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APPLICATIONS OF POLYMERS IN BIOTECHNOLOGY

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Abstract - There is a wide range of opportunities for the application of novel polymeric materials and systems in recombinant DNA and monoclonal antibody processes and products. This paper reviews and highlights such exciting possibilities.

INTRODUCTION

The ability to recombine DNA in precise ways, to insert the novel plasmid into living cells and to produce new proteins with important clinical uses has led to a new and immense scientific field as well as to a modern industrial, "biotechnological" revolution (1-4). In parallel with this significant achievement, immunologists have been able to produce useful quantities of antibodies with high purity and antigenic specificity. These antibodies are called monoclonal antibodies (MAb's). Their availability has created the possibility of many exciting and novel diagnostic and therapeutic applications (5). These two significant developments together make up the exciting new field called biotechnology (Fig. 1). This paper will review the present uses and future possibilities for applications of polymeric materials in biotechnology.

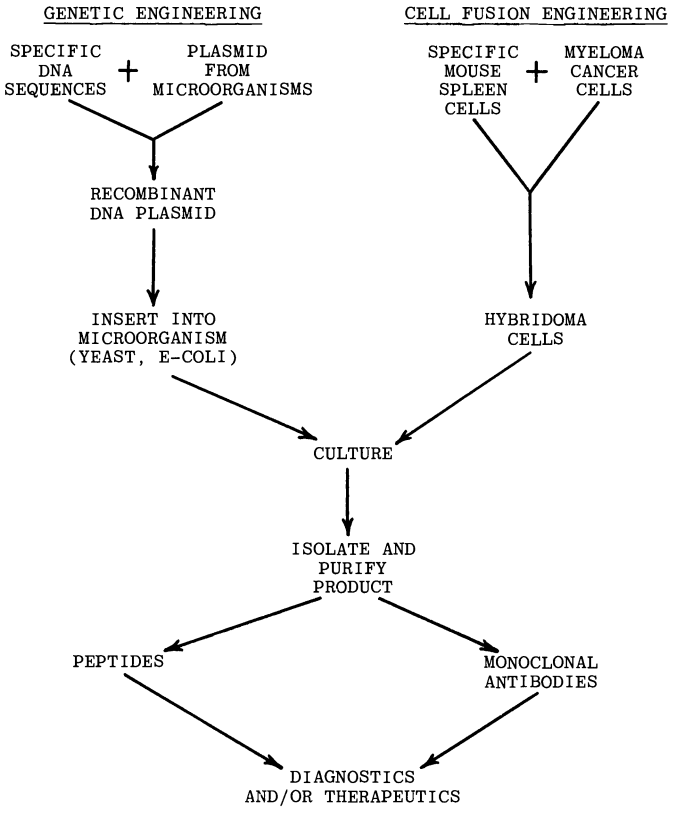

Fig. 1. The two major branches of biotechnology

RECOMBINANT DNA BIOTECHNOLOGY

Recombinant DNA (ReDNA) methodologies are making possible many new and revolutionary diagnostic, therapeutic and industrial applications in clinical medicine, agriculture (plants and insects), veterinary medicine, and animal husbandry (Table 1). This field is often referred

Table 1. Examples of various products which can be made by conventional fermentation and/or by recombinant DNA processing

Clinical Medicine	Agriculture and Food Industries	Veterinary Uses
Amino Acids	Pesticides	Vaccines
Antibiotics	Pest-resistant seeds, plants	Animal Feed (amino acids, single cell protein)
Enzymes	Faster growing plants	Growth hormones
Hormones	Soil inoculants	Antibiotics
Blood proteins	Food processing enzymes	
Regulatory proteins		
Vaccines		
Vitamins		

to as "genetic engineering" and more commonly as "gene-splicing" technology. ReDNA processing is useful for enhancing yields over conventional tissue culture processes (e.g. for antibiotics, enzymes), for larger scale fermentation production in general, or where no other practical means of synthesis is currently available (e.g. for human insulin or interferon). The full range of processes and products of ReDNA biotechnology is far from realization.

Polymers play an important role in processing and applications of ReDNA, genetically-engineered products. Basically, ReDNA technology involves the insertion of a particular sequence of a hybrid ("gene") DNA into living cells, such as yeast or E. coli cells, where it is used as a template for the production of specific polypeptide products. The cells are usually cultured in dishes or in suspension, in a fermentor. These "genetically engineered" products must then be isolated and purified for subsequent uses. To date, most ReDNA products are single gene proteins which can function with little or no post-translational modification, e.g. attachment of carbohydrates.

Figure 2 shows schematically a typical ReDNA process. This figure also shows where polymeric materials are used in many of the important steps in this process. New polymers with or without special surface treatments may be developed to improve process yields, production rates and product purity. For example, it is well known that both cell adhesion and cell motility on foreign surfaces are sensitive to surface chemistry (6-9). It is possible that replication of polypeptides within a cell could also be sensitive to the character of the foreign surface onto which the cell is adhered. Surfaces for cell culture may be used in a variety of forms (Table 2). Such polymer surfaces may be modified by a variety of methods which are based on generation of free radical initiator sites on the surfaces (Fig. 3) (10,11). Plasma glow discharge treatments may be of particular interest for this application.

TABLE 2. Solid forms of polymeric biomaterials

1. Hollow fibers, tubes
2. Films, membranes, discs
3. Microspheres, powders, beads
4. Fibers, rods
5. Molded objects
6. All of the above as:
   a. smooth, homogeneous solids
   b. filled solids
   c. surface-rough solids
   d. porous solids
   e. water-swollen solids
   f. solid suspensions in aqueous solution
7. Coatings (on any of the above)

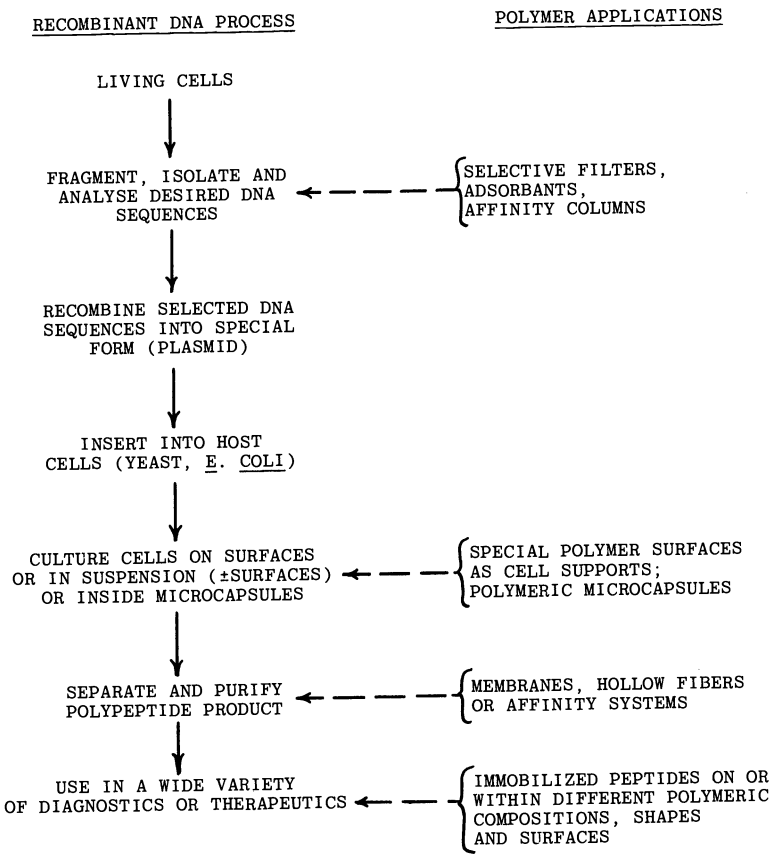

Fig. 2. Applications of polymer materials in a typical ReDNA genetic engineering process

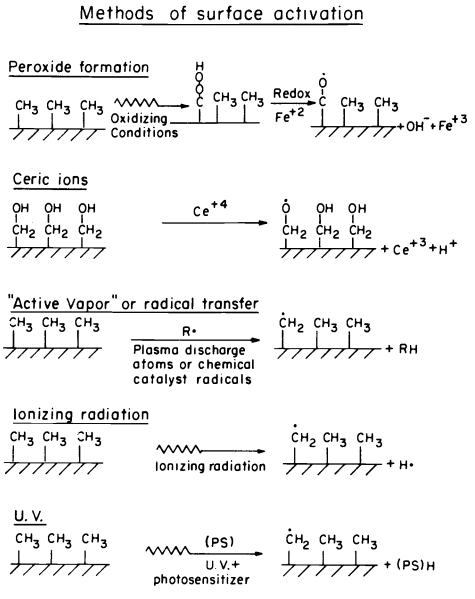

Fig. 3. Methods for generating free radicals on surfaces for subsequent chemical modification via surface graft copolymerization (10)

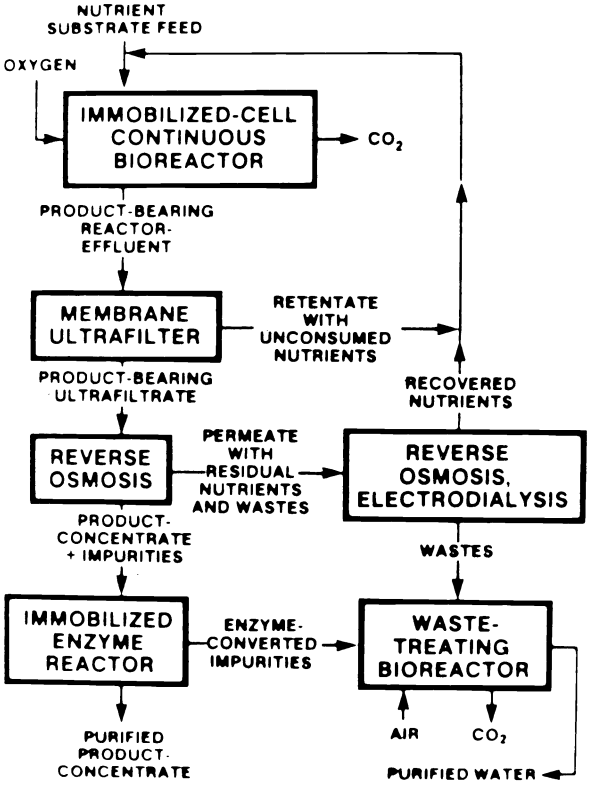

Fig. 4. Idealized, membrane-moderated biochemical manufacturing plant (12)

Polymeric membrane, filter or separation systems are useful for isolation and purification of the polypeptide products of an ReDNA process. Microporous filters may be used to separate solids from a fluid suspension; ultrafiltration may then be used to separate large and small molecules and reverse osmosis can be used to concentrate selected molecules in solution. A variety of polymer separation device configurations are possible, including flat membrane stacks, coiled, flattened membrane tubes, hollow fibers and packed beds. Figure 4 (12) shows schematically an idealized ReDNA fermentation process using several different polymeric membrane filtration systems.

This figure also shows an immobilized cell bioreactor and an immobilized enzyme reactor, to assist in product purification. Cells and enzymes (as well as other biomolecules and ligands) may be immobilized on a variety of polymeric supports, sometimes after these surfaces have been chemically modified, to provide reactable sites for the immobilization step (13-17). Table 3 lists four methodologies for immobilizing cells or biomolecules on

TABLE 3. Immobilization techniques for biomolecules and cells

1. Physical entrapment
2. Electrostatic attraction
3. Chemical bonding
4. Physical adsorption (± chemical crosslinking)

polymer surfaces. Radiation grafting of reactable polymers to more inert polymer supports has been widely used to modify such supports for subsequent covalent biomolecule immobilization (10,11). Immobilized enzymes have certain advantages (Table 4) and there are a number of commercial processes utilizing immobilized enzymes and cells (Table 5).

TABLE 4. Advantages of immobilized enzymes

1. Enzyme more stable
2. Can reuse enzyme
3. Continuous processing possible
4. Product is enzyme-free
5. Can modify microenvironment and/or process conditions
6. Lower cost, higher quality product

TABLE 5. Commercial immobilized enzyme reactors (18)

Enzyme	Product	Immobilization Method	Reactor Type	Operational Mode
Aminoacylase	L-amino acids	Adsorbed	Packed Bed	Continuous
Glucose Isomerase	High fructose corn syrup	Adsorbed	Packed Bed	Continuous
		--	--	--
		Covalent	Stirred Tank	Batch
			Packed Bed	Continuous
Lactase	Lactate-free milk	Entrapped	Stirred Tank	Batch
Penicillin Acylase	6-Amino Penicillanic Acid	Adsorbed	Stirred Tank	Batch
		Covalent	Stirred Tank	Batch
		Entrapped	Packed Bed	Continuous
Aspartase*	Aspartate	Entrapped	Packed Bed	Continuous
Fumarase*	Malate	Entrapped	Packed Bed	Continuous

* Immobilized cells

It may sometimes also be desirable to utilize affinity chromatography to isolate the desired polypeptide product from a complex mixture of proteins, nucleic acids, lipids, glycosaminoglycans, etc. For this purpose specific ligands may be immobilized on polymeric supports (Table 6).

TABLE 6. Examples of affinity biomolecules which may be immobilized on polymer surfaces for separation of ReDNA products

Antibodies
Antigens
DNA (single stranded or hybridized)
Tumor markers
Enzymes, substrates, inhibitors
Drug antagonists
Cells

MONOCLONAL ANTIBODY "IMMUNO-BIOTECHNOLOGY"

The availability of a wide range of monoclonal antibodies (MAb's) has also opened up possibilities for an immense variety of novel and revolutionary diagnostic and therapeutic applications, especially in clinical medicine (5) (Table 7). MAb's may be prepared either

TABLE 7. Uses of monoclonal antibodies

1.	In vitro or in vivo diagnostics
2.	Targeting drugs or drug-containing systems
3.	Therapeutic agents
4.	Affinity chromatography

by fusing specialized mouse spleen cells with myeloma cells to form MAb-producing hybridoma cells, which are then cultured, or by ReDNA processing, with the information for making the MAb inserted into the cell as a DNA plasmid. MAb's may be made against a wide variety of clinically important antigens (Table 8).

TABLE 8. Monoclonal antibodies may be made against many clinically important antigens

Cell surface antigens
Bacteria
Viruses
Tumor markers
Parasites
Drugs (in general)
Hormones
Enzymes
Coagulation factors
Glycolipids
Collagen
DNA (single stranded or hybridized)

TABLE 9. Possible biosensor signals

Optical (visible, fluorescent, luminescent)
Electrical (potential, current)
Radioactive emissions
Chemical (pH, redox)
Biochemical (Ag/Ab)
Mechanical (swelling)
Acoustic
Magnetic

A major use of MAb's is in a wide variety of immunoassay diagnostic tests. Included are assays for drug monitoring, viral diseases, sexually transmitted diseases, respiratory diseases, tissue typing, blood grouping, cell surface antigens and cancer. Most of these assays depend upon one of three types of signals, e.g. radioactivity (radioimmunoassay, RIA), fluorescence (fluorescent immunoassay, FIA), or visible color change (enzyme immunoassay, EIA, such as enzyme-linked immunosorbant assay, ELISA; enzyme-multiplied immunoassay technique, EMIT; and enzyme-membrane immunoassay, EMIA). MAb's may also be used therapeutically, either by themselves or as a targeting marker when conjugated to a drug or to a drug-containing polymeric system (as microcapsules). MAb's or antigens are immobilized to polymeric surfaces (usually by physical adsorption) in many of the immunoassay systems, as well as when they (MAb's) are used as a targeting molecule for a drug delivery system. Specially treated or reactable polymer surfaces are also useful for these applications (10,11).(Fig. 5)

A wide variety of biosensors utilize immobilized antibodies, antigens or enzymes (19). Some of these are miniaturized extensions of conventional assay techniques, while a number are novel fiber-optic or acoustic devices. There are important contributions to be made here by polymer scientists in collaboration with physical scientists, electrical engineers and biological scientists (Fig. 6). Table 9 lists the wide variety of biosensor signals possible. Most miniaturized devices which are intended for in vivo monitoring are still under development.

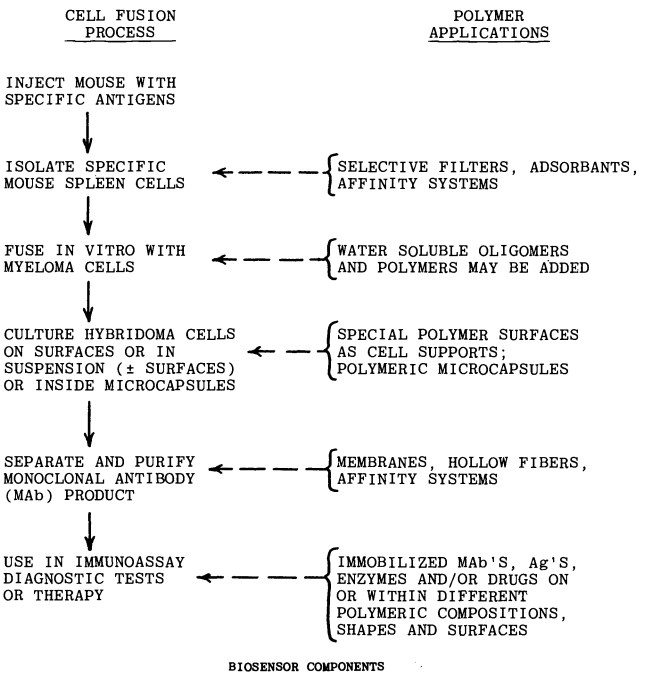

Fig. 5. Applications of polymer materials in a typical cell fusion (monoclonal antibody engineering) process.

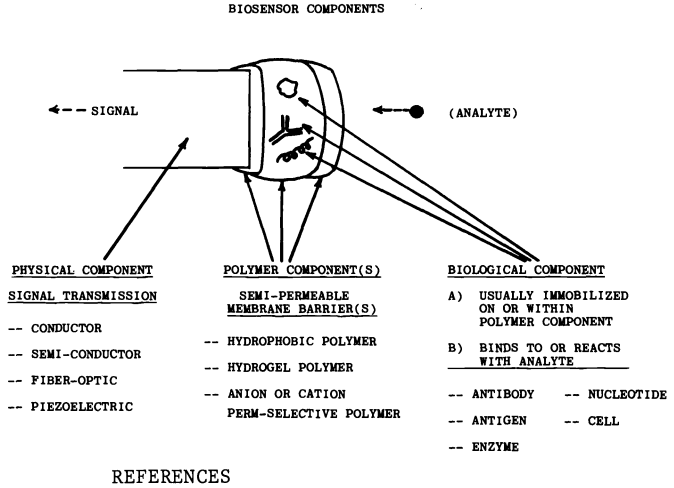

Fig. 6. The three major components of a biosensor

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