form an emulsion. Formalin (5 ml) and glutaraldehyde (1 ml) were added dropwise into the emulsion and were keeping in stir for 1 h. The mixture was filtered through 200-mesh nylon screen to separate the microcarriers, and the microcarriers were suspended in1000 ml distilled water (containing 0.05 g $NaH_4B$ in water) for 12 h. The microcarriers were filtered and rinsed in distilled water, then successively dewatered in 30, 50, 80, 95 and 100% ethanol and ether, respectively.

### 2.3. Characterization of chitosan microcarriers

Scanning electron micrographs (SEM) were obtained with a Stereoscan 250 Mk3 (Cambridge, UK). Chitosan microcarriers were gold-coated in a JEOL JFC-1100 ion-sputter. The IR spectrum of chitosan and chitosan microcarriers were recorded on an FT/IR-430 Fourier Transform Infrared Spectrometer (Jasco Co., Tokyo, Japan) at room temperature based on the method of Shigemasa [17]. The samples were 2 mg in the 100 mg KBr to be made pellet [17]. Thermal stability of chitosan microcarriers was assessed by checking the numbers of the intact microcarrier beads after heating at 120 °C under pressure of 150 kPa for 30 min when the chitosan microcarriers 0.1 g were soaked in 10 ml phosphate buffer solution (PBS, 0.05 M $NaH_2PO_4$–$Na_2HPO_4$, pH 7.2). The size was measured with Laser Diffraction Particle Size Analyzer SALD-3101 (Shimadzu, Japan). The water uptake of chitosan microcarriers was determined by measuring the weight of beads before and after soaking 0.10 g microcarriers in 25 ml PBS buffer for 24 h at 20 °C and expressed in % of the initial bead weight. To obtain the weight after soaking, the chitosan microcarriers were filtered and blotted dry on filter paper (Xinhua 1# Filter paper).

### 2.4. Affinity experiment

Chitosan microcarriers (0.1 g) were soaked in 10 ml distilled water for 5 h to equilibrate moisture uptake, filtered and dried on filter paper. The resulting chitosan microcarriers were soaked in 2 ml BSA solution (2.5%, v/v) at 4 °C for 1, 3, 6, 15 and 24 h and filtered through glass wool, respectively. The protein adsorptive capacity on the microcarriers (Pa%) was calculated using the following equation [18]:

$$\mathrm{Pa\%} = (C_1V_1 - C_2V_2)W^{-1} \times 100\% \quad (1)$$

where $C_1$ and $C_2$ are the protein concentration of the solution before and after adsorption, respectively; $V_1$ and $V_2$ are the volume of the solution before and after adsorption, respectively; and $W$ is the weight of the chitosan microcarriers.

The protein was determined by the Lowry method [19].

### 2.5. Cell culture

The specimens of mouse and human skins, obtained from Prof. Zhang at the Qingdao Hospital (Qingdao, China), were rinsed with D-Hanks solution supplemented with penicillin (1000 U/ml solution) and gentamicin (350 U/ml solution). Each treated skin was minced and explanted into tissue culture flasks (25 ml), incubated at 37 °C, 5% $CO_2$, and 100% relative humidity [20]. The culture medium was RPMI-1640 supplemented with BSA (10%, v/v), penicillin (200 U/ml culture media) and streptomycin (200 U/ml culture media). After 15–20 days, the fibroblasts were subcultured with 0.01% EDTA-0.125% trypsin for the secondary culture. After being subcultured 2–3 times, epithelial cells were disappeared and only fibroblasts were present. The added amount of chitosan microcarriers in culture solution was 1 mg/ml.

### 2.6. Cytotoxicity test

The general cytotoxicity test followed the method of Chen et al. [21]. Cells were inoculated into 96 well plates in 100 μl RPMI-1640 medium with 10% BSA, each well containing $1 \times 10^4$ cells. Chitosan microcarriers were dried, ground into powder, and suspended in the medium at 3 mg/ml. The negative control was surgery seam (30 mg/10 ml medium); positive control was phenol (500 μg/μl) [21]. The suspension sample (10 μl) was added to a well (five replicates per sample) and incubated for 2, 4 and 7 days. At the end of each exposure period, MTT (20 μl) was added to each well and the cultures were incubated at 37 °C for additional 3 h. The cells were then washed gently with PBS of pH 7.5 to remove untransformed MTT and sample residues. DMSO (150 μl) was subsequently added to each well to dissolve the MTT