Free radical homopolymerization, in heterogeneous medium, of linear and star-shaped polymerizable amphiphilic poly(ethers) : A new way to design hydrogels well suited for biomedical applications

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SUMMARY: The synthesis and the properties of selected macromolecular structures based on poly(ethylene oxide), (PEO) are discussed in the present paper. The first part deals with the development of efficient multifunctional anionic initiators aimed to design well-defined functionalizable star-shaped PEO's. Different approaches providing access to branched species of controlled hydrophilic /hydrophobic balance will be considered. The second part is devoted to the homopolymerization of amphiphilic bifunctional PEO macromonomers as an efficient way to yield PEO hydrogels directly in water. The extension of that approach to degradable PEO hydrogels or to the copolymerization of macromonomers with star-shaped PEO's partially functionalized with polymerizable entities will be briefly mentioned. These hydrogels served as semi-permeable membrane for an artificial pancreas and as a template for the growth of nervous cells.

Introduction

Poly(ethylene oxide), PEO, is a non ionic hydrophilic polymer which exhibits specific solution and solid state properties[1)]. PEO is characterized by a good chemical stability and is soluble in water and in a large variety of organic solvents excepted ethers and alkanes. PEO forms complexes at low temperature with organic solvents such as tetrahydrofuran (THF), acetone. Furthermore, the remarkable biocompatible properties of PEO have already led to a wide number of biomedical applications[2,3)]. Various polymeric structures or materials based on PEO such as linear functional polymers including macromonomers[4)], block[5)] or graft[6)] copolymers, branched or star-shaped polymers[7)] or hydrogels[8)] have been synthesized over the years. The purpose of the present work is to discuss selected examples of such structure. Some of them are schematized on Fig. 1. The preparation and some properties of functional star-shaped PEO's based on poly(divinylbenzene), (DVB), cores will be discussed first. Two examples of new efficient polyfunctional anionic initiators for the anionic polymerization of oxirane will be given. In a second part, the synthesis and the properties of PEO hydrogels

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obtained by free radical polymerization of bifunctional PEO macromonomers will be discussed in detail. The extension of that approach to the synthesis of amphiphilic or degradable networks will be outlined. By copolymerization of these bifunctional PEO macromonomers with star-shaped PEO's partially modified at the outer end of the branches with polymerizable groups, hydrogels containing residual functional groups should be accessible.

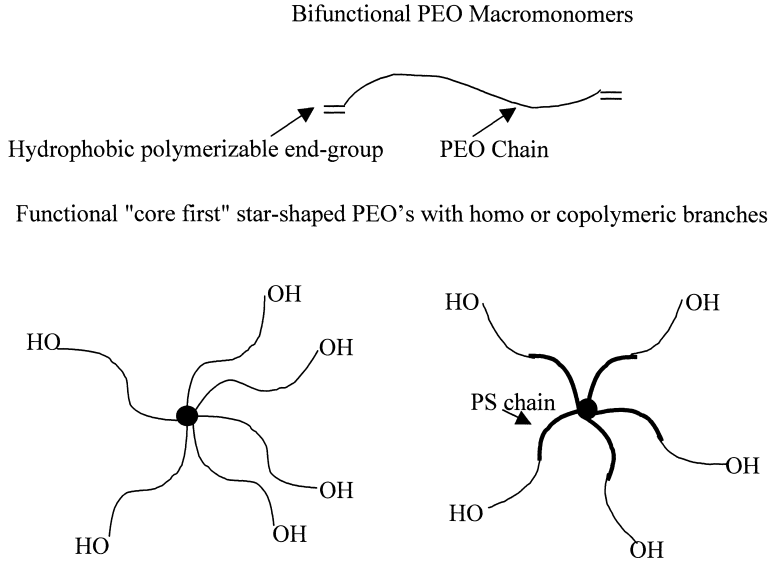


Fig. 1: Examples of homopolymeric or copolymeric linear or star-shaped functional structures based on PEO and accessible by anionic polymerization.

In the last section some preliminary results on the use of these hydrogels for biomedical applications such as a semi-permeable membrane will be presented. Further work is also under progress to test these hydrogels as a template for the growth of nervous cells.

Functional branched PEO's

The interest for star-shaped polymers arises from their compactness and enhanced segment density as compared to their linear counterpart of the same molar mass. Their synthesis is essentially based on anionic polymerization methods whereby star-shaped polymers characterized by branches of controlled molar mass and of defined functionality can be

obtained. Some attempts by cationic polymerization or group transfer polymerization processes have to be mentioned. The different approaches to design star-shaped polymers have been reviewed recently[9] and will not be discussed in details. Here, only those providing access to functionalizable PEO star-shaped polymers will be presented. The synthesis of such species consisting of a core or backbone carrying a controlled number of hydrophilic (PEO) branches represents an important challenge for polymer chemists. The presence of functional end-groups at the outer-end of the branches enables a large scope of functionalization reactions. It can also be anticipated, that among the different parameters governing their solution behavior, the functionality of the core and its chemical nature are determinant.

Functionalizable star-shaped PEO's are now commonly synthesized via "core-first" anionic polymerization especially from polyfunctional divinylbenzene (DVB) cores[10] (Fig. 2).

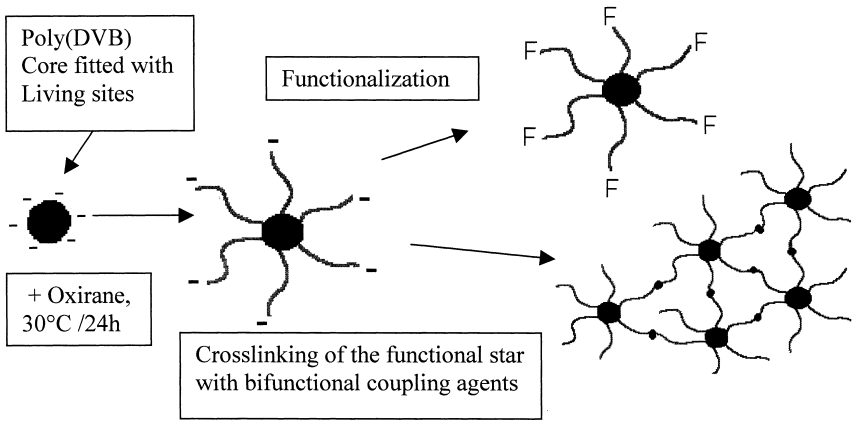


Fig. 2 : Schematic representation for the preparation of functional star-shaped PEO's and hydrogels derived from these PEO's

They are accessible over a large range of molar masses and functionalities. In addition they represent interesting building blocks for structured hydrogels. Their crosslinking can be achieved either directly by deactivation with the appropriate low molar mass bifunctional compound with antagonist functions or in a two step procedure. In the last case, the outer end of the branches bearing hydroxyl functions are reacted with a diisocyanate whereupon the network is obtained. That point will be discussed in the section hydrogels.

Preliminary studies on "core-first" PS star-shaped polymer have shown that the distribution in functionalities is large[11]. This may not always be a drawback, as after appropriate separation

fractions characterized by different functionalities but with branches of identical molar masses can be obtained. Such samples are well suited as models for physico-chemical studies. That method is easy to perform (one-step method) and high amounts of oxirane can be introduced yielding PEO's well suited for biomedical applications. The large polymolecularity, just mentioned above, is not a drawback for such applications where high functionality, good water solubility and biocompatibility are the major requirements. One limitation to an extensive use of that approach may be, that the functionality of the poly(DVB) cores cannot be really controlled in advance even under appropriate experimental conditions.

To learn more about their solution behavior, a systematic investigation of the dilute solution properties of such "core-first" PEO's, synthesized under almost identical experimental conditions, excepted the monomer amount, by static and dynamic light scattering has been done by K Naraghi et al[12]. Some characteristics of these samples are presented on table 1 together with the preparation conditions.

Table 1 : Characterization data of star-shaped PEOs prepared from poly(DVB) cores.

Sample	PEO462	PEO460	PEO467
$\overline{M}_n$ branch calc [a]	4 800	9 100	15 900
$\overline{M}_W$(LS-MeOH) [b]	116 000	415 000	950 000
f	29	45	60
R_g(nm) [c] MeOH	25	38	56
H_2O	98	27	64
R_h(nm) [d] MeOH	7	17	51
H_2O	15	23	67

Experimental conditions: [DVB]/[K^+] = 1,5; addition time of DVB = 10 min; reaction time = 15 min; temperature = -35°C
[a] $\overline{M}_n$ number average molar mass, calculated from ratio [M]/ [K^+], expressed in g/mol
[b] $\overline{M}_w$ weight average molar mass of the PEO star in methanol expressed in g/mol
[c] R_g Radius of gyration
[d] R_h hydrodynamic radius

The results presented on table 1 call for a few comments

- The average molar masses ($\overline{M}_{WLS}$) of the PEO's stars measured by light scattering in dilute solution, methanol being the solvent, increase when the ethylene oxide content in the sample increases.

- The average functionality (f), controlled by the ratio $[DVB]/[K^+]$ is calculated from the ratio of the value of the $\overline{M}_{WLS}$ of the stars to the calculated $\overline{M}_n$ of the branch taking into account the polymerization yield and the concentration of active sites in the medium. As the value of the ratio $[DVB]/[K^+]$ was maintained almost unchanged (1.5) for all the samples, f should be constant. From the results presented on table 1, it can be concluded that f is not constant : f increases with increasing molar mass of the branches i.e. for high ethylene oxide content. This may be explained by the fact, that the values of the molar masses of the PEO's do not correspond to isolated molecules as it should be the case for dilute solution measurements. A possible explanation may be the presence of the polyDVB core neither soluble in methanol nor in water leading to association or aggregation of the star-shaped PEO's.

To clarify that point, the same PEO's were submitted to critical micellar concentration (cmc) determinations. Measurements of the variation of the I1/I3 intensity ratio in pyrene monomer fluorescence as a function of DVB concentration (mol DVB/L) confirmed the existence of a cmc (Fig. 3) and the aggregation or micellization in water. This implied that the hydrophobic poly(DVB) core is not, in all cases, efficiently protected by the hydrophilic PEO branches. These results also showed the same cmc for all the samples of the series ($0{,}6 \times 10^{-4}$ mol DVB/L). For concentrations above that value, association or micellization may take place, corresponding to an increase in the average molar mass as compared to the dilute solution values.

- The R_g and R_h values of the different samples were obtained from static or dynamic light scattering measurements performed in water and in methanol. As it can be concluded from the results presented on table 1 and Fig. 4, these values increased with increasing polymer concentration. That effect was yet more pronounced for the samples with longer branches and in water. In spite of the fact, that these stars contained only low amounts of hydrophobic parts, the poly(DVB) core, (from 1 to 2 weight %), they exhibited for given concentrations association phenomena. These phenomena were observed in water as well as in methanol or in THF dependent on the polymer concentration. In both cases an increase of the average molar mass was noted. Similar observation was made on the evolution of the hydrodynamic and gyration radii.

Further work has been done to study the behavior of these PEO stars at higher concentrations i.e. in the semi dilute regime. The results will be discussed in a forthcoming paper[13].

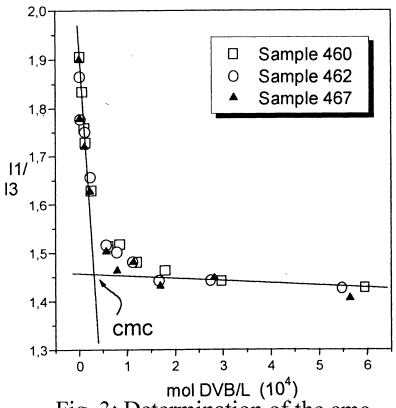


Fig. 3: Determination of the cmc for the three PEO samples

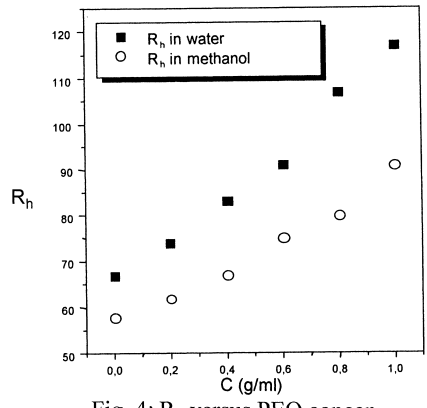


Fig. 4: R_h versus PEO concentration in water and methanol

Much effort has been devoted to develop methods allowing a better control in advance of the functionality of star-shaped PEO's. Among these, it is well established that tris-alkoxide functions obtained by reaction of a stoichiometric amount of a strong base, such as diphenylmethylpotassium, with the primary alcohol functions of trimethylolpropane provided access, after addition of oxirane, to well defined three functional star-shaped PEO's[14]. More recently multifunctional PEO stars were obtained from carbosilane dendrimers applying the same strategy[15] or from an "arm-first" strategy using poly(amidoamine) dendrimers[16]. An other method derived directly from the "core-first" approach has to be mentioned. Star-shaped PEO's can be synthesized from a seed-star prepared by reaction of short polystyrene (PS) living chains with DVB. These stars are fitted on the same core with PEO and short PS branches. The polymolecularity of these samples is markedly reduced with respect to that arising from the "core-first" methods[10]. These well-defined "in-out" star-shaped PEO samples are not suited for biomedical applications: for a given PEO arm length they contain much higher contents of hydrophobic units (the PS chains) than the "core first"ones.

Two other recent approaches to design branched PEO's will be briefly presented below. The first derives again directly from the poly(DVB) approach and the second refers to the use of polyglycerol cores.

In the first case, a linear poly(1,3-diisopropenylbenzene) backbone is prepared by anionic polymerization under conditions leading to linear polymers characterized by one remaining unsaturation per monomer unit in the chain, the number of monomer units incorporated in the chain corresponding to the potential functionality. These unsaturations served, after reaction with a stoichiometric amount of cumyl-potassium, as efficient multifunctional initiators for

the anionic polymerization of oxirane. Accurate characterization of these samples demonstrated that a far better control in advance of the functionality could be reached as compared to the case of poly(DVB) cores[17]. As in the case of stars with poly(DVB) cores, one has to be aware of the presence of an hydrophobic backbone leading to association in water.

This should not be the case for strategies based on poly(glycerol) cores. Knischka et al[18] used such poly(glycerol) as well as poly(glycerol) modified with short apolar oligo(propylene oxide) segments (DP_n = 23 - 52; $\overline{M}_w/\overline{M}_n$ = 1.2-1.4), after deprotonation with diphenyl-methylpotassium, as polyfunctional initiators for the anionic polymerization of ethylene oxide to prepare multiarm PEO star polymers. For the unmodified polyglycerol, after metalation, aggregation due to the highly polar structure occurred and no control of the structural parameters of the PEO stars could be achieved. On the contrary, using the apolarly modified polyglycerols with terminal oligo(propylene oxide) segments, hydroxyfunctional poly(ethylene oxide) (PEO) multiarm star polymers of controlled functionality and arm-length, with molar masses from 34 000 to 95 000 g/mol, and functionalities from 26 to 55 were accessible. ^{1}H- and ^{13}C-NMR evidenced complete conversion of all end groups of the propylene oxide capped end groups of the initiator. Reinitiation of the multiarm PEO-stars by deprotonation was possible and, after addition of a new amount of oxirane, star-shaped PEO's with increased arm-length but identical functionality resulted.

These two approaches represent a decisive step in the synthesis of branched PEO's of controlled functionality. In addition, PEO stars obtained from polyglycerol cores are constituted of hydrophilic PEO chains and an hydrophilic poly(glycerol) core. They are therefore well suited for biomedical applications. Crosslinking of such species will be discussed later.

The different approaches to design star or branched species containing PEO chains, described in the present paper, are always based on anionic polymerization of oxirane. That procedure is efficient but not easy to perform. One possibility to overcome these difficulties could be to start from existing PEO chains to prepare branched species. Ito et al[19] have prepared monofunctional PEO macromonomers, defined as polymers carrying polymerizable units at the chain end. Advantage of the amphiphilic character of such species, water soluble chain

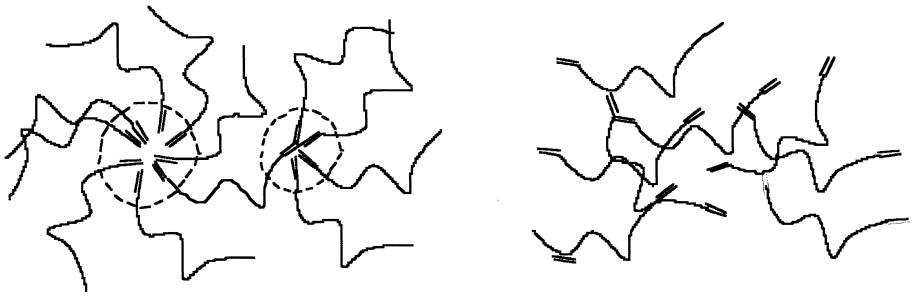
and hydrophobic polymerizable chain end, could be taken to yield, by free radical polymerization processes, directly in water solution, various comb-shaped PEO's.

Hydrogels

Much interest has already been devoted to design materials based on PEO and therefore well suited for biomedical applications. Two preparation ways have to be considered : either by adsorption or grafting PEO onto surfaces or by incorporation of these PEO chains into three dimensional network structures. When a surfaces is treated with PEO chains or copolymers including PEO segments, adsorption of the chains takes place[20] whereby the hydrophilic character of the surface is improved. PEO chains have yet a strong tendency to desorb from the surface especially for high molar masses and to diffuse in the physiological media. PEO. chains can also be chemically grafted onto a surface[21]. This method is devoid of the desorption problem. However, the techniques used (electron-beam irradiation, gamma irradiation, or plasma deposition techniques) are not easy to perform and require expensive experimental set-ups. Chemical methods to covalently attach PEO onto a surface have also been reported[22]. Whatever the method only the surface of the material is modified. This may not be sufficient for biomaterials such as whose aimed to serve as semi-permeable membranes where it is important for the entire material to be biocompatible and hydrophilic. In addition a controlled porosity is also a major requirement.

The crosslinking by formation of bridges between individual chains of well defined molar mass is well known to represent an alternative to insolubilize PEO chains in aqueous or in organic solvents. Irradiation of water solutions of linear PEO chains was until recently the most commonly used method to design PEO networks in water[23,24]. The method could be extended to PEO star-shaped structures whereby materials containing residual functions could be obtained[25]. Due the statistical crosslinking process there are yet large fluctuations in the length of the elastic chains between two junction points. Far better results are obtained when the crosslinking is achieved upon reaction of a linear dihydroxy PEO of controlled molar mass with a stoichiometric amount of a plurifunctional isocyanate under conditions which minimize defects[26]. The reaction requires yet severe experimental conditions to limit the number of pendant chains and unreacted isocyanate groups such as exact stoichiometry, absence of water, presence of a catalyst. That method provides a precise knowledge of the elastically effective network chains. The presence of these functions in the final gel may not be the best for materials aimed for biomedical applications.

To overcome these difficulties we introduced recently[27] a preparation method of hydrogels directly applicable in water via free radical homopolymerization of α,ω–methacryloyloxy PEO macromonomer (Fig. 5). "Macromolecular" monomers, macromonomers, are defined as polymers, usually of rather low molecular weights, carrying a polymerizable group (double bond, heterocycle) at one or two chain ends. Their preparation basically refers to ionic polymerization processes whereby quantitatively functionalized species with narrow molar mass distributions resulted.



a) b)

Fig. 5: Schematic representation of the homopolymerization of bifunctional PEO macromonomers a) in water ($K_2S_2O_8$, 24 °C), b) in organic solvents (benzene, AIBN, 65 °C) the single trace correspond to the PEO chains and the double trace to the polymerizable units

This reaction can be conducted in water solution or in organic solvent. The resulting networks were investigated as gels swollen to equilibrium in THF or in water. In each case, the amount of extractable material, the degree of equilibrium swelling and the uniaxial compression modulus were determined. The influence of the preparation solvent on the properties of the networks was studied[27]. As it can be concluded from Fig. 6 the gel point is reached more rapidly in water than in organic solvents. In addition the mechanical properties of the networks obtained in water are better than those of networks obtained in organic solvents under exactly the same conditions. This can be explained by the preferential formation in water of micellar structures containing high concentrations of polymerizable methyl methacrylate (MMA) units. These results are self-consistent with the lower amount of extractable materials found for the networks synthesized in water. The results are in good agreement with data published by Kazanskii et al[28]. That crosslinking method of PEO macromonomers directly in water represents an important progress in the preparation of hydrogels. Not only, as mentioned earlier, these hydrogels can be prepared in water but polymerization in water eliminates the obligation to exchange the solvent, inevitable when

using in organic solvents an isocyanate as cross-linking agent. In addition that approach presents additional interesting possibilities to control the structure and the properties of networks. As a example the addition of that hydrophobic comonomer improved, at least within some limits, the mechanical properties[29] (Fig. 7.). Kazanskii et al.[30] copolymerized bifunctional PEO macromonomers with ionic comonomers to design hydrogels with charged groups at the network junction points.

Goethals et al[31,32] and later N. Sahli[33] applied a similar procedure to synthesize poly(1,3-dioxolane) (PDXL) networks by homopolymerization of telechelic PDXLs with methacrylate end-groups. Due to the sensibility of the PDXL chains to acidic degradation, access to a new class of degradable hydrogels was provided.

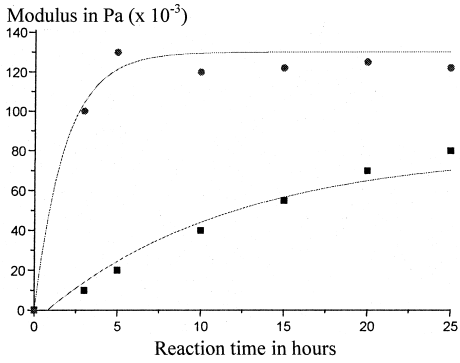


Fig.6:Evolution on the uniaxial compression modulus versus reaction time in benzene (■) and water (•)

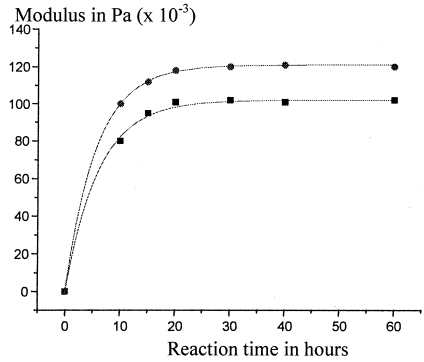


Fig. 7 : Comparison of the evolution of the uniaxial compression modulus in water for homo (■) and copolymerization (•)

Along the same line hydrogels whose elastic chains are constituted of hydrophilic poly(ethylene oxide) (PEO) chains containing a short central (PDXL) block were obtained through free radical homopolymerization of α,ω–methacryloyloxy PEO-b-1,3-PDXL-*b*-PEO macromonomers[34]. Here again advantage of the amphiphilic character of these macromonomers was taken to crosslink them directly in water to high yields. This result can be explained by the formation in water of micellar structures containing in their center the hydrophobic polymerizable units. These networks were investigated as gels swollen to equilibrium in water. In each case, the amount of extractable material, the degree of

equilibrium swelling and the uniaxial compression modulus were determined. As mentioned above, the central PDXL contains acetal groups which are sensitive to acidic degradation. Thus the evolution of the mechanical properties of the networks placed in acid media, was examined by uniaxial compression measurements and by solid state NMR spectroscopy. The degradation of the networks was possible even when swollen in water. That result is in contradiction with data published earlier by Goethals et al[32] .

For some specific biomedical applications it may be on interest to design networks still containing residual chemical functions. One possibility to access such materials could be to copolymerize bifunctional PEO macromonomers with appropriate low molar mass compounds or with monofunctional PEO macromonomers. In both cases the mechanical properties of the resulting networks are strongly reduced as compared to the pure homopolymeric networks. The copolymerization of bifunctional PEO macromonomers with low amounts of PEO star-shaped polymers where the outer ends of the branches are partially modified with polymerizable units constitutes a far better approach[27,35,36]. That reaction is presented on Fig. 8.

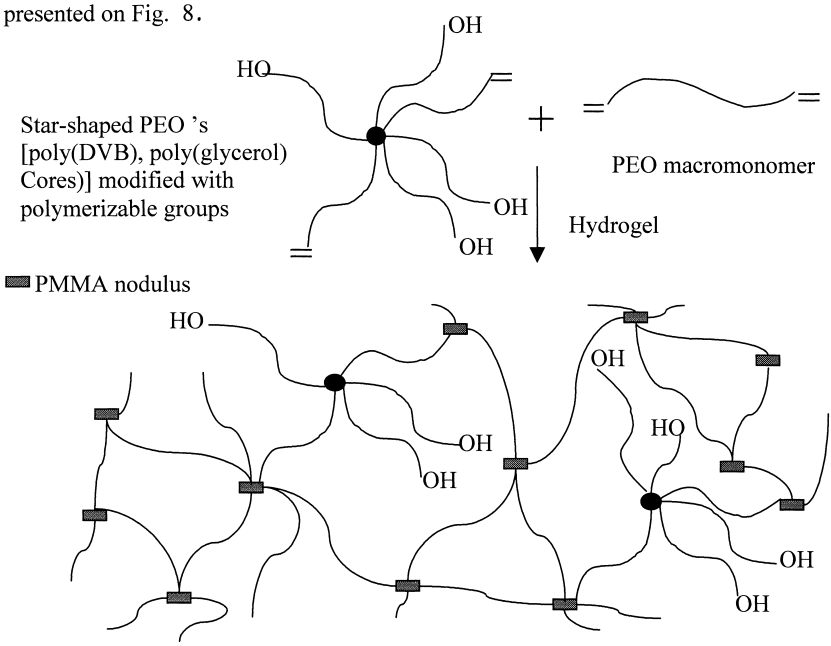


Fig. 8 : Schematic representation of the crosslinking of PEO stars partially modified with polymerizable groups with bifunctional PEO macromonomers

Provided the initial content of PEO stars is maintained low, less than 10 weight %, the physico-chemical properties of the resulting networks are comparable, for a given linear

precursor, to those of networks obtained by homopolymerization[27,36] . The residual hydroxyl functions in the network allow yet further modifications of the properties of the hydrogels.

The different networks just discussed are constituted of PEO or PDXL chains crosslinked by PMMA noduli. Therefore in most cases they are of limited mechanical stability. This may be a disadvantage for biomedical applications such as semi-permeable membrane for an artificial pancreas. The grafting by electron beam irradiation of PEO star-shaped polymers onto poly(tetrafluoroethylene) (PTFE) surfaces has been shown to accces materials combining the good biocompatible properties of PEO with the outstanding mechanical properties of PTFE. The preparation of such hybrid materials is made as follows. Star-shaped polymers of well-know structural parameters were dissolved in water and irradiated by 3 MEV electron beam generator. Crosslinking generally occurred rapidly. Due the hydrophobic character of PTFE, its surface had to be pre-modified first by N-vinylpyrolidone. These PTFE surfaces were examined by ESCA to quantify the amount of star-shaped PEO grafted on the surface. From these measurements it could be concluded that these surfaces are covered by an hydrogel layer. For materials designed for a semi-permeable membranes, porous PTFE had to be used[37].

PEO hydrogels and biomedical applications

The third part of the present work is devoted to the use of selected PEO hydrogels for biomedical applications. Among these, semipermeable membranes for the conception of a bioartificial pancreas have attracted increasing interest. Such membranes have to protect the islets of Langerhans, present in a pancreas, who are controlling the insulin (i.e.glucose) concentration and its formation. A section of a pancreas is presented on Fig. 9, the black domains correspond to these islets of Langerhans. Once these islets are destroyed, one has to supply that function. One way to solve that problem, could to be to implant in the body an artificial pancreas containing islets of Langerhans recovered from an other pancreas. K. Boudjema et al[37] have been able to recover these islets from a pancreas and to keep them active. The membrane surrounding these islets has to exhibit a good biocompatibility and to be non thrombogenic. That membrane had to be permeable to glucose and insulin and ensure the immunoisolation (Fig.10). The aim of our work was to examine the ability of PEO hydrogels obtained by polymerization of bifunctional macromonomers to fulfill these conditions.

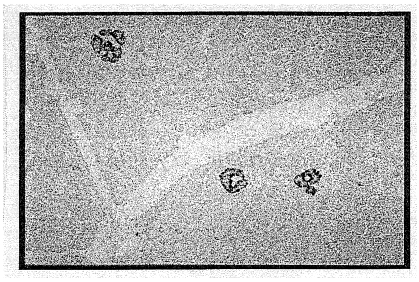


Fig. 9: Section of a pancreas
(Source Ref. 27)

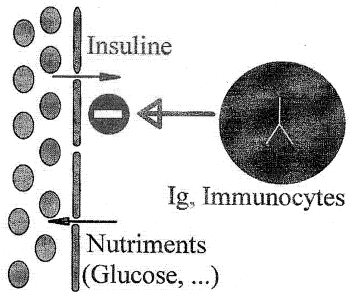


Fig.10:Schematic representation of the artificial pancreas (Source Ref 27)

Before testing the diffusion capacity of such hydrogels, we had to verify their biocompatibility. These PEO hydrogels were tested in vitro for fibroblasts adsorption and implanted subcutaneously and intraperitonealy in rats (during one year). A section of the cave vein of a pig was replaced by an artificial vascular implant based on PEO hydrogel. In vitro experiment results showed that only few amounts of fibroblasts were adsorbed on the surface, and the spherical form of the adsorbed cells evidenced the weak interaction with the surface. Moreover, these PEO hydrogels had not induced thrombotic or inflammation reactions even when implanted for long periods in direct contact with blood[27].

The glucose diffusion properties of these membranes were studied by a lag time analysis method. As expected, glucose diffuses through the membranes. The value of that diffusion coefficient increased with the length of the PEO precursor chain i.e with the decreasing concentration of hydrophobic units, the PMMA nodulus. The insulin diffusion coefficients in the membrane were also studied and the values compared to free diffusion in water [27]. Here again higher values of diffusion coefficients corresponded to longer PEO precursor chains. In some case an adsorption of insulin on the hydrophobic core had to be considered. Further work is now under progress in order to limit the adsorption of insulin. One possibility could be to introduce low amounts of hydrophilic comonomers in the cores.

Further work is now under progress to apply that polymerization method to the preparation of hydrogels containing longer precursor chains in order to improve the diffusion properties. One limitation may be the mechanical properties of these PEO hydrogels. This is why surface treatment with linear or star-shaped PEO's is actually developed.

PEO hydrogels obtained by polymerization of macromonomers in water have also been tested regarding their capacity to serve as a template for the cell growth[39]. The network structure should directly affect cell attachment and differentiation. Best results have been obtained with a sample characterized by an elastic chain length of 15 000 g/mol and prepared at a polymer concentration of 20 weight %). This sample is characterized by the highest equilibrium swelling and the longest elastic chain. After a few days *in vitro* dissociated cells are able to form aggregates firmly attached to that hydrogel surface. Immunocytochemical labellings reveal in these clusters a mixed population of glial cells, neurons and pituitary endocrine cells, the melanotrophs. More details concerning the use of such PEO hydrogels for the growth of nervous cells will be published in a forthcoming paper[40] .

Conclusion

The main aim of the present work was to examine various ways to design PEO's of controlled structural parameters. For star-shaped PEO's among the different approaches discussed, the development of poly(glycerol) cores of controlled functionality as multifunctional initiators for the anionic polymerization of oxirane represents probably the most important recent contribution to the domain of well-defined PEO stars. The homopolymerization of macromonomers offers a new way to prepare hydrogels directly in water and its interest goes far beyond the present paper. The availability of these PEO based structures enlarges the number of PEO's structure well-suited for biomedical applications.

The author wishes to express his acknowledgments to all his colleagues and coworkers who have contributed to various research topics dealing with the control of macromolecular architectures by various polymerization processes.

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