HPMA and HEMA Copolymer Bead Interactions with Eukaryotic Cells

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Two different hydrophilic acrylate beads were prepared *via* aqueous suspension polymerization. Beads produced of a hydroxypropyl methacrylate (HPMA) and ethyleneglycol methacrylate (EDMA) copolymer were obtained using a polyvinyl alcohol suspending medium. Copolymers of 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate (MMA) and ethyleneglycol methacrylate (EDMA) beads were obtained using magnesium hydroxide as the suspending agent. Following characterization by scanning electron microscopy (SEM), nitrogen sorption analysis (NSA) and mercury intrusion porosimetry (MIP), the beads were cultured with monkey fibroblasts (COS-7) to evaluate their ability to support cell growth, attachment and adhesion. Cell growth behavior onto small HPMA/EDMA copolymer beads and large HEMA/MMA/EDMA copolymer beads is evaluated regarding their hidrophilicity/hidrophobicity and surface roughness.

1. Introduction

Extensive research has been carried out to attain biocompatibility and/or to modify the surface interactions between cells and artificial polymeric materials. Cellular behavior such as adhesion, morphological change and proliferation on various polymeric materials has been investigated by several groups[1-4]. It has been observed that the surface conditions for cell attachment and proliferation change with the cell type and the material composition. The role of scaffolding has received increased attention recently due to the issues involved with cell attachment and compatibility when using biocompatible materials for organ replacements[2].

Interactions between polymeric materials and eukaryotic cells have been demonstrated in previous studies, in which the effect on the cell growth, function and metabolism were assessed[2-5]. These observations laid the framework to study and design better materials for contact, implant, scaffold media, membrane or tissue engineering. For instance, Koyano, Minoura, Nagura and Kobayashi[4] demonstrated that the attachment and growth of cultured mouse fibroblasts was dependent on the chitosan polymer concentration of hydrogels. In a similar study, the effect of surface roughness, the chemical structure and the contact angle of polymeric films on cell growth was evaluated in mouse fibroblasts[5]. The authors observed a greater influence of the film texture on the cell growth compared to contact angle of the film. The surface texture of the biomaterial controlled cell adhesion, shape, proliferation and function.

Ma *et al.*[3] discussed the effect of porosity, apparent pore volume and distance between adjacent fibers on the cell proliferation of skin fibroblasts of a polymeric fibrous matrix. They reported that the porosity of the matrix was an important structural parameter for regulation, distribution and growth of cells forming the tissue scaffolding architecture. Kaufmann *et al.*[2] evaluated the interaction of biodegradable highly porous polymer sponges with hepatocytes. They showed that both cell-cell and cell-matrix interactions may be potentially regulated to optimize the phenotype of cultured cells. In addition, they investigated the role of these variables in hepatocyte gene expression.

The present study examines cell interactions with two different bead shaped, porous, hydrophilic cross-linked co-

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polyacrylates in an effort to analyze the role of porosity on cell attachment and proliferation upon synthetic matrices. In addition, the possible use of these mechanically resistant beads as a support media for cell growth was explored using monkey kidney epithelial fibroblasts cells.

2. Materials and Methods

2.1. HPMA and HEMA copolymer bead preparation

The synthesis of different batches of HPMA copolymer beads (hydroxyethyl methacrylate/ethyleneglycol methacrylate) has been described elsewhere[6,7]. Briefly, they were prepared by using a free radical suspension polymerization in an aqueous medium with the use of an organic suspending agent, polyvinyl alcohol (PVA). Dodecanol (DOD) was used as the porogen solvent at varying concentrations. Polymerization was carried out at 70 °C and stirring at 375 rpm in the presence of the initiator 2,2,azobis-2-methylpropionitrile (AIBN). The HPMA copolymer beads in this study were produced with HPMA:EDMA:DOD in a molar ratio of 1:0.73:1.09. DOD concentration was 41.25% w/w of the total organic weight.

The syntheses of the HEMA copolymer beads (2-hydroxyethyl methacrylate/methyl methacrylate/ethyleneglycol methacrylate) have also been described elsewhere[6]. They were prepared using a free radical suspension polymerization in the presence of an aqueous inorganic magnesium hydroxide gel medium prepared *in situ*. Dodecanol (DOD) was used as a porogen solvent at several concentrations. The polymerization was carried out at 70 °C and stirring at 250 rpm in the presence of AIBN as initiator. The HPMA copolymer beads used in this study were produced with HPMA:EDMA:DOD in molar ratio of 1:1.30:0.19:0.40. DOD was used in the concentration of 20% w/w of the total organic percentage.

2.2. Scanning electron microscopy (SEM) preparation

The beads morphology and porosity were evaluated by a 551 A scanning electron microscope (15 KV, Phillips Electronic Instruments, Inc., Mahwah, NJ) coupled with a Polaroid camera model 545 (Polaroid Corp., Cambridge, MA) and 4 × 5 inches film holder. Dry samples were assembled in a double-coated tape mounted on an aluminum stub and gold sputtered for 4.0 min at 10 mA of current and 200 μm Hg of vacuum. Micrographs were exposed for 1/8 s and shot at various magnifications.

2.3. Cell culture preparation

Monkey kidney epithelial fibroblasts (COS-7) were purchased from ATCC (American Type Culture Collection, Rockville, MD). The cells were maintained in Dullbeco's Modified Eagle Medium (DMEM, Gibco BRL Laborato-

ries, Grand Island, NY) and supplemented with 10% fetal bovine serum (FBS), antibiotics (penicillin and streptomycin) and incubated in 5% CO_2 incubator at 37 °C in triplicate. After the fibroblasts reached confluence, the medium was removed and they were washed with sterile phosphate buffer solution (PBS) then, detached using trypsin-EDTA (ethylenediaminotetracetic acid) and suspended in regular medium. To determine their number the cells were exposed to tryptan-blue (Aldrich Chemical Co., Milwaukee, WI) and counted using a hemocytometer. HPMA and HEMA copolymer beads (2.5 μg/mL aqueous suspension, 100 μL) were sterilized in an autoclave, mixed with cells (3×10^5, 100 μL), and seeded into a 6-well polystyrene plate in duplicate and incubated for 72 h. In control experiments, the cells and the beads were separately placed in the medium and incubated. The interactions between the beads and the cells were evaluated under a phase-contrast light microscope (G300 Series Binocular, UNICO, Dayton, NJ).

2.4. Porosity evaluation

Nitrogen sorption analysis (NSA) and mercury intrusion porosimetry (MIP) were used to evaluate the specific surface area (SSA, m^2/g), total pore volume (TPV, 10^{-3} mL/g), average pore radius (APR, Å) and maximum pore size measured (MPS, Å) of the beads. The gas sorption analyses for specific surface area and pore size determination were performed using the NOVA 1000 analyzer (Quantachrome Corp., Boynton Beach, FL). The vacuum adsorption system consisted of a vacuum pump; a gas supply; a sample container; a calibrated volume; manometer and a coolant. MIP analyses were performed using a mercury contact angle of 140° in the Poremaster 60® (Quantachrome Corp., Boynton Beach, FL). Data was generated by the Quantachrome Poremaster for Windows® software, version 2.03.

3. Results and Discussion

SEM results show that the HPMA copolymer beads (Fig. 1) obtained using the PVA suspending agent were a white fine powder of very small particles (10-20 μm). In contrast, the HEMA copolymer beads (Fig. 2), produced using the $Mg(OH)_2$ suspending agent, appeared as larger white spheres (200 μm). The presence of mesopores (20-500 Å diameter) was verified for the HPMA copolymer beads while macropores (> 500 Å diameter) were found in the HEMA copolymer beads.

Both methods of bead preparation rendered hydrophilic copolymers. The HEMA copolymer beads, however, are less hydrophilic than the HPMA copolymer beads because of the presence of the pore size controller MMA, a hydrophobic monomer. Peluso *et al.*[8] have suggested that the hidrophilicity/hydrophobitcityratio of the material surfaces would play an important role in cell growth. The differ-
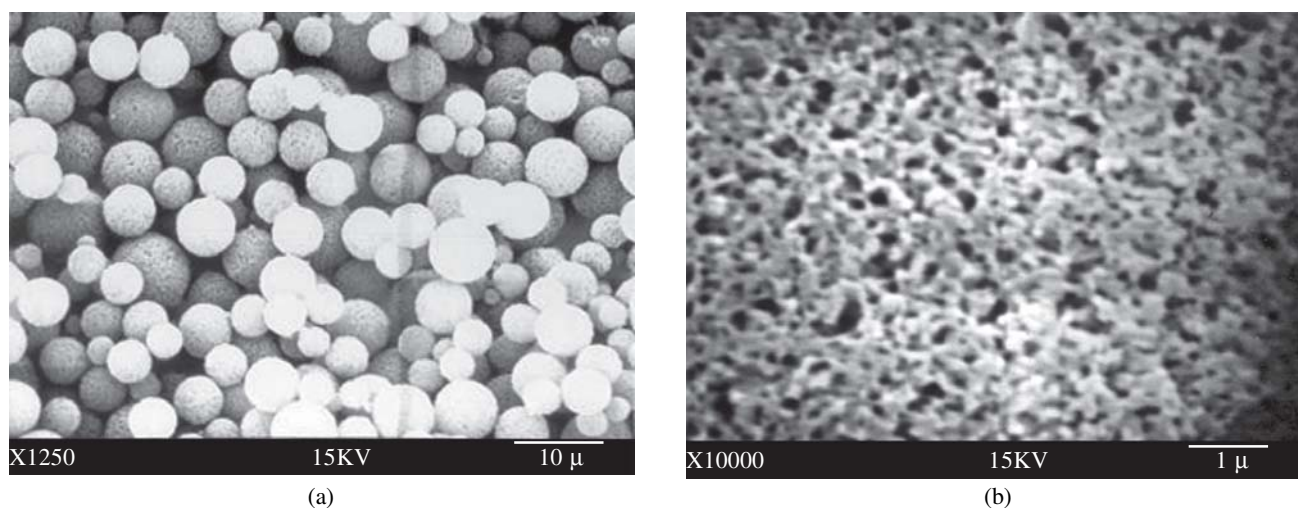
Figure 1. SEM micrograph of copolymerized HPMA/EDMA (1:1) beads with 41.25% addition of porogen solvent, DOD, before simple extraction (a, 1.250 ×, scale bar 10 μm) and after Soxhlet extraction (b, 10.000 ×, scale bar 1 μm).
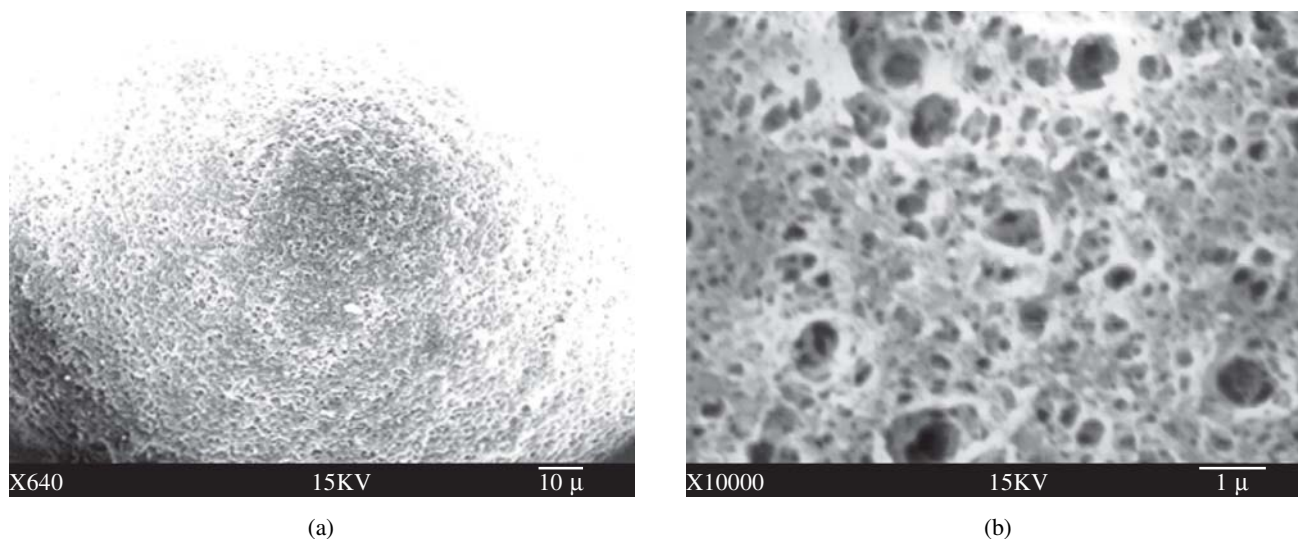

Figure 2. SEM micrograph of HEMA/MMA (1:1) and 10% EDMA copolymerized beads with 35% addition of porogen solvent, DOD, before (a, 640 ×, scale bar 10 μm) and after (b, 10.000 ×, scale bar 1 μm) Soxhlet extraction.

ences in the porous structure of the beads were certainly related to the method of preparation of these particles together with the presence of a cross-linker and a porogen.

NSA and MIP results (Table 1) showed greater specific surface area (SSA, m^2/g) and total pore volume (TPV, 10^{-3}mL/g) for the HPMA copolymer beads than the HEMA copolymer beads. An average pore radius (APR) of about ten fold is verified for the HEMA copolymer beads. The results shown in Table 1 are consistent with the SEM results for pore diameter ranges. Both types of beads appear to be porous.
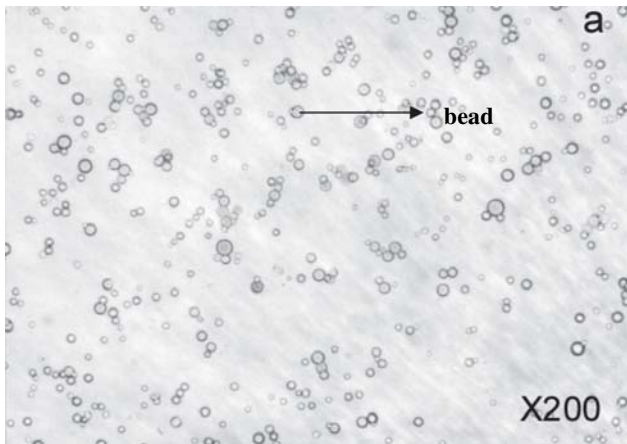
The interactions between the cells and the beads after

Table 1. NSA and MIP results (specific surface area, SSA, m^2g^{-1}, total pore volume, TPV, average pore size, APR) for HPMA and HEMA copolymer beads, respectively.

Polymer	SSA (m^2/g)	TPV (10^{-3}mL/g)	APR (Å)
HPMA copolymer beads	142	351	49
HEMA copolymer beads	31	74	441

incubation (n = 3) were visualized using the light micro-scope and are illustrated in Fig. 3 and Fig. 4 for HPMA and HEMA copolymer beads, respectively. As described earlier, the particle size (~ 20 μm) of HPMA copolymer beads was smaller than the HEMA copolymer beads (~ 200 μm). In comparison to the size of the cells, COS-7 fibroblasts showed a similar particle size relative to HPMA copolymer beads, whereas HEMA copolymer beads were larger than the cells. The density of the cells (3×10^5, 100 μL) was purposely kept low in order to focus on the behavior between the cells and beads.

Figure 3a represents the control for the HPMA copolymer beads. As shown in Fig. 3b, the proliferation of cells revealed that they were able to attach and grow in the presence of the beads and indeed surrounded these particles with
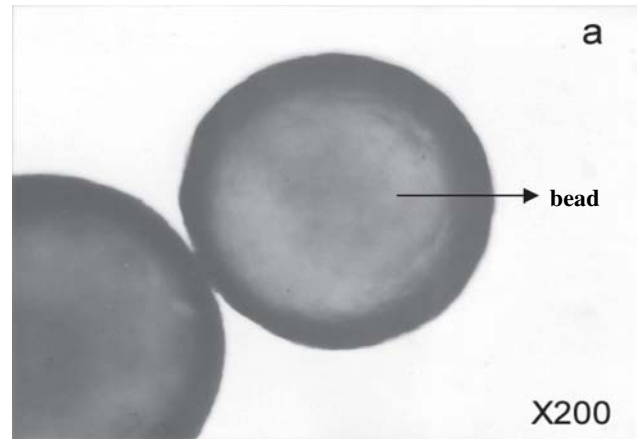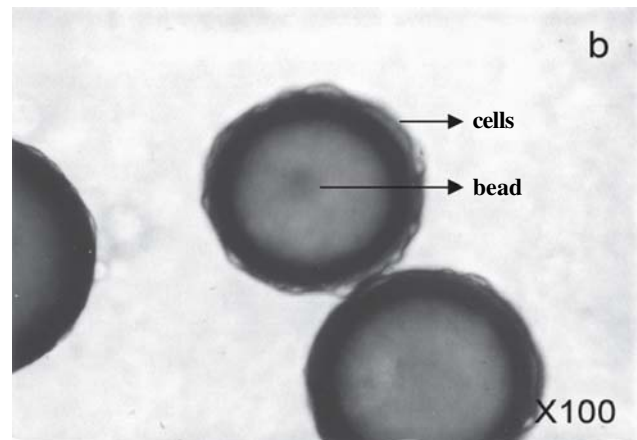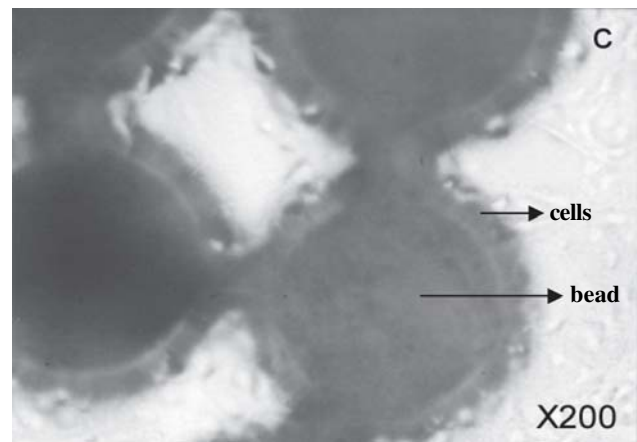

(a)

(a)

(b)

(b)

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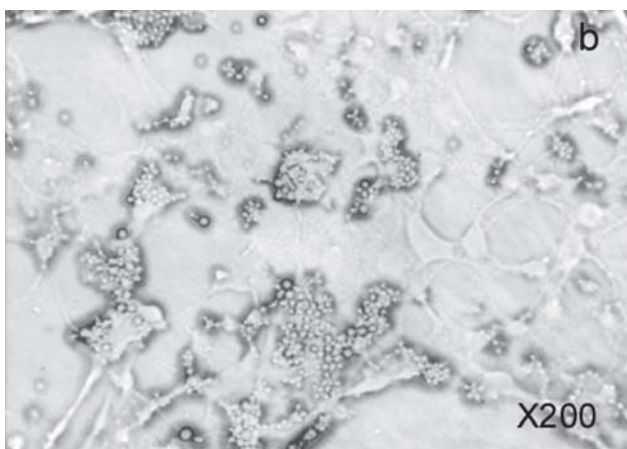
Figure 3. HPMA copolymer beads control (a, 200 ×) and their interaction with the COS-7 cells (b) seeded in regular medium and incubated at 37 °C for 72 h (200 ×). Note the cell attachment to the beads and cell growth in the background.

Figure 4. HEMA copolymer beads control (a, 200 ×) and their interaction with the COS-7 cells (b, 100 ×) and (c, 200 ×) seeded in regular medium and incubated at 37 °C for 72 h. Note the cell attachment on the beads in the form of a coating layer and the cell growth in the background (c).

a particular agglomerated, confluent pattern. This pattern, nevertheless, was not observed with the large size HEMA copolymer beads (Fig. 4a, control). It is clear from Fig. 4b that the cell-bead association is different from the HPMA copolymer beads. At a higher magnification, as depicted in Fig. 4c, a cell layer coating the bead surface can be visualized. In this case, the cell background density for the HEMA copolymer beads was less when compared to the HPMA copolymer beads (Fig. 3b). This can be explained not only by their larger particle size, but also, by the greater porous texture (larger pore size). The superior adhesive characteristics of the cell onto HEMA copolymer beads may be attributed to the lower degree of hydrophilicity of the polymer beads used, i.e., the additional hydrophobic properties of the chemical structure of the beads, due to the presence of the pore size controller, MMA. Therefore, not only the chemical structure but also the surface roughness affected cell growth, likewise Higuchi *et al.* have reported[5].

Kiremitci, Gürhan, and Piskin[1] have observed cell culture in a copolymer using HEMA as a basic monomer, except that it was on a swellable gel based matrix. They showed that cell attachment and growth can be controlled by changing the degree of hydrophilicity, by the addition of MMA, as well as the degree of charge. Jayakrishnan and Chithambara Thanoo[9] have reported the manufacture of beads made with the hydrophilic monomer (HEMA) only and cross-linked with EDMA. They reported that the control of porosity is accomplished by the addition of a polymeric diluent in the dispersed phase. In fact, HEMA monomer has been proven to be non-toxic to human cells and affords preparation of beads in a wide size range, which is crucial for many biomedical applications. Nevertheless, the same authors did not report on any cell-bead interactions.

4. Conclusion

The mechanically resistant, cross-linked hydrophilic, copolymers beads of HPMA and HEMA are able to support cell growth. The surface area and pore volume are intimately related to the porosity of the material and greatly influence cell growth. Porosity is an important structural parameter to assess cell growth and attachment. These materials may be used as a cell substrate or for other biomedical applications by selecting proper bead and pore size. Furthermore, toxicity in the form of cell death was not detected in the described conditions, hence, residual solvent or bead synthesis by-products seem to be absent.

Acknowledgments

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