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SHORT COMMUNICATION

Adhesion and growth of goat mammary epithelial cells on novel polyelectrolyte complex

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Abstract Alginate/poly(acryloxyethyl-trimethylammonium chloride-*co*-2-hydroxyethyl methacrylate) [poly(Q-*co*-H)] microcapsules were prepared by ionic gelation (Ca^{2+}) for adhesion and growth of goat mammary epithelial cell culture. In the procedure of microcapsule formation, alginate was first pumped into a $CaCl_2$ solution and then transferred into a poly(Q-*co*-H). The poly(Q-*co*-H) was prepared by free-radical polymerization in aqueous solution at 60 °C using potassium persulfate as initiator. The microcapsules obtained were sterilized using gamma radiation according to International Standards Organization (ISO)/TR 13409. Scanning electron microscopy studies indicated the high porosity and rough surface marked by large wrinkles of the microcapsule surface, and the diameter of the microcapsule was approximately 497 μm, and diameters ranking 480–515 μm were obtained. Optical michrography shows the epithelial morphology acquired by goat epithelial mammary cells (GMEC) on poly(Q-*co*-H)/NaAlg microcapsule surface after 8 h of culture.

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Introduction

Tissue engineering is an emerging multidisciplinary biotechnology that combines the principles of engineering and cell biology to facilitate the formation of novo tissues and organs [1]. This new scientific discipline is primarily targeted towards the creation of functional biological substitute as opposed to inert implants. As it is now well accepted, a tissue-engineered implant is a biological/biomaterial combination in which some component of tissue has been combined with biomaterials to create a device to treat the loss or malfunction of a structural tissue or organ such as skin, cartilage, bone, and small blood vessels [2, 7].

The development of this specific approach of tissue engineering is based on several observations: (1) as it was stated before, most of the tissues undergo constant remodeling due to apoptosis and renewal of constituent cell, (2) isolated cells tend towards forming tissue structures in vitro if the conditions are favorable, and (3) although isolated cells have the capacity to remodel and form the proper tissue structures, they require a template to guide their organization into the proper architecture [3]. At this point, we have defined the main factors necessary to achieve this tissue engineering approach: (1) cells, (2) growth factors, and (3) their scaffolds.

Hydrogel-based material, specifically polyelectrolyte complex (PEC) formed by mixing polymers of opposite charge, have recently attracted attention because of their potential for use in various biomedical applications

including drug or bioactive molecule-delivery vehicle, due to their many advantageous features [4, 5]. Recently, PEC have been increasingly utilized as cell-immobilization matrices to produce various biological products (e.g., vaccines, proteins, and antibodies), and in tissue engineering to recreate tissues intended to replace those damaged or lost [6].

Therefore, this study concentrates on adhesion and growth behavior of goat mammary epithelial cells in cultures on novel polyelectrolyte complex prepared by copolymerization of 2-hydroxyethyl methacrylate and acryloxyethyl-trimethylammonium chloride.

Methodology

Cell and materials

Goat epithelial mammary cells (GMEC) cell line was kindly donated by the Genetic Engineering and Biotechnology Centre of La Havana (Cuba).

2-Hydroxyethyl methacrylate (H, Merck), potassium persulfate ($K_2S_2O_8$, Fluka; 0.001% N), acryloxyethyl-trimethylammonium chloride (Q, AQUATECH; see Fig. 1), acetonitrile (CH_3CN, Merck), and silver nitrate ($AgNO_3$, Merck) were used as received.

Sodium alginate (NaAlg) extracted from *Macrocystis pyrifera* (medium viscosity) was purchased from SIGMA Chemical. Its viscosity average molar mass was 3.83×10^4 g/mol, which was determined by viscometry in 0.2 M NaCl at 25 °C. The polysaccharide samples were carefully purified with our own procedure as described elsewhere [11].

Twice-distilled water ($\sigma\approx1.3$ μS cm^{-1}) was employed during the experiments. All other reagents were of extra-pure grade and used as purchased.

Synthesis of copolymer

The copolymer poly(Q-*co*-H) was synthesized by polymerization of H and Q, respectively, in water solution at 60 °C, using $K_2S_2O_8$ as initiator. Comonomer and initiator concentrations were 1 and 0.01 mol/l, respectively. After the proper reaction time, the flask content was poured into a large excess of acetonitrile. The precipitated copolymer was rotoevaporated to separate the acetonitrile and was dried under vacuum until constant weight was attained.

Microcapsule elaboration

Sodium alginate was dissolved in distilled water (pH 6.5) at a concentration of 2.0 wt%. The solution (5 ml) was poured into a spray dryer nozzle unit using a continuous, automatic infusion/withdrawal pump at a rate of 50 ml/h and sprayed into 15 ml of a 0.4% $CaCl_2$ solution under magnetic stirring with the aid of a N_2 jet under pressure. The microcapsules thus formed were allowed to stand in the $CaCl_2$ solution for 10 min, and the $CaCl_2$ solution was decanted. The microcapsules were washed three times with 200 ml of distilled water and transferred to 15 ml of a poly(Q-*co*-H) solution (1.5 wt%) and incubated at room temperature under gentle magnetic stirring.

Microcapsule morphological characterization

The shape, size, and the size distribution of the microcapsules was characterized by Optical Microscopy (OM, Model Leitz Metallux 3, Germany). For morphological studies, microcapsules were affixed on aluminum studs and coated with platinum using a vacuum evaporator (JFC-1100, Japan). The microcapsules were then examined using a scanning electron microscope (SEM, JEOL JSM 6300, Japan) using a 15 kV accelerating voltage.

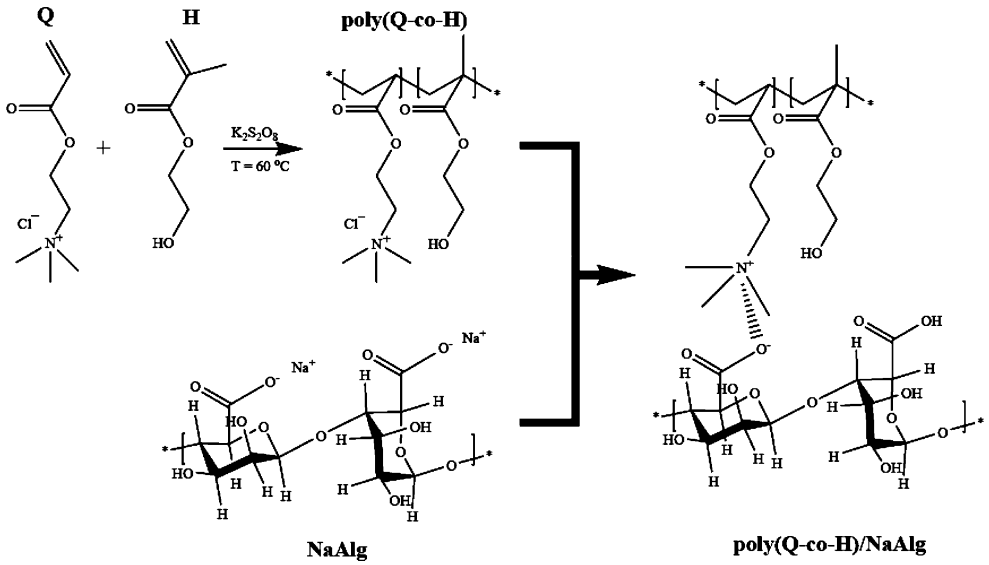

Fig. 1 Schematic representation for the ion complex formation reaction between the anion group ($–COO^-$) of sodium alginate and the protonated cation group ($–NH_3^+$) of poly (Q-*co*-H)

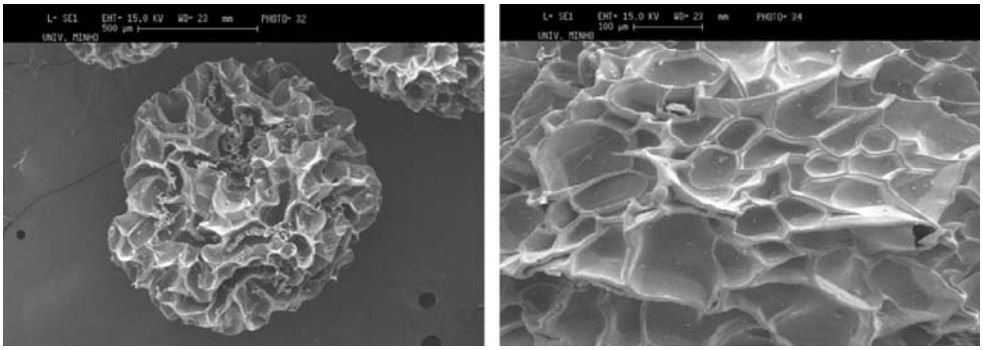

Fig. 2 SEM micrograph of poly (Q-*co*-H)/NaAlg polyelectrolyte microcapsules. (**a**) Microcapsule of poly(Q-*co*-H)/NaAlg. (**b**) Surface morphology of poly (Q-*co*-H)/NaAlg

Microcapsule irradiation

Microcapsule samples were irradiated in sealed glass bulbs in vacuum or under oxygen atmosphere in a ^{60}Co gamma source, at the Center of Technological Application and Nuclear development, Havana, Cuba. The activity of the radiation chamber was 10 kCi, and the dose rate was 25 kGy/h according to Fricke and ceric sulfate dosimetry. The applied doses were in the range of 1 to 100 kGy, according to International Standards Organization (ISO)/TR 13409 norm.

Preliminary study of cultured cells

GMEC cells were initially cultured in a growth medium consisting of Dulbecco's modified essential medium (DMEM). The culture mediums were supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution, maintained at 37 °C in a humidified 5% CO_2/95% air atmosphere for cell proliferation. After 2 days, cells were harvested and grown in a suspension culture with homogeneous mixing. The cell suspension, containing 2×10^5 cells, was added to 0.25 g of Cytodex 1 microcarriers (Pharmacia, Upsala, Sweden) used as a control, previously suspended in 500 ml of DMEM medium with 10% fetal bovine serum. The suspension was incubated in a spinner bottle and continuously stirred at low speed. After 48 and 96 h, GMEC cells attached to Cytodex were analyzed under optical microscopy (Model Olympus, Japan). The same procedure was utilized in the GMEC culture on poly (Q-*co*-H)/NaAlg microcapsules case.

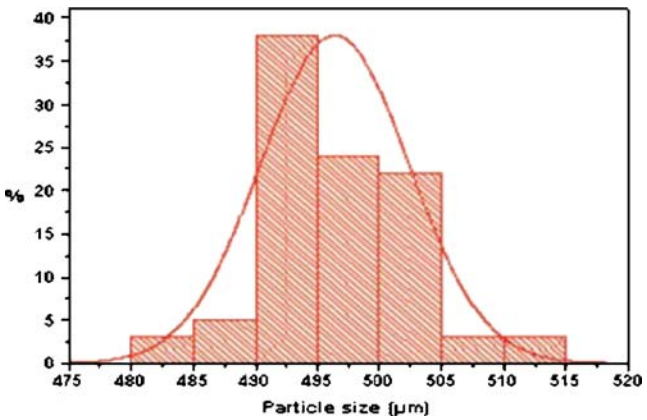

Fig. 3 Size distribution of poly(Q-*co*-H)/NaAlg microcapsules

Results and discussion

Preparation and characterization of poly(Q-*co*-H)/NaAlg microcapsules

Few researchers have investigated the ion complex formation between natural and synthetic polymer with opposed charge. The complexation mechanism involved the formation of electrostatic attraction between amino group on the poly(Q-*co*-H) and carboxyl group on acrylic acid. In our case, poly(Q-*co*-H)/NaAlg polyelectrolite complexes were prepared by inducing the gelation of a sodium alginate solution with $CaCl_2$. By controlling the stoichiometry of sodium alginate to $CaCl_2$, the desired amounts of sodium carboxylate groups were left unreacted, and these were complexed with poly(Q-*co*-H) to generate ionic cross-links between the two polymers [12]. Figure 1 exhibits the

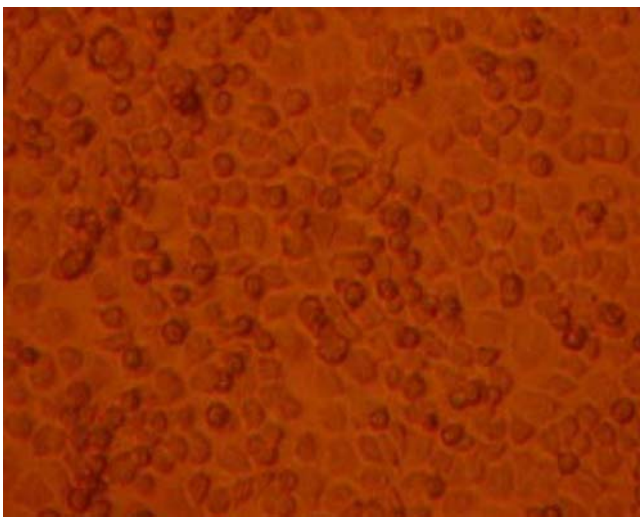

Fig. 4 Photomicrograph of GMEC cell control in the culture medium used (original magnifications ×48)

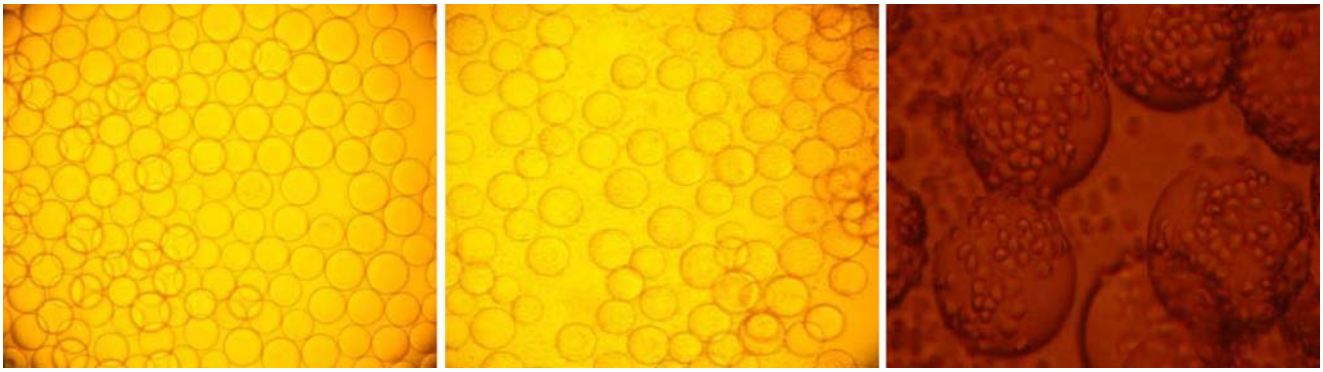

Fig. 5 Images of the growth profile of GMEC cell on Cytodex. **a** Cytodex in the culture medium without cells (original magnification ×12). **b** GMEC cultures on Cytodex past 8 h (original magnification ×12). **c** GMEC cultures on Cytodex past 8 h (original magnification ×48)

complexation reaction schemes between cationic group ($–NH_3^+$) of poly(Q-*co*-H) and anionic group ($–COO^–$) of sodium alginate polymer. These microcapsules were shown to have good mechanical resistance and stability.

Scaning electron micrographs of poly(Q-*co*-H)/NaAlg polyelectrolyte microcapsules and their surface morphology are shown in Fig. 2. The pores form as a consequence of the lyophilization process since during cross-linking the polymers swell in a continuous liquid phase, which is removed during lyophilization. Another remarkable characteristic of the microcapsule surface was its high porosity and rough surface marked by large wrinkles.

The microcapsules have a narrow size distribution, and 95% of the microcapsules have diameters ranging from 480.0 to 515.0 μm (see Fig. 3).

Cell adhesion and growth

Cell growth on culture medium and scaffold (Cytodex) controls

Figure 4 shows the optical micrograph for the GMEC culture cell control. It was observed that there was a prevalence of fibroblastic cells, and small quantities of epithelial cells was observed too. The cellular single-layer seed reached a 50% of sump coating after 8 h. Omelchenko et al. [10] described a fibroblastic cell as a fusiform appearance or tapered along a parallel axis. Therefore, the epithelial cells are described with a spherical appearance.

MacDonald et al. [8] report the cellular heterogeneity observed in this culture which constitutes a characteristic feature in the primary cell culture.

Regarding the growth pattern of GMEC cells in both cultures [Cytodex and poly(Q-*co*-H)/NaAlg], it was interesting to observe whether influence of the scaffold surface would affect its growing profile and morphology. Cytodex was employed as control for evaluation of cell morphology attached to microcapsules [9]. As shown in Figs. 5 and 6, GMEC cells grew initially in both types of scaffolds, but the growing appearance of the cells was distinctive for each type of microcapsule.

GMEC culture on sterilized microcapsules of poly(Q-co-H)/NaAlg

The cell culture on sterilized microcapsules of poly(Q-*co*-H)/NaAlg grew in the same way as the controls used. Micrography demonstrated the surprising form of the cell attached to the matrix scaffold.

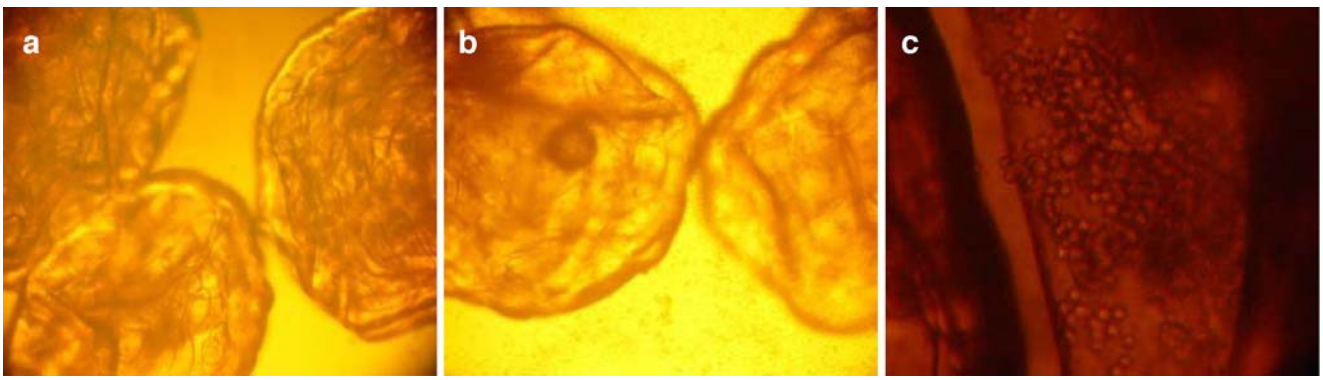

Fig. 6 Images of the growth profile of GMEC cell on sterilized microcapsules of poly(Q-*co*-H)/NaAlg. **a** Microcapsules of poly (Q-*co*-H)/NaAlg in the culture medium without cells (original magnification ×12). **b** GMEC cultures on microcapsules of poly (Q-*co*-H)/NaAlg past 8 h (original magnification ×12). **c** GMEC cultures on microcapsules of poly(Q-*co*-H)/NaAlg past 8 h (original magnification ×48)

Following the same analysis described above, Fig. 6 shows two images; Fig. 6a corresponds to sterilized microcapsules of poly(Q-*co*-H)/NaAlg in the culture medium without cells. Figure 6b and c shows the GMEC culture past 8 h; in this image, the morphology of the cell attached to sterilized microcapsule can be appreciated. The epithelial morphology acquired by cells is probably a consequence of the poly(Q/H)-NaAlg microcapsule surface.

Conclusions

We proposed a new type of porous polyelectrolyte complex microcapsules from alginate and poly(Q-*co*-H) which was used as scaffold for adhesion and growth behavior of goat mammary epithelial cell culture. The microcapsule was prepared by complex coacervation and characterized by optical microscopy and scanning electron microscope. The results indicated the high porosity and rough surface marked by large wrinkles of the microcapsule surface; the diameter range of the microcapsule was 480–510 μm.

The study of culture GMEC cells on sterilized microcapsules of poly(Q-*co*-H)/NaAlg by optical microscopy showed the epithelial morphology acquired by goat epithelial mammary cells on microcapsule surface after 8 h of culture.

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