Biocompatible polymers for medical application

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SUMMARY: The principal ways of creating biocompatible, biodegradable and hemocompatible polymers for application in medicine have been considered. The conditions determining biocompatibility, regeneration of the natural tissues after polymer implantation and role of the polymer micro- and macrostructure in hemocompatibility are discussed.

Introduction

Polymeric materials are widely used in medicine. Of the greatest interest are those polymers that are applied for prosthetic appliance in the direct contact with living tissues. They should possess one general property, namely biocompatibility. Polymeric implants may be divided into two groups: 1) implants (endoprothesis) of the life-long action (cardiac valves, crystalline lens) and 2) implants of temporary actions. The latter after execution of a definite function should be destroyed in the body and products of their biodegradation should be fully exterminated. A typical example of the implants of the temporary action are medical glues. After fulfillment of the glue function in the living body, the adhesive seam must be destroyed and replaced by regenerating tissue. Biocompatibility is the polymer property by introduction into the living body to execute a definite function, not interfere (or even enhance) the functioning of a given organ, exerting neither local nor general toxicological action on the body. If the polymer should be biodegradated after accomplishing a definite function, the products of biodegradation have to be fully eliminated from the organism and the natural tissues should be regenerated instead of implant.

From our point of view, the most interesting are those polymers that are capable of biodestruction in the living body, being replaced by the natural tissues during biodegradation. The present paper considers the new approaches to the creating of biocompatible and hemocompatible polymers that can be used for medical application.

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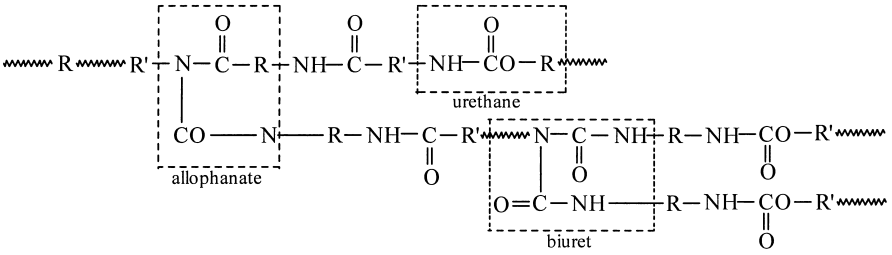
Mechanism of destruction of polyurethanes in the living body

One of the most suitable classes of polymers for endoprothesis are polyurethanes (PU) and related compounds.

Chemical mechanism of glue destruction in model media and living body

Let us consider the mechanism of biodegradation of PU using as an example the polyurethane glue KL-3[1)]. This glue is a blend of the macrodiisocyanate from toluilene diisocyanate (TDI) (a mixture of 2,4- and 2,6-isomers) and poly(propylene glycol). As an accelerator of the glue curing dimethyl-tri-(aminomethyl) phenol is used. After hardening this glue represents microporous elastic mass having the definite ratio of open and closed pores. Glue KL-3 has the following chemical structure that was established by the IR-spectroscopy and by chemical analysis[2)]:



To elucidate the mechanism of biodegradation of the polymer in the living organism, it was necessary to study the biodegradation in the media modeling organism, in the very living organism and to compare the results. As the modeling media extracts of rabbit liver, kidneys and sceletal muscles; 0.01 wt % solution of trypsin, chymotripsin, saline and Ringer-Lock solution were taken. Besides, the specimens were placed into the animal body. The destruction mechanism was studied using IR-spectroscopy and from the data on the cross-linking density determined by the Flory-Rehner method. It was established that the main reaction of the KL-3 glue destruction is hydrolysis of different links of the PU chain. The maximum reaction rate was observed in the animal organism. Proteolitic enzymes slow down the destruction. In condition of the experiments on the animals the cell mechanism of destruction was eliminated, because every three days the specimen was removed from one

animal and placed in another. It was essential to establish the reason of the highest rate of the destruction in the animal organism. For this purpose the morphology of cured glue KL-3 was studied using transmitting electron microscopy. It was found that cured glue is characterized by the globule-like structure with a definite distribution of globules by their size[3]. When destruction proceeds in saline, the distribution curve is characterized by the main maximum with average dimensions of globules 80-100 nm and a wing with globule dimensions above 1200 nm. This wing disappears when degradation takes place in the animal organism and for this case the globules with dimensions above 300-400 nm do not appear.

The study of the cured KL-3 glue in saline at 37 ºC under dynamic and static loading has shown that in this case the formation of the globules with dimensions above 300 nm is absent as well. Thus the loading promotes hydrolysis that may be connected with higher rate of removing of the polymer fragments from the specimen. The vital capacity of tissues contacting glue leads to the same effect on globules dimensions.

Cellular mechanism of degradation and resorption of the glue

Together with chemical degradation, the cell mechanism of the destruction of a polymer in organism plays an important role. It would be very interesting to determine what cells participate in the process and how they do so in the degradation and resorption of the adhesive layers[4]. For this purpose the glue masses were dyed by Sudan III-IV (mixture) that is sorbed selectively by KL-3 preparations. The intensity of dyeing to a reddish-orange colour was so strong that it was possible to determine particles of the adhesive in preparation up to a size of 3 μm. Experiments were performed in gluing wound surfaces of different animal organs (kidney, liver, small and large intestine, gluteral muscles). Reaction of tissues and healing are described[4].

The investigation of the cellular mechanism of KL-3 degradation showed that the process starts soon after implantation and is tightly connected with the productive reaction, starting at the place of adhesive contact with the tissue. The pore walls of the glue are destroyed due to hydrolysis. As a result lacunas are formed and on the 14^{th} day the connective tissue gemmas growth into them. Together with the tissue liquid, leucocytes and macrophages penetrate into lacunas. Macrophages destroy the adhesive surface. A microphotograph (Fig.1) shows inner pore and macrophages contacting the surface. Inside macrophages there

are situated the adhesive particles [Fig.1(a)]; the macrophages remote from the surface are also seen [Fig.1(b)] that contain inside an adhesive particle. The conclusion can be drawn that macrophages capture ("digest") the adhesive particles. The number of giant cells increases during 2-3 months after the start of the experiments and giant cells of foreign bodies appear in connective tissue incorporating polymer. The cells are located in depressions which are formed on the polymer surface in contact with the tissue. Many giant cells penetrate polymer cavities six months later.

Generally, the process may be described as follows. At first, a small depression (a lacuna) forms in the part of the polymer neighboring the giant cell to where a minor amount of cytoplasm and one, rarely two, nuclei are displaced simultaneously. The size of lacuna increases owing to the lysisis by the giant cells and a greater amount of cytoplasm displaces there to. At last, the lacuna gains such dimensions that giant cell may enter it completely.Its outgrowths penetrate deeply into the newly formed cavities in the polymer mass. Under the action of giant cells polymer destroys due to their lytic activity (Fig.2). In such a way, the cell mechanism of biodegradation includes the phagocytizing activity of the macrophages and lysisis by giant cells of foreign bodies.

As a result of action of different factors mentioned above, the adhesive is destroyed and fully eliminated from the organism. This was proved by application of glue labeled by C^{14} in experiments with animals[4)]. The antigenic reaction of the organism may be considered as the result of the regeneration process. This reaction reflects the appearance of some factors influencing the growth and differentiation of the tissues in the course of the regeneration process. In such a way, repeated application of the medical adhesive is harmless.

The investigation of the destruction of monolithic specimens of linear PU has shown that their destruction in model media and in the living body always begins from the surface. The determining role in degradation play easily hydrolyzing groups. The initial hydrolysis of the polymer functional groups manifests itself in three months after implantation as the surface erosion.

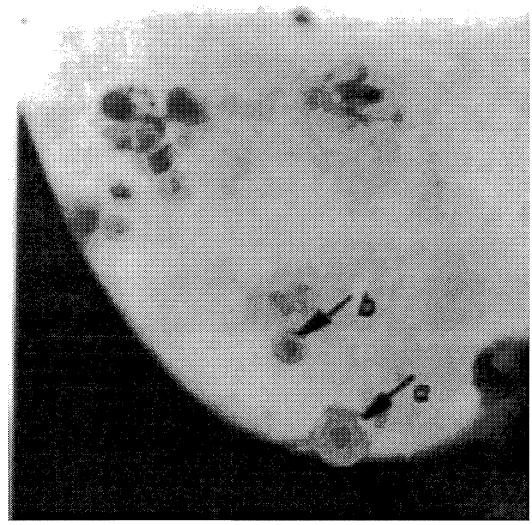


Fig. 1: Microphoto of the part of micropore of the KL-3 glue. Fragment of the adhesive in cytoplasm of macrophages on polymer surface (a) and at some distance from it (b)

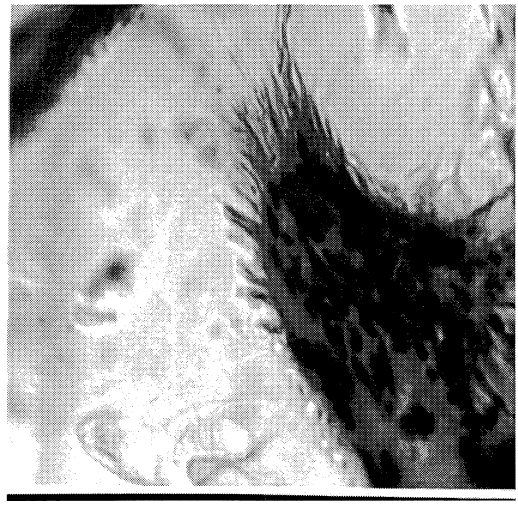


Fig. 2: Giant cell of foreign bodies and numerous outgrowths of it penetrating polymer KL-3

In polymers capable of hydrolysis, the layers in contact with tissues have loose porous structure that allows the cell mechanism of biodegradation to begin. At that time some not deep wedge-like defects appear (the depth up to 20 μm). The elements of the newly formed connective tissue penetrate these defects and at last (to 12^{th} month) the process envelops all the volume due to germination of thick interlayers of the connective tissue in the form of wedges (Fig. 3).

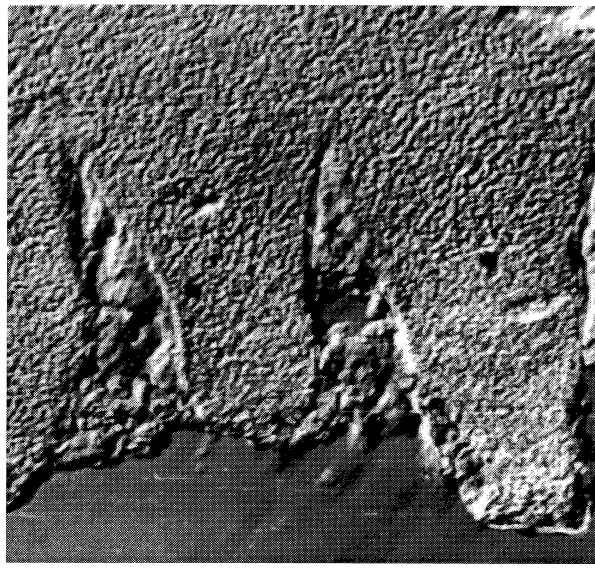


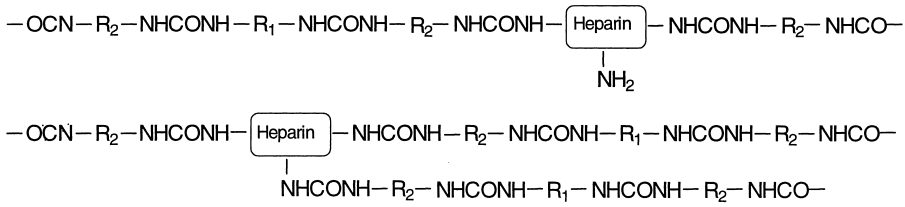
Fig. 3: Wedge-like growing in of connective tissue in KL-3 polymer. Cut section. Interference-polarization microscopy

The cell activity in biodegradation displays when all the polymer is divided into fragments; in particular, the giant cells of foreign bodies appear. Thus to realize the cell mechanism of biodegradation, the polymer has to be of porous structure, i.e. to have lacunas convenient for the action of the giant cells. Such structure appears in monolithic specimens due to the initial chemical degradation of the polymer. The term of the onset of the various stages of biodegradation may be regulated by introduction into the polymer chain of the easily hydrolyzing fragments and by creating the porous structure on submicrolevel.

Hemocompatible segmented polyurethanes containing heparin fragments

During last 25 years a great deal of work has been published on the problems of polymer hemocompatibility. In our laboratory we have studied the problem of hemocompatibility using polyurethanes as one of the most perspective polymers. Our aim was the search of the way to bond heparin chemically with PU chain. It is well known that heparin is a highly active anticoagulant. In most works dedicated to hemocompatible materials, heparin was used only to modify the polymer surface[5,6]. In our Institute heparin was bonded with PU chains by covalent bonds to realize its uniform distribution throughout the whole polymer volume[7]. The creation of artificial blood vessels capable of biodegradation and replacement by the natural tissue were the aims of work; namely for this purpose heparin should be bonded in bulk not only at the surface.

Such polymers were obtained by emulsion reaction of PU formation using various chain extenders. The scheme of the PU chain modified by heparin may be presented as follows:



where

R_1 is the residue of chain extender and

R_2 is the residue of macrodiisocyanate

The polymers were obtained from different diisocyanates, oligoetherglycols and chain extenders. For materials obtained some physical properties have been investigated. The ultimate mechanical properties of polymers corresponded to the same properties of the native vessels[7]. The structure of the polymers was investigated using small and wide angle X-ray scattering[8] and IR-spectra of the polymer at the surface and in the deep layers of a polymer[9]. Dynamic mechanical spectroscopy allowed to calculate the degree of microphase separation in the specimens[10,11].

Hemocompatibility is connected with the microheterogeneity of the surface in direct contact with blood. It is explained that the natural tissue has a mosaic structure consisting of hydrophilic and lypophylic fragments, but the mechanism of the action of heterogeneity is not clear.

The heparin-containing segmented PU obtained by us possesed various amount of covalent-bonded heparin and were characterized by the different degrees of microphase separation, determined from the SAXS data and dynamic mechanical spectroscopy. Specimens for investigations were obtained by casting from solution on the polished fluoroplast surface. IR spectra have shown that the difference in the types of hydrogen bonds from the different sides of the films (at the interface with air and fluoroplast at which the films were cast) is leveling off whereas for pure PU there was observed the great difference. These heparin containing PU were tested for hemocompatibility according to the following parameters (*in vitro*): relative index of adhered platelets (RIAT)[12], relative time of the blood coagulation (RTC) and hemolysis degree. For the polymers studied, hemolysis was absent, whereas the relative time of blood coagulation was in the range 3±0.6 to 2. 3±0.5. The results of tests are given in Fig. 4).

The measurements of the surface tension of the films have shown that this parameter passes through the minimum with increasing amount of heparin in the polymeric chain. Introduction of small amount of heparin to the main chain of PU diminishes their surface tension of all polymers (curves 3-5). Each curve corresponds to a series of experiments. For each series the polymers were synthesized from one and the same initial compounds but with different amount of heparin. It is seen that the surface tension passes through the minimum in the region of the heparin content up to 1 wt % and then increases, not reaching the value of pure PU. The degrees of segregation in two series of specimens (curves 1 and 2), also are characterized by the minimum at the same heparin content and then again increase up to the values exceeding the values for pure PU. At last, value of RIAT also goes through the minimum in the region of the heparin content approx. 0.2 wt % (curves 6-8). The further increase in heparin content leads to the increasing RIAT up to the values above those for PU. It's important that all the minimum manifests themselves at the heparin

content approx. 0.2 wt %. In such a way there exists some correlation between the degree of microsegregation, surface tension and hemocompatible properties of the polymers.

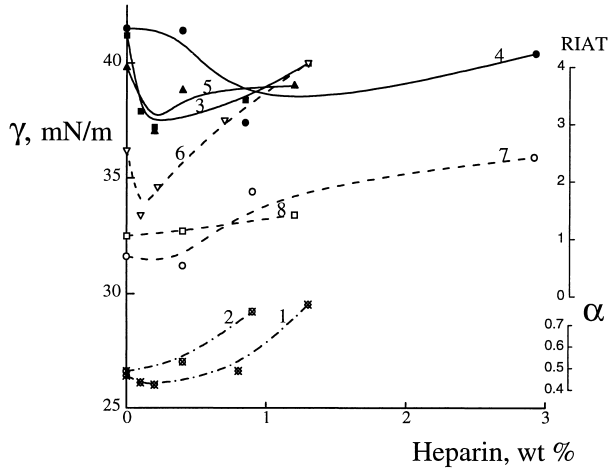


Fig. 4: Dependencies of the segregation degree α (curves 1,2), surface tension γ (curves 3-5) and relative index of adhered platelets (RIAT) (curves 6-8) on the heparin content in PU chain. Composition of PU: curves 1,3,6-diphenylmethanediisocyanate (DPM), poly(tetramethylene glycol) (PTMG) M=1000, dihydrazide of adipic acid; curves 2,4,7- DPM, PTMG M=1000, ethylene diamine; curves 5,8-DPM, PTMG M=1500, ethylene diamine

Let us consider now the curve's branches in the region of the heparin content above 1 wt % (Fig. 4). All dependencies are linear and all values increase insignificantly. The most interesting transformations in the polymer structure proceed at the heparin content 0.2-0.4 wt %. Increasing heparin content gives no additional effect in relation to hemocompatibility. If one consider the effect of the microsegregation degree, from the curve 2 (Fig. 4) it is seen that RIAT increases with increasing segregation degree. It allows to conclude that this characteristics plays an important role in hemocompatibility.

It may be supposed that hemocompatibility is determined by one more factor, namely the roughness of the polymer surface. The relief scale should correspond to the dimensions of the blood elements, platelets, in particular. To prove this supposition we have studied the connection between hemocompatibility parameters and surface structures. Using the test[13)]

for the determination of the hemocompatibility parameter, the latter was determined for the surfaces of the polymers under investigation and for standard surfaces with poor (glass) and good (hydron) hemocompatibility. According to the test mentioned, the higher is the prothrimbin index (time of the platelets formation in contact with the surfaces), the worse is the parameter of hemocompatibility (for hydron the prothrombin index is equal to 31.4±2.5 s). The surfaces of the polymers were investigated from the side of the contact with fluoroplast and from the air side. Polymer based on hexamethylene diisocyanate, poly (tetramethylene glycol) M=1000 and ethylene glycol as chain extender has the following values of the prothrombin index: 44.5±2.1 s for the air and 180 s for fluoroplast. The initial surfaces and surfaces after the contact with blood were investigated using scanning electron microscopy. It was observed that the surface elements from the air side are much greater as compared with those for the fluoroplast side. The comparison of the adhesion of the platelets on both surfaces shows that on the "air" surface the platelets form small scattered islands, whereas "fluoroplast" surface is fully covered by the platelets. The dimensions of the hollows for the "fluoroplast" surface are comparable with the platelet dimensions and may serve as original traps, leading to the full covering the surface.

Thus, the dimensions of the relief element and their shape play the determining role in the platelet adhesion. If the depressions are small as compared with the platelet dimensions, or very large, the "trap" mechanism does not operate.

An example of such a surface gives the surface of PU formed from hexamethylene diisocyanate, PTMG M=1000 and dihydrazide of adipic acid. The surface elements on the side turned to air are very small as compared with the platelets dimensions and because of it the prothrombine index is equal to 33±7 s.

Discussion

All the polymers under consideration are capable of biodegradation and in the course of the process polymer are replaced by the regenerating tissue. What may happen with the artificial blood vessel made of PU and implanted to the living organism? This problem was investigated in some works[14-16]. The part of the abdominal aorta of rats was replaced by the porous polyurethane endoprothesis. In the work[17] porous PU endoprothesis were implanted

instead of large blood vessels of dogs. The results were favourable when annulate structure of the smooth muscle of the vessel was formed. In other cases the aneurismes and sclerosis of the vessel walls was observed[17,18]. In all the cases the inner vessel layer is formed. However, full regeneration of the vessel proceeds only when the annulate structure of the middle smooth muscle layer was formed (30 % of cases).

In our special experiments it was shown that by plasty gluing of different tissues using biodegradable PU one can reach their regeneration with restoration of the normal tissue structures[4, 18-20].

By gluing of the skeleton muscles of the rabbits and rats using polyurethane adhesive KL-3 the full regeneration of the muscle fibers with further innervation took place. This fact plays the determining role in the functioning of the organ. By closing the kidney wounds using KL-3, their tissue structure and excretoric function also were regenerated[19, 20]. The good results gives the plasty gluing of the cut sciatic-ischiatic nerves of rabbits using combination of the KL-3 with porous supports made of KL-3, ends of the cut nerves being placed on this support[4]. In this case the full anatomic regeneration of the nerve took place.

Our data show that microporous structure of the polymer promotes regeneration of the normal tissues. The lack of the hystotoxicity seems to exclude the fibrous reaction, i.e. the normal tissue is regenerated not fibrous one. Besides, architectonics of endoprothesis should correspond to hystoarchitectonics of the tissue. Thus, to create an artificial vessel capable of being replaced by the native one, it is necessary to use microporous material with the definite ratio of the open and closed pores and directing orientation of the microchannels.

Conclusion

Thus, the experimental data allow the following conclusions to be drawn. To create the hemocompatible polymeric materials it is necessary to create a definite structure of the surface of the material that should be a mosaic with dimensions of the surface elements corresponding to the elements of the surface of the native vessel. Introduction of a small amount of heparin leads to structural changes of PU and promotes improving the parameters of the hemocompatibility. In this case the role of heparin is not the role of anticoagulant, because at high content of heparin the properties of the polymers in relation

to hemocompatibility become worse. It is possible that properties of heparin as anticoagulant may play their role at the prolonged contact of the material with blood.

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