SYNTHETIC POLYMER-COLLAGEN COMPOSITES FOR BIOMEDICAL USE

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INTRODUCTION

Chemical purity and inertness of classical implantable synthetic materials warrants their non-toxicity and non-antigenicity in respect to the recipient’s organism (Bruck, 1973). However, chemically inert plastics are rather poor substrates for attachment of animal cells. If implanted into the organism, they are surrounded by a fibrous capsule.

Practically all animal cells need attachment to a solid surface for their life. Their normal metabolism, growth and expression of differentiated characters are anchorage-dependent (Grinell, 1978).

One of the ways, how the adhesivity of cells to substrate can be enhanced, is combination of artificial and biological materials.

A group of synthetic hydrogels was developed by Wichterle and Lim (1960). The poly(2-hydroxyethyl methacrylate)-polyHEMA is frequently used as a prosthetic material. It is translucent, non-toxic, non-antigenic, fairly hydrated, permeable for ions and small molecules, very resistant to enzymatic digestion. However, animal cells do not attach, grow and differentiate satisfactorily on its surface (Folkman and Moscona, 1978; Štol et al., 1985).

We have shown that copolymers of polyHEMA with methacrylic acid were able to support growth of transformed cell lines in vitro (Tolar and Štol, 1968; Tolar et al., 1969). Civerchia-Perez et al. (1980) incorporated soluble collagen into the polyHEMA hydrogel structure by mixing dissolved components before the free-radical crosslinking polymerization. The growth of fibroblasts and smooth muscle cells (Toselli et al., 1984) was greatly enhanced, although the content of collagen in the composites was rather low (maximally 3-4 %, w/v). Elastin incorporated in a similar way (Toselli et al., 1983) improved adhesion and growth of endothelial cells.
Several years ago, a new technique was suggested by us (Stol et al., 1980; Stol et al., 1985), which enabled incorporation of any amount of acid soluble collagen (ASC) or insoluble collagen (ISC) into the composite with polyHEMA, ranging from 0 to 100 % (w/w).

The biological properties of the composites were tested in vitro (Stol et al., 1985) and in vivo (Stol et al., in preparation). Skeletal myogenesis was chosen for testing in vitro, because formation of myotubes from myoblasts is dependent on presence of collagen (Hauschka and Konigsberg, 1966). The same composites were implanted into a popliteal region of rats and their interaction with surrounding tissues was followed. The aim of this study was to specify quantitatively the combinations of polyHEMA and collagen suitable for the in vitro and in vivo use.

MATERIALS AND METHODS

Preparation of liquid mixture of polyHEMA and collagen

Uncrosslinked polyHEMA was prepared separately by free-radical solution polymerization of HEMA monomer (SPOFA, Prague) and it was then thoroughly purified (Wichterle, 1971).

The insoluble fibrillar collagen was prepared according to Steven (1976). The acid soluble collagen was prepared following the procedure described by Bazin and Delaunay (1976). In both cases, bovine hides were used as a source of collagen.

The procedure of mixing polyHEMA and collagen in any desired ratio consisted of several steps:

(a) Preparation of 10 % (w/v) polyHEMA stock solution in 33 % (v/v) aqueous acetic acid.

(b) Preparation of 2 % (w/v) stock dispersion of insoluble collagen fibrils or stock solution of acid-soluble collagen in 1 % (v/v) aqueous acetic acid.

(c) Mixing of the two polymeric components in a desired ratio. Before mixing, the content of acetic acid in the collagen dispersion or solution was raised to 33 % (v/v) to avoid precipitation of polyHEMA from the mixture.

More detailed information on materials and methods used can be found in Stol et al. (1985).

Coating of cultivation surfaces

The resulting liquid mixture was used to form a thin layer on the cultivation surface of plastic Petri dishes 60 mm in diameter (Koh-I-Noor, Czechoslovakia) under sterile conditions. The solvent was evaporated from the layers at room temperature. Then the layers were rinsed with sterile double-distilled water and dried out. The layers were rehydrated by adding some cultivation medium several hours prior to seeding of the dissociated muscle cells. Sterility of the dishes can be maintained with help of a germicide lamp.