Adhesion and morphology of fibroblastic cells cultured on different polymeric biomaterials

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Cell adhesion is influenced by the physical and chemical characteristics of the materials used as substrate for cell culturing. In this work, we evaluated the influence of the morphological and chemical characteristics of different polymeric substrates on the adhesion and morphology of fibroblastic cells. Cell growth on poly (l-lactic acid) [PLLA] membranes and poly[2-hydroxy ethyl methacrylate] [polyHEMA], poly[2-hydroxy ethyl methacrylate]-cellulose acetate [polyHEMA-CA] and poly[2-hydroxy ethyl methacrylate]-poly(methyl methacrylate-co-acrylic acid) [polyHEMA-poly(MMA-co-AA)] hydrogels of different densities and pore diameters was examined. Cells adhered preferentially to more negatively charged substrates, with polyHEMA hydrogels being more adhesive than the other substrates. The pores present in PLLA membranes did not interfere with adhesion, but the cells showed a distinctive morphology on each membrane.


Introduction
Biomaterials play an important role in tissue engineering by serving as scaffolds to guide tissue regeneration by releasing medicines and growth factors to stimulate the tissue response, or to create a new functional structure when damaged tissue does not regenerate. Biomaterials can also be used in cell culture techniques to create tissue-like structures that simulate the mechanics and physiological characteristics of tissues in vivo.

Materials used in implants can be classified as biostable or permanent and bioabsorbable or temporary [1]. Biostable materials include prostheses, which are used to substitute damaged body parts for an unspecified period of time [2]. The materials used in these cases need to retain their mechanical and chemical properties in vivo for years or decades [1]. In many cases, however, only the temporary presence of a biomaterial support is required during tissue replacement or to guide tissue growth during wound healing. Bioabsorbable polymers are components that are degraded in vitro and in vivo, and disappear with time as the tissue is being repaired [2]. In both cases, a knowledge of the pattern of cell adhesion, growth, and differentiation on these biomaterials is important.

Poly(l-lactic acid) (PLLA) is biodegradable polyester frequently used as a support in cell culture or for the experimental treatment of damaged tissues, mainly because of its good biocompatibility [3–6]. PLLA degradation occurs by hydrolyses, which results in a gradual release of the degradation monomers [7]. PLLA devices have been prepared by techniques such as phase inversion [8], addition of salt particles [9], addition of plasticizer[10], incorporation of CO₂ [11], and by solution casting [12].

Another biomaterial commonly used in tissue engineering is poly(2-hydroxy ethyl methacrylate) (polyHEMA). This compound forms a hydrogel consisting of a three-dimensional polymeric network that swells in water without dissolving [13]. PolyHEMA shows similarities with the extracellular matrix, and thus represents a good model for cell culture studies of tissue restoration. Indeed, polyHEMA copolymers that simulate the articular cartilage matrix have been tested as alternative permanent prostheses. Chemical modification have been used to evaluate the hydrophobic/hydrophilic properties of polyHEMA in order to establish which changes affect cell adhesion and growth [14–18]. The presence of a –COO⁻ H⁺ group in the copolymer structure introduces a higher negative charge changes the cell–polymer interactions [19, 20].

In this work, we examined the influence of structural variations in PLLA and polyHEMA on the adhesion,
growth and morphology of cultured fibroblastic cells. The materials tested included dense and porous PLLA membranes of known in pore diameters, or polyHEMA and its copolymers poly(2-hydroxy ethyl methacrylate)-cellulose acetate [polyHEMA-CA] and poly(2-hydroxy ethyl methacrylate)-poly(methyl methacrylate-co-acrylic acid) [polyHEMA-poly(MMA-co-AA)].

**Materials and methods**

**Preparation of biomaterials**

**Dense PLLA membranes**

Poly(l-lactic acid) [PLLA] membranes were prepared by dissolving PLLA in high purity for medical use, $M_w = 300,000$ Da, obtained of Medisorb Technologies International LP, Cincinnati, OH, USA) at room temperature in 10% chloroform solution (w/v) (from Merck KGaA, Darmstadt, Germany). The polymer solution was then transferred to Petri dishes which were dried by casting in a closed chamber connected to a flow of compressed dry air (0.01 nm$^3$/h) for 24 h.

**Porous PLLA membranes**

The porous PLLA membranes were prepared by dissolving PLLA in 10% chloroform solution (w/v) at room temperature. Forty grams of sodium citrate with grain diameter <$45 \mu m$; 180–250 $\mu m$ and 250–350 $\mu m$ (Fluka Chemicals, Netherlands) were added in portions of 10 g to 70 mL of PLLA–Chloroform solution to produce a film with a pore volume of approximately 80%. The drying procedure was as described for the nonporous film. After drying, the films were washed in demineralized water for 24 h to remove the salt and subsequently washed for 6 h in ethanol. In both cases, the polymers were vacuum dried and maintained in a dessicator for 5 days to guarantee the total removal of solvent.

**PolyHEMA hydrogels**

Three hydrogels, namely, poly(2-hydroxy ethyl methacrylate) [polyHEMA], poly(2-hydroxy ethyl methacrylate)-cellulose acetate [polyHEMA-CA], and a poly(2-hydroxy ethyl methacrylate)-poly(methyl methacrylate-co-acrylic acid) [polyHEMA-poly(MMA-co-AA)] were used in the form semi-interpenetrating networks (sIPN). Hydrogel samples were obtained as 2 mm thick sheets by thermal polymerization. The monomer, crosslinking agent (1.0% w/w) and thermal initiator (0.5% w/w) were mixed by stirring and poured into a glass mold for polymerization. In the case of mixed sIPN, the linear polymer, cellulose acetate or poly(MMA-co-AA) was dissolved in the HEMA monomer to give a solution with a final polymer concentration of 5% (w/w). After synthesis, the hydrogels were washed in distilled/deionized water to remove residual monomer and initiator, and then washed in 0.15 M NaCl until a constant pH 7.0 was reached. The uptake of NaCl the different hydrogel samples was measured and expressed as the percentage of saline solution within the gel at equilibrium. The fixed negative charge density (mEq COO$^-$/gel gram) was measured by titration.

For use in cell culture, the biomaterials were sterilized and the sterility confirmed. The samples were washed three times in Ham F-10 medium without fetal calf serum and incubated in culture medium for 24 h at 37°C before cell addition.

**Cell culture**

Vero cells, a fibroblastic cell line established from the kidney of the African green monkey (*Cercopithecus aethiops*), were obtained from the Adolfo Lutz Institute, São Paulo, Brazil. The cells were cultured in Ham-F10 medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% fetal calf serum (FCS, from Nutricell Nutrientes Celulares, Campinas, SP, Brazil) at 37°C. Vero cells are recommended for studies of cytotoxicity and cell-substratum interactions in biomaterial research [21, 22].

![Figure 1](image-url)  
**Figure 1** Cell adhesion to different polymeric biomaterials after 2h incubation. (a) Cell adhesion on hydrogels of different negative charge densities: polyHEMA (0.017 mEq/g), polyHEMA-CA (0.02 mEq/g) and polyHEMA-(MMA-co-AA) (0.11 mEq/g). Polypropylene and silicone were used as a positive and negative controls, respectively. (b) cell adhesion on porous PLLA membranes of different diameter pores.