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Biomedical application of pullulan - A review

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ABSTRACT

Pullulan, an extracellular α -glucan, having botanical name as *Pullularia pullulans*, a natural polymer was discovered in 1900's. It is recently being investigated for biomedical applications in various fields like targeted drug delivery, gene delivery, diagnostic imaging using quantum dots etc. Pullulan being biocompatible gives a great scope in the biomedical applications. Pullulan is highly capable of replacing currently commercially used polymers in biomedical application in the form films, blend or nanocomposite. The main aim of the review is to make the readers aware of the various modifications done in Pullulan to make it more suitable for critical applications like gene delivery, controlled drug release etc.

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KEYWORDS

Pullulan;
Drug delivery;
Gene delivery;
Biomedicine;
Biocompatible.

INTRODUCTION

The structure of pullulan, an extracellular α -glucan elaborated by *Pullularia pullulans*, has been shown to be a polymaltotriose polymerized through (α -1, 6-bonds on the terminal glucose residues of the trisaccharide^[1,2]. Wallenfels et al.^[2] have presented evidence for 6'- α -glucosylmaltotriose and 6'- α -maltotriose as minor tetrasaccharide components of pullulan, and presumed their location to be solely at the termini of the polymer (Figure 1a). Catley et al.^[3] proposed the presence of a small number of malto-tetraose units located within the basic polymaltotriose structure, and showed them to be linked through α -1,6-bonds on their terminal residues (Figure 1b). It had been observed^[3] that there is, perhaps, no unique structure for pullulan, since it is the term used to describe any extracellular α -

glucan elaborated by *P. pullulans* cultured under a variety of conditions on a variety of substrates. The location and content, therefore, of the tetrasaccharide described by Wallenfels et al. and Catley et al. are not held to be in conflict.

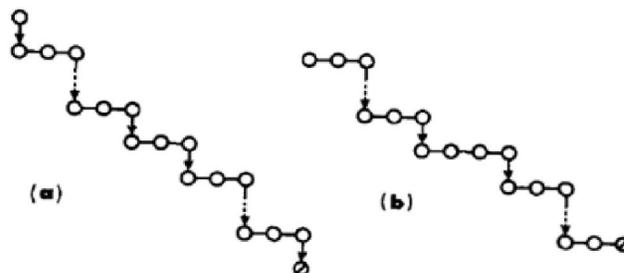


Figure 1 : (a) The structure of pullulan proposed by Wallenfels et al. with terminal tetrasaccharide units. (b) The structure proposed by Catley et al. with maltotetraose units located within the polymer.

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Recently pullulan are being investigated for its biomedical applications in various fields like targeted drug delivery, gene delivery and diagnostic imaging using quantum dots etc^[4-7].

PULLULAN NANOGELS AND AMPHIPHILIC POLYMERS IN BIOMEDICAL APPLICATION

The study of nanogel (i.e. hydrogel nanoparticle) has intensified during the last decade due to enormous potential applications in the development and implementation of new environmentally responsive or smart materials, biomimetics, biosensors, artificial muscles, drug delivery systems and chemical separations^[8]. The nanogels can be designed to undergo large changes in their chemical, mechanical, optical and/or electrical makeup in response to a chemical stimulus, biomolecular

interaction or electromagnetic field^[9]. Such materials can be viewed as biomedical amplifiers or sensitizers of the environmental event^[10].

Akiyoshi's group has made significant advances through the formulation of amphiphilic polymers with the propensity to self-aggregate, referred to as nanogels^[11-13]. Monodisperse and colloidally stable nanogel particles (20–30 nm) form upon the self-assembly of partly hydrophobized (less than 5 wt%) cholesteryl group-bearing pullulans (CHP) in water (see Figure 2). These CHP nanoparticles forms complexes spontaneously with it^[14]. In this regard, a cationic hydrogel nanoparticle (CHPNH₂) designed for intracellular protein delivery was developed^[15]. CHP nanoparticles have protein-folding and refolding activity *ex vivo*^[16], their behaviour being quite reminiscent of the artificial chaperone model proposed by Rozema and Gellman^[17].

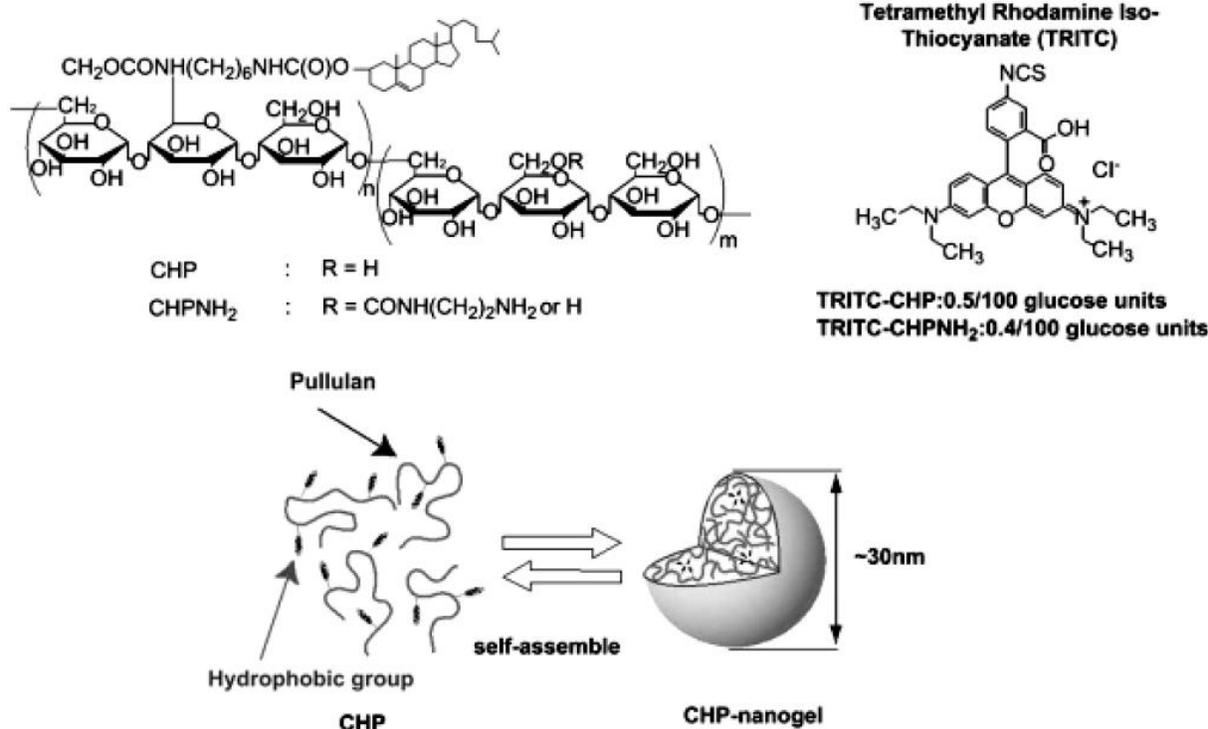


Figure 2 : Structure of cholesterol-bearing pullulan (CHP) and cationic CHP (CHPNH₂).

Given that the down regulation of chaperone expression and/or activity is thought to be heavily implicated in the pathogenesis of various neurodegenerative diseases^[18], nanogels could be of particular value as artificial chaperones in neuro-nanomedicine^[19].

Sebastien et al. tested the effectiveness of CHP nanogels to bind on b-amyloid peptide monomers, and to the more deleterious b-amyloid peptide oli-

gomers, demonstrating their ability to decrease cytotoxicity in primary mixed cortical and microglial cells in culture. By employing fluorescent CHP analogs with different charges it was found that: (i) Both neutral and positively charged CHP nanoparticles interact with b-amyloid monomers and oligomers, (ii) Neutral CHP is non-toxic, but positively charged derivatives (CHPNH₂) are toxic, particularly in pri-

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mary cortical cultures, and (iii) Binding of both monomeric and oligomeric b-amyloid to CHP significantly reduces b-amyloid toxicity in both the primary cortical and microglial cells.

These results suggest that CHP nanogels could provide a valid complementary approach to antibody immunotherapy in neurological disorders characterized by the formation of soluble toxic aggregates, such as those in Alzheimer's disease^[20].

Jeong et al. synthesized amphiphilic macromolecules composed of pullulan and poly(dl-lactide-co-glycolide) (PLGA) (PuLG) to give amphiphilicity and biodegradability as novel drug carriers (Figure 3 and 4). Introduction of PLGA into a water-soluble polysaccharide, pullulan, successfully induced amphiphilicity and provided unique physicochemical properties. PuLG

nanospheres had a round shape with a particle size of about 75–150 nm.

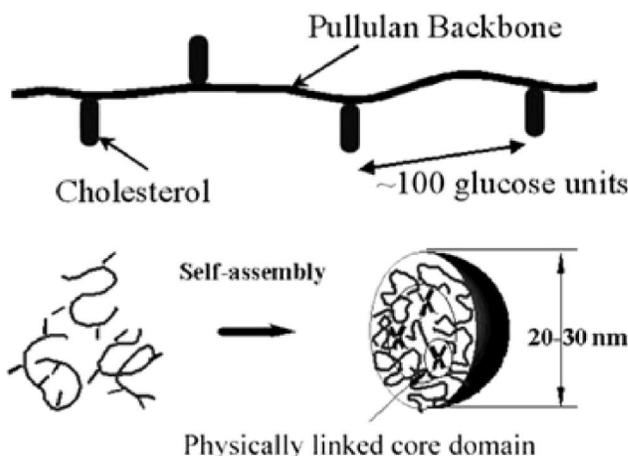


Figure 3 : Schematic depiction of a cholesterol-bearing pullulan (CHP) and the hydrogel nanoparticle.

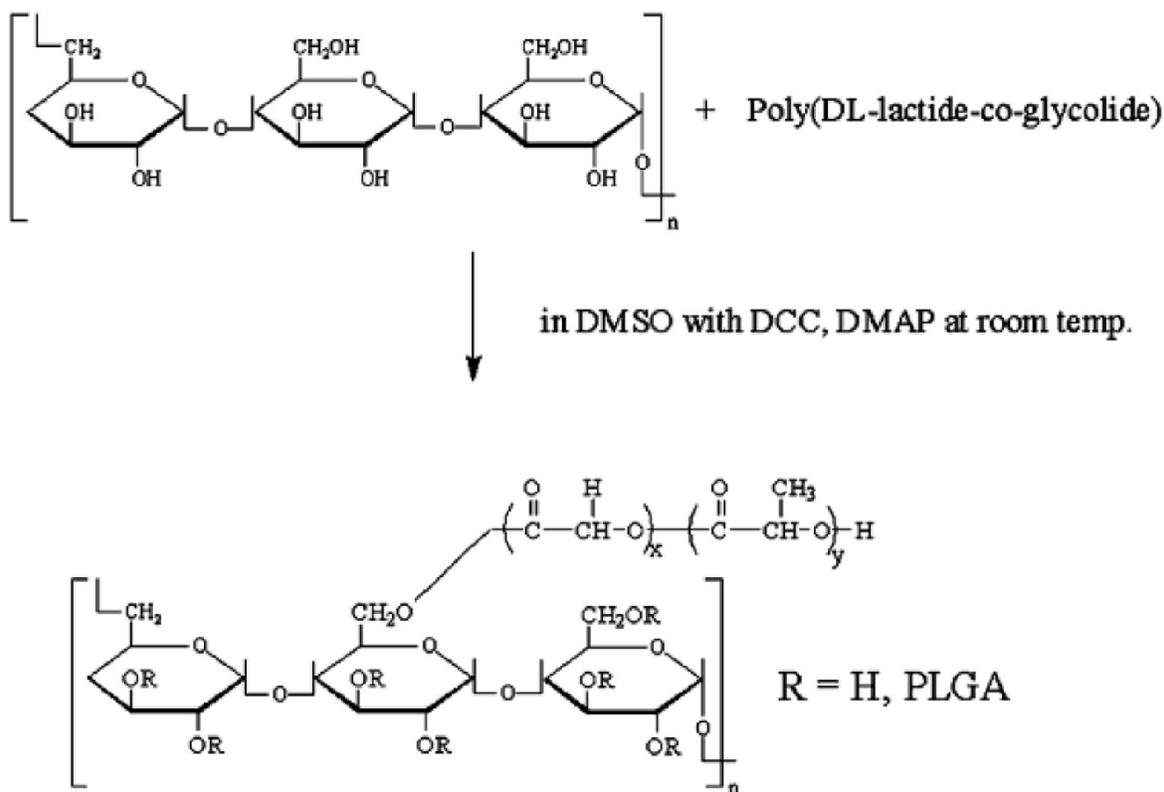


Figure 4 : Synthesis scheme of PuLG graft copolymer

The drug contents of the PuLG nanospheres were approximately 20–30% (w/w). As the drug contents of PuLG nanospheres increased, the drug release rate from nanospheres decreased. The drug release rate from PuLG nanospheres was delayed as the molecular weight of PuLG increased. PuLG copolymer with higher graft ratio of PLGA showed slower degradation rate rather

than that with lower graft ratio^[21].

PULLULANS IN PROMOTING HORMONE SECRETION

During the last decade, there have been several attempts to produce artificial pancreas for the treatment

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of diabetes mellitus, with an emphasis on hybrid structures that combine insulin-secreting cells with biomaterials^[22-25]. A major problem with this concept is the impact that the immune-modulating membranes and extracellular matrix have on the physiological functioning of the structure, including oxygen supply, and the large number of pancreatic islets that are needed for such a structure^[26-29].

Sungwon et al. attempted to promote insulin secretion from insulinoma cell lines or pancreatic islets through a number of mechanisms, including direct stimulation of cells by interaction with sulfonylurea (SU) conjugated to macromolecules^[30-32] and glucagon-like

peptide-1 (GLP-1)^[33] and by incorporating cross-linked haemoglobin^[34]. An SU/polymer (SUP) conjugate was prepared (see figure 5) using pullulan, a polysaccharide that has been used previously in drug delivery because of its solubility and good biocompatibility^[35,36]. In vitro static experiment showed that sulfonylurea concentration in SUP over 50 μm was required to stimulate the rat islets. The long-term (1 month) culture experiment demonstrated that the microcapsules of islets with SUP, with well-preserved morphology, presented higher insulin secretion level and better ability in responding to glucose changes than those without SUP.

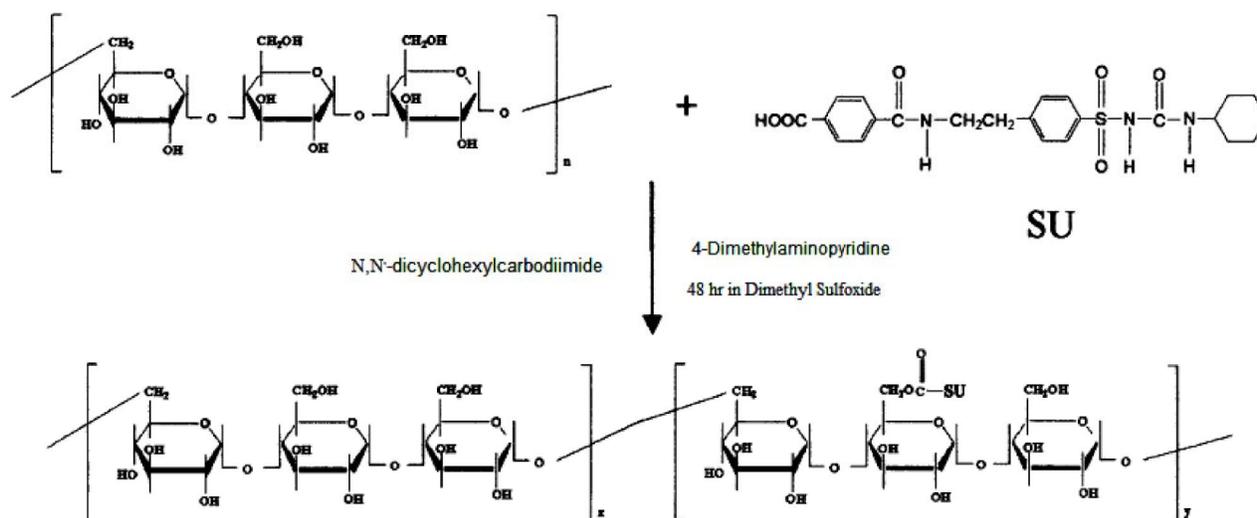


Figure 5 : The synthetic scheme of a SUP

PULLULANS IN DRUG DELIVERY SYSTEMS

Drug delivery system enables to precisely control the release rate or target drugs to a specific body site having an enormous impact on the healthcare. Among them, the administration of drugs to small intestine and colon by the oral route still remains one goal towards which numerous biotechnology companies are striving for^[37-40]. The main advantages presented by oral drug delivery are the ease of target accessibility, enhanced patient compliance owing to the non-invasive delivery method, and the possibility of local and systemic therapy. Carrier technology offers an intelligent approach for oral drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc., which modulates the release and the absorption char-

acteristics of the drugs^[41-44]. Polymeric microspheres represent an important part of these particulate drug delivery systems due to their small size and efficient carrier characteristics. For instance, polymeric microspheres can be easily functionalized with anionic or cationic groups allowing high loading of drugs possessing opposite charges^[45-48]. In addition, the electrostatic interactions between drug and polymer increase the chemical stability or mask the unpleasant taste of the drugs^[49].

Constantin et al. prepared poly(vinyl alcohol) (PVA) microspheres by dispersion reticulation with glutaraldehyde and further aminated it (see figure 6). These microspheres were firstly loaded with diclofenac (DF) and then entrapped in cellulose acetate butyrate (CAB) microcapsules by an o/w solvent evaporation technique for intestinal delivery of drug.

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able under physiological conditions. Moreover, due to the insertion of acrylic acid units in the copolymer, even if in very small amounts, the continuous sequence of NIPAAm is disturbed, and the cumulative hydropho-

bic interactions between isopropyl groups are much more reduced at a temperature higher than LCST. Therefore, the thermo-sensitivity of these copolymers is weaker.

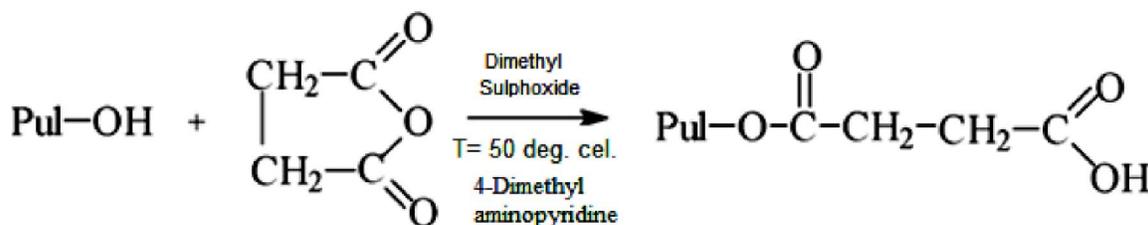


Figure 7 : Reaction of pullulan with succinic anhydride

Recently, a method to introduce a larger amount of carboxylic groups in the main chain of poly(NIPAAm), without weakening thermo-sensitivity has been developed, it is based on the new acidic monomer N-isopropyl-maleamic acid^[62]. This unique pH/ thermo-sensitive character of the acid was attributed to the existence of a continuous isopropylamide sequence in hydrogels. Unfortunately, neither these polymers are biodegradable and biocompatible. In order to obtain biodegradable gels that preserve also pH/ temperature sensitivity, PNIPAAm has been grafted onto natural polymers possessing natively pH-sensitive units as alginic acid^[63] or chitosan^[64]. After grafting, the continuous sequence of NIPAAm is not disturbed and gels maintain thermo-sensitivity. Furthermore, the presence of carboxylic or amino groups confers pH-sensitivity to gels.

Gheorghe reported the preparation of pullulan microspheres by suspension cross-linking with epichlorohydrin of an aqueous polymer solution. Firstly, the thermo-responsive units were inserted by grafting poly(N-isopropylacrylamide-coacrylamide) (poly(NIPAAm-co-AAm)) onto pullulan microspheres (figure 8a). Secondly, the pH-sensitive functional groups were introduced by reaction of succinic anhydride with the remaining -OH groups of pullulan (figure 8b). Volume phase transition temperature of microgels was close to the human body temperature in isotonic phosphate buffer at pH = 7.4. The pH/thermo-responsive microspheres maintain a sharp volume phase transition both below and above pKa of carboxylic acid at low values of the exchange capacity. This unique characteristic is attributed to the existence of a continuous sequence of isopropylacrylamide in copolymer^[66].

K. Akiyoshi et al. described the complexation of a protein drug, Ins, with the hydrogel nanoparticle of hydrophobized pullulan. The complexed nanoparticles (diameter 20–30 nm) formed a very stable colloid. The thermal denaturation and subsequent aggregation of Ins were effectively suppressed upon complexation. The complexed Ins was significantly protected from enzymatic degradation. Spontaneous dissociation of Ins from the complex was barely observed, except in the presence of bovine serum albumin. The original physiological activity of complexed Ins was preserved in vivo after i.v. injection. The hydrogel nanoparticle formed with hydrophobized polysaccharides behaved as an excellent protein drug carrier^[67].

Yoshiharu et al. investigated the biodisposition of pullulan in rats focusing especially on receptor-mediated hepatic uptake. FITC-labeled pullulans were prepared by method of de Belder and Granath^[68]. The degree of substitution by FITC of pullulan (FP-60; MW 58,200) was 0.004 in FITC mol/ glucose unit. The tyramine derivative of pullulan (P-60; MW 58,200) was prepared according to the cyano-transfer method^[69]. Marked dose-dependency was seen in the hepatic uptake of FP-60 which was markedly reduced by the coadministration of both asialofetuin and arabinogalactan. The binding of [¹²⁵I]P-60 to isolated parenchymal cells was significantly inhibited by arabinogalactan and asialofetuin, however dextran, the same glucan as pullulan, did not affect the binding of [¹²⁵I]P-60. It was found that pullulan, which is bound to the asialoglycoprotein receptor with high affinity, is subsequently internalized to the hepatocyte via receptor-mediated endocytosis^[70].

Interferon (IFN)- β is widely used for the elimina-

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tion of hepatitis C virus in patients with chronic liver disease, but its clinical efficacy is unsatisfactory. Targeting IFN- β to the liver might enhance its efficacy without increasing its side effects. For clinical use of this targeting technology, pullulan conjugate can be conjugated with human groups of IFN- β as IFN- β has poor cross-species activity. Y. Suginoshta et al. attempted

to synthesize pullulan-conjugated human IFN- β through metal coordination and succeeded in enhancing 2-5AS induction specifically in the liver by i.v. injection of the conjugate more potently than by free human IFN- β . Thus, human IFN-b-DTPA-pullulan conjugate appears to be applicable for clinical use, which is promising for treatment of patients with chronic hepatitis C^[71].

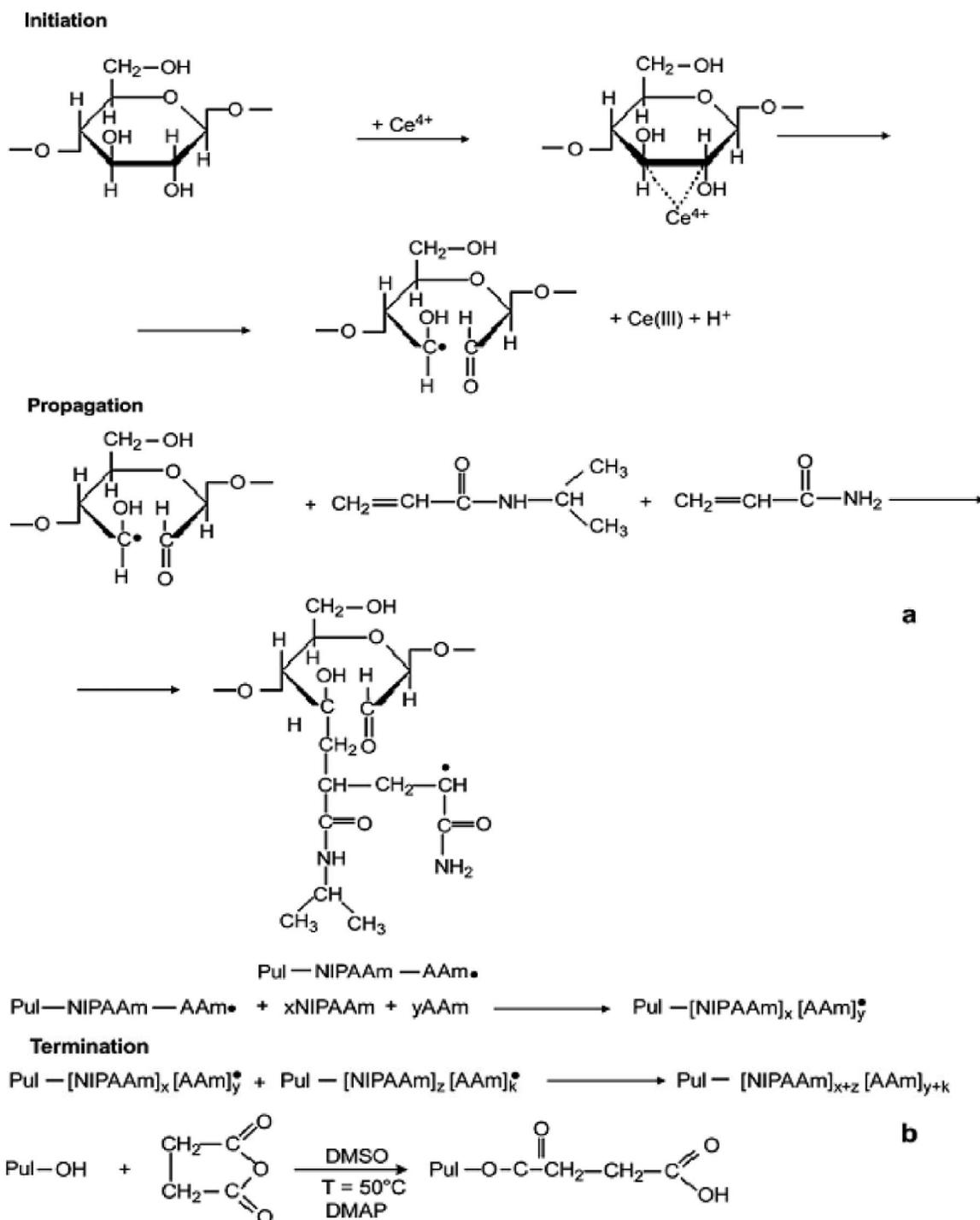


Figure 8 : Scheme for insertion of thermo-responsive (a) and pH responsive (b) groups onto pullulan

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PULLULANS IN PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) has been intensively studied and employed as a modality for the treatment of various tumors and non-oncological disorders due to its unique properties. This modality involves a noninvasive process that causes minimal nonspecific damage to adjacent healthy tissues while remaining cost effective and requiring only a short term period of re-treatment^[72-74]. PDT is based on the delivery of a photosensitizer (PS) by local and systemic administration followed by selective irradiation with appropriate laser light to generate highly reactive oxygen species, such as singlet oxygen, at the disease site.

In tumor treatment, singlet oxygen generated in this manner by PS localized at the tumor site directly or indirectly treats target cells. Tumor destruction can be achieved both by cell death and by photodestruction of the tumor vasculature, which results in local hypoxia and indirect cell death^[73,74]. PDT is also expected to be a potential method of overcoming multidrug resistance (MDR) because PS in the plasma membrane exhibits cytotoxicity to cancer cells that is different from that of other chemotherapy agents^[75]. However, there are some difficulties with the use of PS, such as water-insolubility and low selectivity for the target site, which often limit its broader clinical applications in PDT.

To solve these problems, various nano-carrier systems including lipid-based nanoparticles, polymer conjugates, polymeric micelles, and polymeric nanoparticles have been investigated. These systems have improved the solubility of PSs by incorporating a hydrophobic core, thereby prolonging circulation in the body, and have improved their targeting efficiency through an enhanced permeability and retention (EPR) effect at the tumor site^[76-79]. Unfortunately, most conventional PSs incorporated into polymeric nano-carrier systems easily form aggregates due to their π - π interactions and hydrophobic characteristics, resulting in incomplete release and significant reduction of singlet oxygen by self-photo quenching^[80].

Byoung-chan Bae, Kun Na reported the development of polysaccharide-based nanogel with self-quenchable photoactivity prepared from pullulan/folate-PS conjugate. Folate was introduced in order to endow this nanogel with a homing property; the nanogel

possesses superior cancer cell selectivity induced by a specific interaction with folate receptors overexpressed on the surface of several types of human cancer cells^[81-84]. The pullulan/folate conjugate was synthesized by one-step chemistry and spontaneously formed a self-organized nanogel in an aqueous environment. Pheophorbide-a (Pheo-A), which is a derivative of chlorophyll a, was then grafted to the pullulan/folate conjugate. Pheo-A been used as a PS, generating PDT effects both as a monomer and in polymeric form, and producing antitumor effects in human lung, hepatoma, uterine sarcoma and liver cancer cells^[85,86]. Pullulan/folate-PS (Pheo-A) nanogels exhibit controlled photoactivity. During circulation in the blood, nanogel photo activity may be suppressed by a self-quenching effect between PS molecules grafted to the pullulan/folate backbone similar to the fluorescence resonance energy transfer (FRET) effect^[87]. As the nanogel penetrates tissue and internalizes in cancer cells, photo activity may be recovered due to loss of the FRET effect by cleavage of the pullulan/folate backbone and the ester bond in the PS graft through enzymatic attack in the extracellular matrix and cellular compartments such as lysosomes^[88,89] (See Figure 9).

PULLULANS IN GENE THERAPY

Gene therapy holds great promise for treating diseases ranging from inherited disorders to acquired cancers^[90,91]. Viruses are the most common vectors in gene delivery due to their inherent function of cell invasion that is followed by expression of the gene into the host cell. However, the structure and stability of transferred gene is restricted by the character of virus genome. Moreover, viral vectors can only introduce genes, not other macromolecules like siRNA or antisense nucleotide. Furthermore, side reactions such as host immune response and insertional mutagenesis leading to death, carcinogenesis or germ line alterations have led to serious concerns about the use of viruses as gene transfer vectors^[92-94]. The mechanistic pathway for gene expression is limited by at least five major barriers that must be overcome for successful gene delivery: in vivo stability, cell entry, endosome escape, cytosolic transport and nuclear entry. The nuclear membrane restricts the transport of the plasmid DNA and the efficiency of the

DNA transfer from the cytoplasm to the nucleus has been estimated to be about 10^{-4} . Therefore, in order to obtain a high gene expression, the genes introduced into the cells must be reduced to a compact size, which can pass through nuclear pores. The collapsing of DNA into nanoparticles of reduced negative or increased positive charge (i.e. DNA condensation)^[95-98] has received considerable interest in recent years due to its biological importance in DNA packaging in virus heads^[99-102].

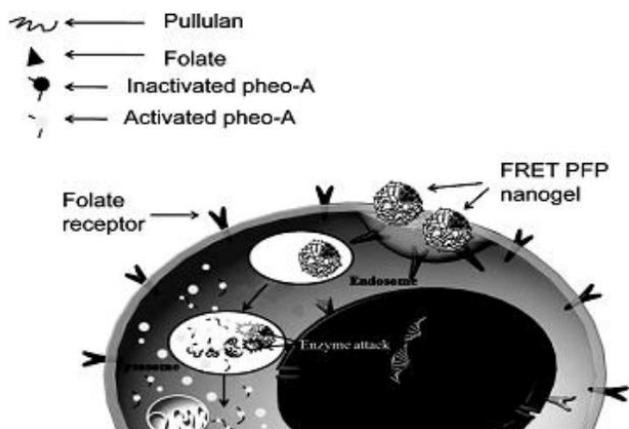


Figure 9 : Conceptual image of the change in photoactivity of PFP nanogels in cells

Cationic polymers seem to produce more stable complexes, offering more protection during cellular traffic^[103-106]. Gene delivery usually takes advantage of the endocytic pathway of the cell. The cells continually ingest a part of their plasma membrane via endocytosis to form endocytic vesicles^[107]. Solvent and solute can be taken up by the cells by the endocytosis activity of the latter. Endocytic vesicles incorporating DNA-bearing particles are transferred to endosomes and then to lysosomes, where liberation of DNA somehow takes place^[108,109].

Mona Gupta and Ajay Gupta prepared hydrogel nanoparticles of pullulan with hydrophilic core that can encapsulate water-soluble materials like DNA for intracellular delivery. Pullulan nanoparticles in narrow size range encapsulating DNA (pBUDLacZ) had been prepared inside the aqueous core of the reverse micelles formed by aerosol-OT/n-hexane (without microemulsion). Particles are spherical in shape with size of 45 ± 0.80 nm diameter. The nanoparticles were internalised and the cells exhibited vacuoles in the cell body due to nanoparticle internalisation. Endocytosis of nanoparticles resulted in disruption of F-actin and h-

tubulin cytoskeleton of human fibroblasts. The efficacy of transfection in vitro on HEK293 and COS-7 cells demonstrated cell type dependence, with COS cells having a higher gene expression. The h-gal expression in COS-7 cells by pullulan nanoparticle was comparable to commercially available Lipofectamine 2000^[110].

M.R. Rekha and C.P. Sharma developed a conjugate of pullulan and PEI (for scheme of synthesis see figure 10) for gene delivery applications. Conjugation of PEI with pullulan improved the transfection efficiency and reduced the toxicity associated with PEI. The cytotoxicity, blood component interactions such as red blood cell/white blood cell aggregation, platelet and complement activation, and protein interaction of the pullulan-conjugated PEI was drastically reduced in comparison to PEI-based nanocomplexes. The conjugation of pullulan with PEI did not hinder the plasmid nuclear localization ability of PEI. The transfection efficiency of pullulan conjugate was similar to PEI, with the added advantage of hemocompatibility and non-cytotoxicity. Thus pullulan-PEI conjugate seems to be a promising gene delivery vector with good hemocompatibility and low toxicity but without compromising the transfection efficacy of PEI^[111].

They also developed a liver targeting cationic pullulan which was blood compatible. Cationic groups were introduced by reacting glycidyl trimethyl ammonium chloride with pullulan. The cationic derivatives readily formed polyionic complexes with DNA. Pullulan has high specificity for liver and it is well established^[112] that it is internalized by the hepatocytes by receptor mediated endocytosis through specific binding of pullulan to the asialoglycoprotein receptor. They reported, that carboxymethyl pullulan unlike pullulan has low affinity for asialoglycoprotein receptors and the liver uptake clearance of pullulan was decreased by more than hundred fold^[113]. The high solubility and chain flexibility of pullulan may be one of the contributing factors for its blood compatibility^[114].

I. Kanatani et al. investigated pullulan-spermine for gene delivery to cells that do not express the asialoglycoprotein receptor (ASGPR). Pullulan-spermine-mediated transfection of plasmid DNA resulted in greatly reduced cytotoxicity and a 10-fold increase in the level of gene expression when compared to Lipofectamine 2000, a commercially available cationic

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lipid and the in vitro proliferation of T24 cells was significantly reduced. Pullulan-spermine is a promising carrier for gene transfection, and that cellular uptake of

pullulan-spermine-plasmid DNA complexes is mediated by clathrin- and raft/caveolae-dependent endocytotic pathways^[115].

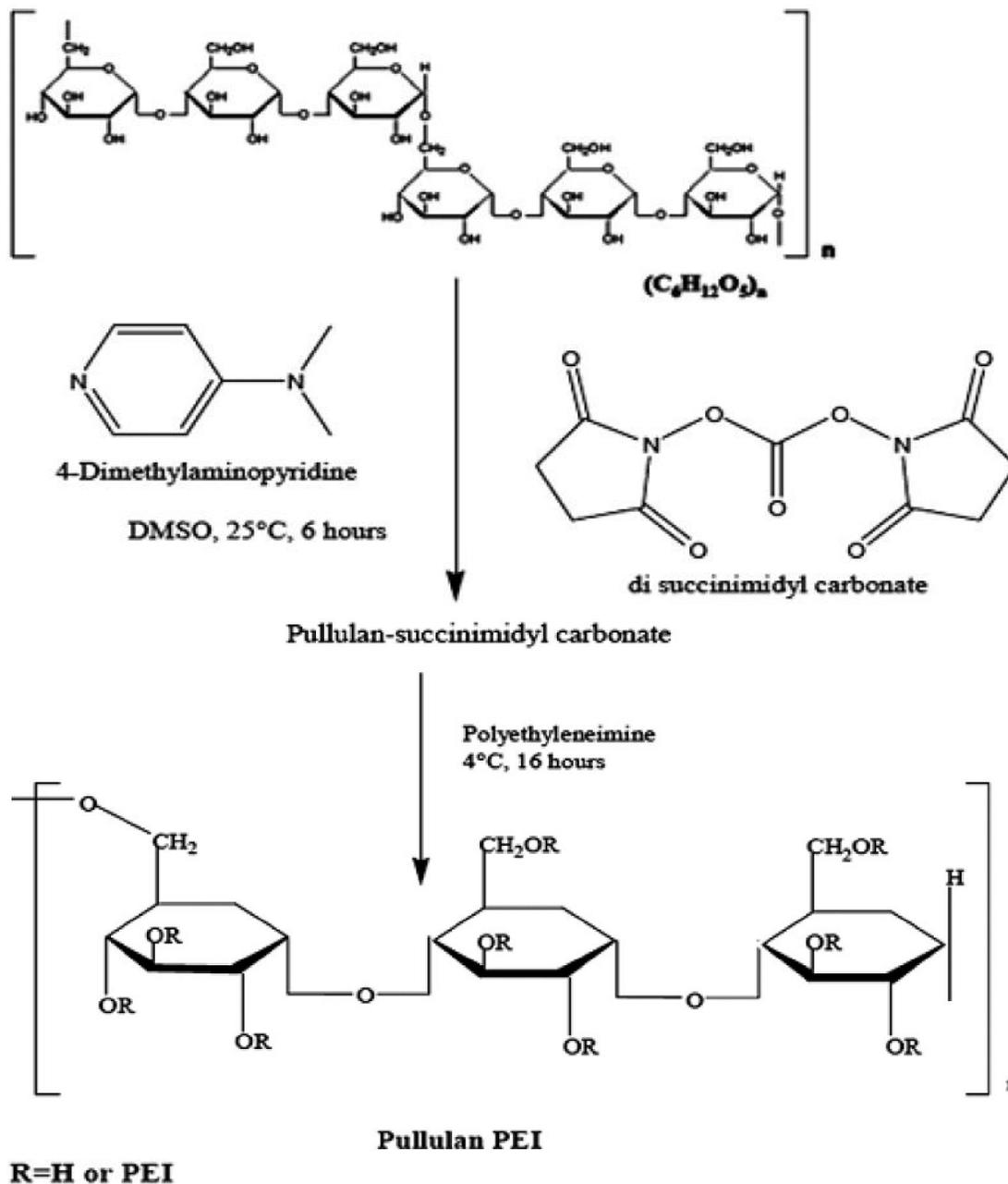


Figure 10 : Synthesis of pullulan-PEI

CONCLUSION

Pullulans is having very important applications, but are rarely heard or spoken of. Pullulans have wide scope of applications from drug delivery to gene delivery. Applications of pullulans in the fields are just a start with lot to add up in future. More of research is re-

quired to open up the vast field.

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