PEGylation, the Glorious Past, Present Dilemma and Future

Chenyang Lou *

Shanghai Weiyu High School, Shanghai, China

* Corresponding author: dcolwellz50975@student.napavalley.edu

Abstract. Polyethene glycol (PEG), with first application approved in 1990, is a widely used polymer in the modification of nanometer level drug particles until now due to its outstanding ability to give nanoparticle the "stealth" property in drug delivery in circulation system thus enhancing the half-life. Therefore, this paper aimed to deconstruction the mechanism how PEG works on liposome with a present example by literature review on recent and previously published papers. Except the advantages of PEG in drug delivery, accelerated blood clearance (ABC) phenomenon as a massive problem in PEG application is been discovering by other researchers. This paper collected the relevant papers about the recent discovery on ABC phenomenon about how the immune system is stimulated to produce anti-PEG IgM which causes the rapid clearance of PEGylated nanoparticle in second dosing, and some alternative polymers are selected among the alternatives by the capability to replace PEG and preserving the "stealth" ability. However, the fact is that the alternatives have their disadvantages to some extent cannot perfectly replace PEG without any side effects.

Keywords: PEG, Drug delivery, PEGylation, PEG alternatives.

1. Introduction

Since the more and more substances that might effectively discovered in the 20th century. The conventional way that releases all drug particle unreserved in circulation system cannot satisfy the standard to effectively ensure the drug particles reaches the nidus. At 1950, the first controlled release drug delivery system is invented, called the Spansule® 12-h release technology by Smith, Kline & French Laboratories [1]. After that, more mechanisms for controlling the drug release have been discovered such as the diffusion control mechanism, osmotic pressure controlled and the ion exchange mechanism. Their advantage compared to conventional drugs is that they largely prolong the circulation time to 12-24 hours, and maintain the drug concentration in patients' blood in a stable level during the circulation time, but the prolonged time is also the deficiency of the primary controlled release drug delivery system, as some drug requires vivo circulation time ranging from weeks to month, 24 hours of circulation time is not enough to fulfill the goal. After the invention of those mechanisms, the main drug delivery technology of nanoparticle decoration was invented at 1990's, and the first applicated polymer is PLGA system. It prolonged the circulation time to a maximum of 6 months by changing the lactide: glycolide ratio. After the PLGA based system, the topic of this paper, PEGylation technology is invented and firstly applicated on Adagen®. Compared to PLGA based system, PEG could provide "stealth" property to the conjugated nanoparticle to avoid rapid clearance due to immune reaction based on the serum protein attachment on nanoparticle surface. The synthesis of PEG is relatively simple, which is the polymerization of thylene oxide, ethylene glycol, or diethylene glycol with the aid of alkaline catalyst. The reaction time can be controlled to produce PEG chain with different molecular weight for different types of applications. After the production, the conjugation of PEG varies a lot due to the properties of the conjugated nanoparticle. Normally, PEG is modified into different variants such as methylPEG, branched PEG and other PEG chain with different functional groups added. After the modification, the conjugation of PEG molecule is mainly about building linkages to amino group with the functional groups on the modified or unmodified PEG chain [1-3]. Due to the benefit of PEGylation on nanoparticles such as liposomes which is the prolonged circulation time and the stealth effect that increased the efficiency of drug delivery further, PEG has become a widely used drug particle decorator on the surface of the nanoparticle providing stealth effect. However, as the application of PEG modified products, a severe problem affects the application of PEG is discovered which is called the accelerated blood clearance

(ABC) phenomenon. Since the present dilemma of PEG application, this paper is going to introduce the mechanism of PEGylation with some application examples. After the introduction, this paper will introduce the detail mechanism of the ABC phenomenon that affecting the applications, and lastly, some alternative polymer aimed to replace PEG is reviewed and collected through past papers will be introduced in the last part of the main body.

2. Mechanism and Application of PEGylation

2.1. Principle and process of PEGylation

As introduced, PEG is a polymer of linked ethylene oxide as its basic units, which means that PEG and be formed differently by the size, mass or the configuration (linear or branched). The most common modification example is monomethoxy polyethylene glycol (mPEG) [4]. The addition of a methyl group in the head of PEG could reduce the hydrophilic effect in the head of the PEG chain. PEGylation on proteins or other drug molecules are mainly to achieve these goals: to increase the solubility and the circulation time in patient body. These two goals are achieved by the different properties of PEG chain.

The enhanced solubility of PEGylated nanoparticles is related to the hydrophilic effect. Due to the structure of PEG molecule, it has the nature of attract water molecule due to its polarity, and this property allows PEG to easily disperse in water solvent. For example, in recent application, PEG chains are decorated on lipidic surface which is originally hydrophobic. With the appropriately decorated PEG chain, the nanoparticle obtained the ability to attracted water molecule to disperse.

The enhanced circulation time is due to the famous "stealth" effect that PEGylation can give to the decorated nanoparticle. As the PEG chain decoration reached the optimal, the chain could sterically prevent the nanoparticle adsorbed on unwanted surfaces, such as proteins which might accumulate in the reticulo-endothelial system where part of the immune system that will destroys the exotic substances, or also by the steric effect that repulses the attachment of serum proteins, the minimized attachment will decrease the probability of phagocytosis [5, 6]. These two effects bring stealth effect to the PEGylated nanoparticle and increase the success rate of drug delivery. The steric effect also repels the assemble of the decorated nanoparticle, prevents the unwanted accumulation on other tissues.

3. Application of PEG Chain on Different Nanoparticles

3.1. PEG on liposomes

Recently, liposome has become an increasing popular choice on solving the drug delivery problems. With the aid of liposome layer, many therapy methods achieved a route to success such as small molecule therapeutics, gene therapy and immunotherapy, etc. Old topics like conventional drug delivery and vaccine injection also developed as the invention of liposome. In the application of liposomes, PEG is a common ligand on phospholipid bilayer to prolong the half-time of nanoparticle in patient body, and also given the stealth property to protect the nanoparticle by decreasing or disable the attachment of plasma proteins and lipoproteins [2].

An example of PEGylated liposome used for conventional drug delivery is doxorubicin. The application of PEGylated liposome used on doxorubicin delivery was firstly approved by the US FDA in 1995, which is the earliest approved application of liposome with PEG modification on the surface [7].

The existence of PEG chain on liposome offers multiple benefits. The doxorubicin liposome is modified by DSPE-mPEG2000. As introduced, the PEG could sterically prevent the accumulation of nanoparticles in certain region, which increases the efficiency of drug delivery. Additionally, the liposome can be modified twice by editing the ligand on end of PEG chain, and the surface functionalization of liposome can be diversified in multiple ways based on the primary modification

of PEG chain conjugation. Among the advantages PEG chain given on liposome, the most important and notable one is the largely increased circulation time. After the modification, the half time has increased to 40 hours. This has solved the problem of rapid clearance, and also as the prolonged circulation time, the accumulation of drug on tumor region is available now.

3.2. PEG on mRNA vaccines

In 2019 a new type of virus called coronavirus disease 2019 (COVID-19) was discovered in Wuhan China and infected people worldwide afterwards. Until February 2022, COVID-19 had caused more than 390 million infected people and more than 5.73 million of deaths and still remains an increasing trend to threat human live. The COVID-19 vaccine is now under research in lab worldwide. Hundreds of vaccines have been developed and can be sorted into 4 types: whole-virus vaccine, viral vector vaccine, protein subunit vaccine and nucleic acid vaccine. Among them, nucleic acid vaccine is the major type in the invention of COVID-19 vaccine. Nucleic acid can be classified into two types: DNA vaccine and RNA vaccine. DNA vaccine has high level of stability but lower practicability on application of COVID-19 vaccine. RNA vaccine usually refers to mRNA vaccine. Compared to DNA vaccine, mRNA vaccine has higher efficiency with higher security by preventing the foreign DNA from integrating into the host DNA chain since it starts translation at the ribosome.

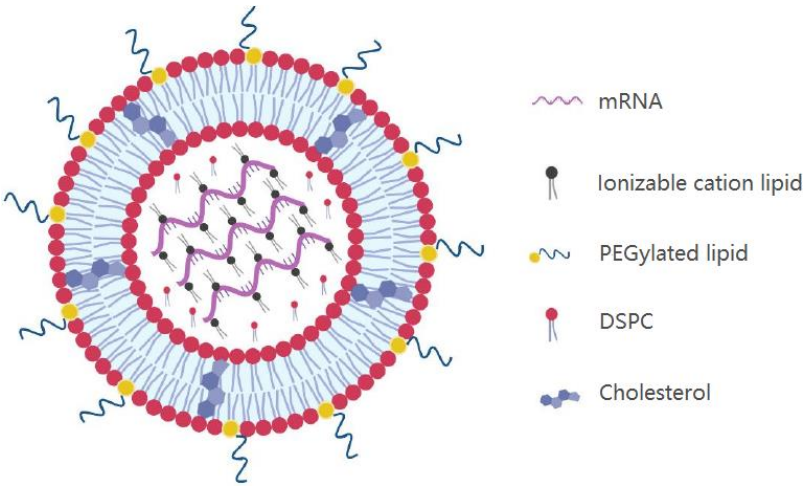


Figure 1. The structure of mRNA vaccine liposome nanoparticle [8]

Although mRNA vaccine has many benefits, it has some problems on drug delivery due to these reasons: the existence of ribonuclease in blood will decompose mRNA before the vaccine reaches the target cell; the mononuclear phagocyte system will attack the mRNA vaccine by phagocytosis; the negatively charged mRNA chain will be repelled by plasma membrane to result in the low penetrability of mRNA vaccine [8]. These challenges on drug delivery are solved by nanoparticles, especially by liposomes. The liposome used for producing mRNA vaccine is shown in Fig.1. The mRNA binds with ionizable cationic lipid by coulomb force, and the periphery is coved by the bilayer of DSPC which imitates the phospholipid bilayer. Just like plasma membrane to carry out the endocytosis while target cell is reached, cholesterol is used to maintain the structure of the membrane and enhance the biocompatibility. The PEG chain decorated in the outer surface of the liposome prevents the accumulation of liposome, stops the adsorption of serum protein and the phagocytosis of mononuclear phagocyte system which is the key problem in drug delivery. Due to these substances, the nanoparticle could escape the decomposition of ribonuclease, the adsorption of serum protein and phagocytosis of mononuclear phagocyte system, penetrate plasma membrane by endocytosis and break the phospholipid bilayer of endosome by the ionizable cationic lipid. Thus the initiation of translation starts to cause wanted immune reaction to form immunological memory cell.

3.3. PEG on peptides

PEGylation on peptides was another topic in the previous decades, but now as the requirement on drug delivery is more advanced, the application of PEGylation is mainly focused on nanoparticles instead of peptides. Similarly, PEGylation on peptide also gives it longer circulation time and higher solubility depending on the number and the position of the PEG chain conjugated on the peptide chain. Adagen® is an example for PEGylated peptide. In the production of Adagen, a mPEG chain is conjugated on Adenosine deaminase, which provides the solubility [9]. However, in the other hand, Adagen has some possible side effects such as shortness of breath; swelling of face, lips, tongue, or throat and other lighter effects. PEGylated proteins are not popular in present application might due to these factors: the conjugation position for PEG is various for each type of protein, and the conjugation route is also various which requires different catalyst and chemical environments which increases the cost to produce different PEGylated peptides.

4. Present Problems on PEG Application

4.1. ABC phenomenon

At present, the application of PEG chain as a decorator on drug nanoparticles faces some challenges that causes the effectiveness of redoes of PEGylated drug extremely decreases, and this phenomenon is called the ABC phenomenon. The first report of the discovery of ABC effect is reported in 2000 by Dams et al. [10,11]. By experimenting dosing PEGylated products on rats and monkeys, the team discovered that the speed of clearance of PEGylated product remains in a very high level if the second injection is in 5-21 days after the first dose. The ABC phenomenon includes two phases according to Laverman et al. [12]. The first stage is the induction stage, which means after the first injection of PEGylated nanoparticles, the biological system is ready. The second stage is the effectuation phase, as the second injection is done, the PEGylated particles are immediately cleared in the circulation system. This is because the production of anti-PEG IgM after the first injection of PEGylated products. As shown in Fig.2, the spleen is stimulated after the first injection, and produces anti-PEG IgM depends on the extent of the ABC phenomenon induction [13,14]. As the simulation of first injection or even subcutaneous injection of PEG on spleen, the immune reaction of producing anti-PEG IgM takes 2-3 days, which is similar to the splenic marginal zone B cells as the first fighter to defense infection which has the capability to produce large number of antibodies in a short period of time [15].

Until now, ABC phenomenon largely affects the application of PEGylated nanoparticles, which compulsorily created a time interval of around 20 days between the first injection of PEG products and the second injection. Because the increasing of PEGylated products, the percentage of people having pre-existing anti-PEG IgM has increased from 0.2% to 70% from 1984 to 2016, indicating that ABC phenomenon is indeed becoming an increasingly problem on PEG application and urging requires a solution due to the present condition of pandemic.

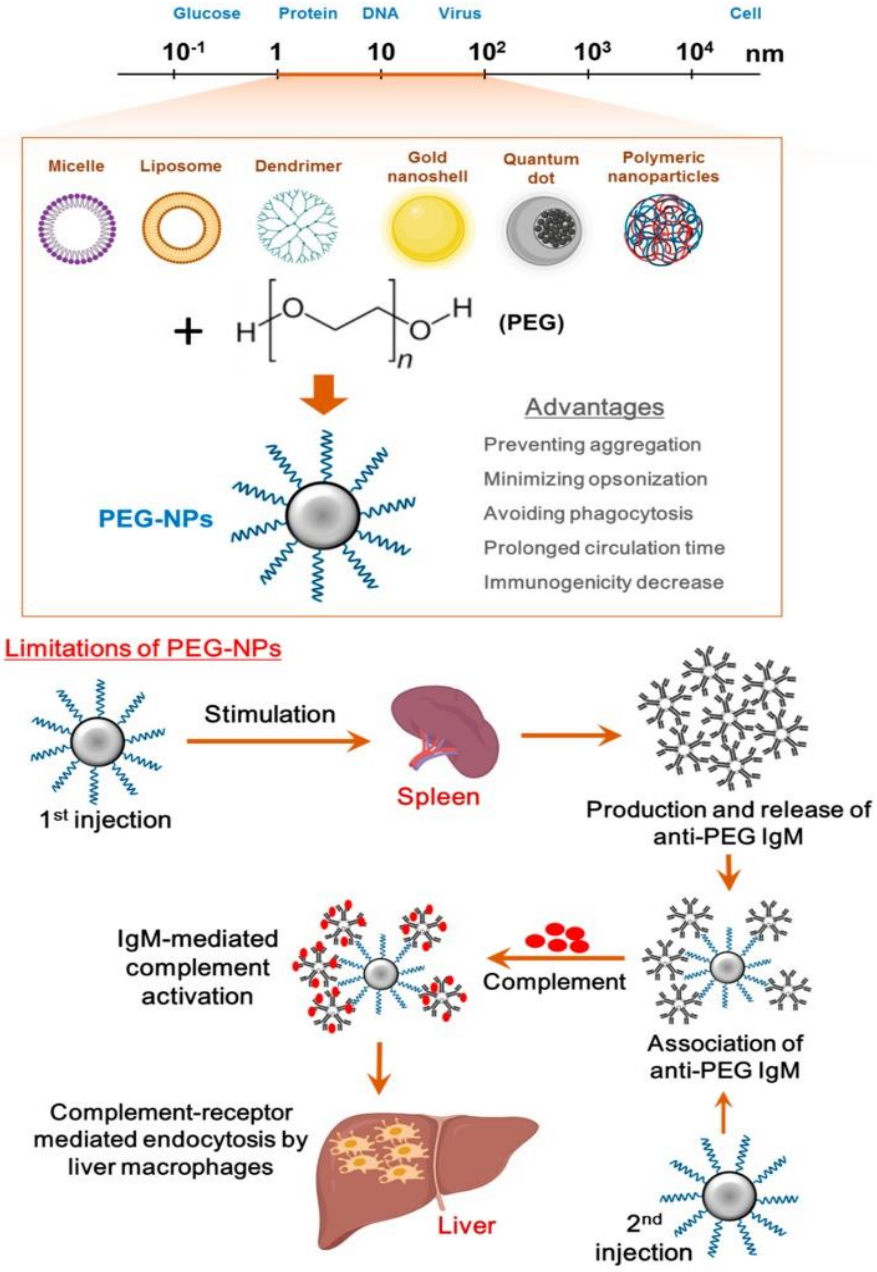


Figure 2. The visualization of ABC phenomenon [6]

5. Present Possible Alternatives for PEG

Many possible solutions are proposed in previous publications, but due to the limited length of this paper, only the alternatives that are believed to have better effect are introduced.

5.1. Polyglycerol

Polyglycerol (PG) has similar structure to PEG, which determines that it could applicate similarly as PEG does. The repetitive hydroxyl group present in PG makes the chain able to reform in to hyperbranched glycerol (HPG), simultaneously. PG itself is easily synthesized through anionic ROP route from epoxide [16].

Both PG and HPG endow liposomes with stealth effect. According to research, when comparing the protein repulsion effect between 1.5-5 kDa PEGylated liposome and HPG decorated liposome, the HPG decorated liposome showed equivalent or even better effect than the PEGylated one [17]. Compared with, HPG showed better effect on increasing the circulation time because the variable

structure of HPG enhanced its hydrophilic effect and was even better than PEG due to some expectation [16]. The best characteristic of PG and HPG is that there is no ABC phenomenon occurs after the second dosing in a short period of time, but the other problem is that HPG accumulates largely in liver and spleen. However, on the other hand, the modification on HPG might solve this problem such as adding an ether linkage to stress up the degradation [13,17].

5.2. POEGMA

Poly (oligo (ethylene glycol) methyl ether methacrylate) (POEGMA) is another alternative of PEG which has the same back bone as PEG, but it is branched to form a "bottlebrush" structure. It is believed that POEGMA could offer an alternative of PEG that has even better stealth effect than PEG. Through a test of fabrication of POEGMA by SI-ATRP method, it is confirmed that SI-ATRP method could produce POEGMA with required properties such as thickness, predictable interfacial hydration and chemical composition [18]. Furthermore, by experimenting on comparing Adagen and other mPEGylated therapeutics with POEGMA modified therapeutics in human specimens, POEGMA showed decreasing in antigenicity, therefor more efficiently evaded from immune system [18]. However, about whether POEGMA would cause ABC phenomenon or other similar immune reaction, it is yet to be determined.

5.3. Zwitterionic polymer

Zwitterionic polymer r shows positive charge and negative charge in different part of the molecule, and it is used on the modification of proteins. By conjugation of zwitterionic polymer such as poly(carboxybetaine) (pCB) on micelle forming a charged layer, a better antigenicity than PEG was obtained through a different mechanism called anti-fouling property. By conjugating high density of zwitterionic polymer and PEG on uricase, the zwitterionic modified uricase showed a higher level of preventing the unwanted immune reaction. Han et al. observed that zwitterionic modified drug caused weaker ABC phenomenon in second dosing [13]. Although zwitterionic polymer shows multiple advantages compared to conventional PEGylated nanoparticles, zwitterionic polymer has its own challenge on the difficulties of synthesis and conjugation compare to PEG chain.

6. Conclusion

This review presented the stealth mechanism of PEG and its application on liposomes such as the present COVID-19 mRNA vaccine using in many countries. After that, the present problem in the application of PEG on nanoparticles due to the ABC phenomenon occurs as the second dosing of PEGylated drug between the time interval of 5-21 days, which largely weakens the effectiveness of drug delivery. Tthus an alternative polymer of PEG is required to solve the problem while keep the stealth effect. This paper introduced some alternatives like polyglycerol, POEGMA and zwitterionic polymer, then discovered that the alternatives have different difficulties on replacing PEG such as the accumulation of macromolecules, or potential ABC phenomenon. This paper may not include the present perfect alternative of PEG or mistaken the level of negative effects the alternatives bring about. In the future, the standard and the method of research can be refined and altered to get the best result to compare the effectiveness of the polymers on drug delivery techniques.

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