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CURRENT STATUS OF THE APPLICATIONS OF PULLULAN AND ITS DERIVATIVES IN BIOMEDICAL FIELD

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Abstract

This review highlights the applications of pullulan in biomedical field, focusing on drug delivery. Pullulan is a microbial exo-polysaccharide produced by yeast like fungus Aureobasidium pullulans and it has been declared safe by FDA in United States and has GRAS status. Pullulan has biocompatible, biodegradable, non-mutagenic, non-toxic, non-carcinogenic, non-immunogenic properties, as well as other functional properties. Furthermore, pullulan can be easily derivatized by several chemical reactions such as etherification, amidification, esterification, oxidation and co-polymerization in order to widen its applications. Due to its unique features pullulan and its derivative is being explored for various biomedical applications like drug and gene delivery, tissue engineering, wound healing, diagnostic imaging, etc. This research was supported through Nucleu project PN 1941-04 01.

Key words*: pullulan, biomedical applications, drug delivery, microbial exo-polysaccharide.*

INTRODUCTION

Pullulan is a microbial exo-polysaccharide produced by yeast like fungus *Aureobasidium pullulans*. There are other microorganisms that produce pullulan, such as: *Tremella mesenterica, Cytaria harioti, Cytaria darwinii, Teloschistes flavicans, Rhodotorula bacarum, Rhodosporidium paludigenum, Eurotium cheyalieri* and *Cryphonectria parasitica* (Singh et al., 2008; Forabosco et al., 2006; Chi and Zhao, 2003; Reis et al., 2002). Pullulan consists of α-(1,6)-repeated maltotriose units via an α-(1,4) glycosidic bond. The molecular formula of pullulan is $(C_6H_{10}O_5)_n$ with a molecular weight ranging between 4.5×10^4 - 6×10^5 Da. It presents like a white to off-white powder, and it is soluble in water, dimethylsulfoxide, formamide, and dilluted alkali.

Pullulan has biocompatible, biodegradable, non-mutagenic, non-toxic, non-carcinogenic, non-immunogenic properties, etc, and it has been declared safe by Food and Drug Administration (FDA) in United States and has GRAS (Generally Recognized as Safe) status. Pullulan has good mechanical strength, film-forming ability, and adhesiveness.

Pullulan has been researched for food, cosmetic, pharmaceutical and biomedical applications (Ran et al, 2017; Leathers, 2003). Some applications of pullulan in food industry are: edible coating material, material in packaging industry, adhesive material (e.g. to bind nuts to the cookies), low-calorie food additive in solid or liquid foods, low viscosity filler in sauces and juices, intensifier in baked foods, confectioneries and beverages, etc (Singh et al, 2019; Trinetta et al, 2011). Pullulan can be used in cosmetic industry specifically in skincare products, because possess good skin adherence and can provide an instant skin-tightening effect (Coltelli et al, 2020). Furthermore, pullulan can be easily derivatized by several chemical reactions such as etherification, amidification, esterification, oxidation, sulfation and co-polymerization in order to widen its applications. Due to its unique features pullulan and its derivatives is being explored for various biomedical applications including drug delivery, gene delivery, tissue engineering, medical imaging, etc.

This review highlights the applications of pullulan and its derivatives in biomedical field, with a special focus on drug delivery.

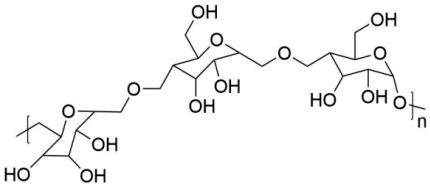

Figure 1. Structure of pullulan

MATERIALS AND METHODS

A bibliometric search was carried out in Scopus database in February 2022, using keywords like "pullulan" and "pullulan drug delivery". The search was conducted taking into consideration title, abstract and keywords of publications. Only papers published in the period of 1990-2021 were included in the evaluation of trends in publications. After the exclusion step, the distribution of papers by year, type of publication, language, top five fields and top 10 publishing journals were extracted directly from Scopus.

RESULTS AND DISCUSSIONS

1. Drug delivery applications

1.1. Trends in publications of pullulan use in drug delivery applications

The evaluation of trends in publications showed that from 4796 papers were published regarding pullulan, a number of 616 publications addressed drug delivery. Majority of these publications were written in English (603) and just a few in Japanese and Chinese. The evolution over time of these publications is presented in Figure 2. The publications increased over the years, especially between the years 2018 and 2020. Papers involving pullulan applications in drug delivery concerned 23 fields of knowledge. The top five fields with the highest number of publications are Pharmacology, Toxicology and Pharmaceutics (291), Biochemistry, Genetics and Molecular Biology (198), Materials Science (176), Chemistry (139) and Chemical Engineering (115). Mainly, the publications were original articles (400), followed by reviews (166) and conference papers (24), as shown in Figure 3. The top 10 journals in which published the papers were: International Journal Of Biological Macromolecules (32), Carbohydrate Polymers (27), International Journal Of Pharmaceutics (20), Journal of Controlled Release (17), Advanced Drug Delivery Reviews (14), Biomaterials (13), European Journal Of Pharmaceutical Sciences (13), Journal Of Drug Delivery Science And Technology (10), Materials Science And Engineering (9) and Current Pharmaceutical Design (8).

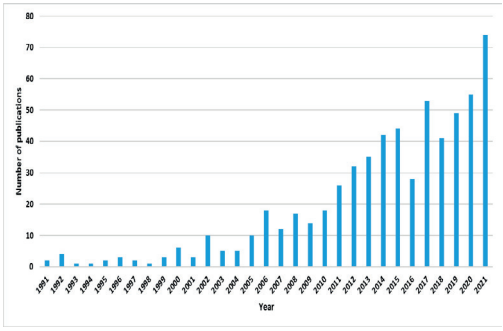

Figure 2. The evolution over time of the papers published regarding pullulan in drug delivery

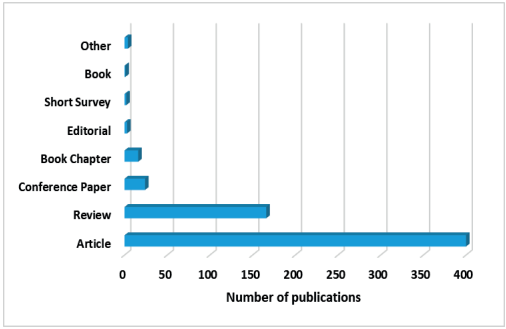

Figure 3. Type of papers published regarding pullulan in drug delivery

1.2. Pullulan based drug delivery systems

Various formulations based on pullulan like hydrogels, micro/nano-particles, nanogels, micelles can be effective drug delivery systems possessing increased permeability and enhanced drug retention capacity. Also, the incorporation of therapeutic agents in pullulan based drug delivery systems reduces their toxicity.

Various pullulan systems acted as good drug carriers to different active substances such as: anticancer agents, doxorubicin (Li et al., 2014; Zhang et al., 2011; Balasso et al., 2017),

docetaxel (Satoh et al., 2008), mitoxantrone (Yang et al., 2014, Yuan et al., 2019), mitoxantrone - doxorubicin (Lu et al., 2009), cisplatin (Wang et al., 2015), paclitaxel (Huang et al, 2017), 5-fluorouracil (Ganeshkumar et al., 2014), antibiotics (Adriamycin) (Guo et al., 2014), epirubicin (Zhang et al., 2009; Zhang et al., 2010), indomethacin (Constantin, et al., 2017), antiinflamators, diclofenac (Constantin et al., 2007) and diclofenac - α-tocopherol (Mocanu et al., 2014), naproxen (Bishwambhar et al., 2012), antiviral agents, lopinavir (Ravi et al., 2014), antipepileptic agents (clonazepam (Jung et al., 2003; Jung et al., 2004), oral antidiabetics, insulin (Lin et al., 2019), antiasthamatic agents, salbutamol sulfate (Xu, et al., 2015), biophosphonates administered for slow down or prevent bone loss, risedronate (Velasquez et al., 2014), natural antioxidant agents for liver health, silymarin (Kumar et al., 2012), etc. Furthermore, pullulan and its derivatives were used in targeted drug delivery for different diseases like cancer, hepatitis C virus, autoimmune diseases, atherosclerosis, graft rejection, ischemia, asthma (Constantin et al., 2007; Masuda, 2001; Satoh et al., 2008; Suginoshita et al., 2002).

Extensive research for the utilization of pullulan and its derivatives was made in cancer therapy. Several papers described stimuli sensitive systems based on pullulan (temperature, pH, charge, redox, light). Liang et al. (2019) designed pH-responsive injectable hydrogels based on chitosan-grafted-dihydrocaffeic acid and oxidized pullulan for localized drug delivery. Lu et al. (2010) prepared pH sensitive pullulan-doxorubicin conjugates which displayed enhanced drug accumulation in tumors and reduced cardiotoxicity. Fundueanu et al. (2010) developed pullulan combined with poly (N-isopropyl - acrylamide - co - acrylamide) microspheres with temperature dependent delivery capability. Mocanu et al. (2011) designed thermosensitive carboxymethyl pullulan nanoparticles for controlled drug release.

Various pullulan systems can be used to target breast, kidney, lung, brain, or ovary cancer (Raychaudhuri et al., 2020). For example, Huang et al. (2017) designed pullulan nanoparticles loaded with paclitaxel for liver-targeting. In another study, Li et al. (2014) designed carboxymethyl pullulan based nanoparticles with pH sensitivity for liver targeting. Also, Satoh et al. (2008) showed the *in vitro* and *in vivo* ability of cholesterol bearing pullulan nanoparticles modified with amine containg docetaxel to target lung cancer cells.

Apart from cholesterol bearing pullulan and carboxymethyl pullulan, pullulan acetate was as well used in cancer treatment. Pullulan acetate micelles have can be used for longer circulation of drugs in blood and their delivery to the targeted tumor cells/tissues. Pullulan acetate combined with carboxymethylated poly(ethylene glycol) possess good delivery properties like enhanced hydrophobic active substances release as well as avoidance of macrophages (Jung et al., 2003). In another study, Suginoshita et al. (2001) described diethylenetriamine penta acetic acid - pullulan for cancer treatment possessing higher uptake of active substances in tumors. Also, Seo et al. (2012) reported poly(L-lactide) and poly(DL-lactide-co-glycolide) grafed on pullulan thermosensitive nanogels with enhanced cytotoxicity against the tumor cells.

Furthermore, folate modified pullulan systems were used for active targeting of cancer cells exploiting the fact that in general folates are taken up into cells by folate receptors *via* receptor-mediated endocytosis. Chenab et al. (2018) described pullulan nano-micelles decorated with folate loaded with doxorubicin and shRNA of Beclin1 effective in tumor targeting in HeLa and HepG2 cells. Zhang et al. (2010) developed folate modified pullulan acetate nanoparticles loade with epirubicin with better drug entrapment efficiency and release properties for active targeting of cancer cells. Li et al. (2013) described pH sensitive folate-decorated maleilated pullulan for cancer therapy with increased cellular uptake of active substances and enhanced cytotoxicity.

Although a large part of the literature regarding drug delivery systems based on pullulan address cancer therapy, there are some papers focused on the treatment of other diseases like Alzheimer's disease, hepatitis, autoimmune diseases, atherosclerosis, graft rejection, ischemia, asthma, etc (Shi and Li, 2005; Masuda, 2001; Suginoshita et al., 2002; Boridy et al, 2009). For example, cholesterol bearing pullulan can be used for insulin delivery with

improved biological activity, better stability, reduced side effects (Shi and Li, 2005). In another study, Boridy et al. (2009) reported the use of hydrophobically modified cholesterol bearing pullulan as a replacement of antibody immunotherapy for Alzheimer's disease treatment.

2. Other biomedical applications

2.1. Gene delivery

Formulations of modified pullulans have significant potential for targeted gene delivery. The genes can be encapsulated inside the pullulan formulations and protected from DNase destruction while being delivered to the desired cells or organs. Pullulan with a cholesterol group within creates a hydrophobic core with self-aggregation characteristics (Singh et al., 2015). Cholesterol-bearing pullulan is used for targeted distribution of various hydrophobic proteins or genes due to these features (Lee and Akiyoshi, 2004). It has also been used to transport proteins to immune cells, such as shortened HER2-147 (Ikuta et al., 2002). Pullulan hydrogels have high plasmid DNA loading efficiency and provide sustained DNA release to cancer cells (Gupta and Gupta, 2004). Using cationic pullulan formulations such as polyethylenimine pullulan, desired genes can be targeted in the liver (Kang et al., 2010). Polyethylenimine pullulan reduces the side effects of DNA or genes and is commonly used to target tumor cells (Ambattu et al., 2015; Kang et al., 2010; Rekha and Sharma, 2011; Wang et al., 2014). Pullulan based on diethylaminoethylamine can be manufactured in tubular or three-dimensional matrices to deliver genes to local arteries or muscle cells while also preserving them from DNase destruction (Juan et al., 2007). Folate modification improves gene silencing and gene transfection effectiveness (Wang et al., 2014). Pullulan spermine has been shown to deliver the intracellular gene and promote dopamine release in the treatment of Parkinson's disease (Nagane et al., 2009). It is also used for neuronal gene delivery and gene targeting to human bladder tumor cells (Kanatani et al., 2006). Pullulan derivative formulations, such as pullulan-g-poly(l-lysine) (Park et al., 2012), pullulan-protamine (Liu et al., 2014; Priya et al., 2014; Yang et al., 2014), and succinylated pullulan (Kim and Nan, 2010) are effective for targeted gene delivery while minimizing cytotoxicity.

2.2. Tissue engineering

From data literature, various derivatized forms of pullulan have been used for tissue engineering applications i.e. phosphorylated pullulan, carboxylated pullulan, pullulan-cellulose acetate, etc. Tissue engineering may be a handle to improve self-healing potential of the harmed tissues or organs by making a reasonable cell environment using a fitting manufactured 3-dimensional (3D) scaffold (Tabata, 2003; Singh et al., 2016). Various biopolymers which have properties same as characteristic tissues can be molded into different shapes like hydrogels, scaffolds, micro-molded frameworks, micro-beads and nanoparticles for tissue engineering application (Mallick and Cox, 2013). The application of these biopolymers in the field of tissue engineering primarily includes surface adjustments. The surface properties of pullulan can be effectively improved by substitution of some specific chemical moieties on its hydroxyl groups (Kumar et al., 2012). Pullulan presents amazing mechanical properties, a high hydration capacity and an excellent cell compatibility (Chaouat et al., 2006; Shingel, 2004). Due to these properties, pullulan based scaffolds play a main role in encouraging cell-based dermal substitution, tissue designing of vascular cells and bone recovery. Therefore, pullulan hydrogel scaffolds help assemble cell-loaded microtissue complexes and encapsulate damaged cells for regeneration and proliferation purpose (Bae et al., 2011), and due to the structure of hydrogels, they support the controlled release of active substances to the target site. Pullulan hydrogels have applications in maxillofacial surgery and orthopedics, because they help stimulate the differentiation of bone cells from mesenchymal stem cells (MSCs). In addition, coating with nanocrystalline hydroxyapatite particles (nHAP) improves the mechanical properties of these 3D hydrogels and also improves the ability of cells to attach to these constructs. They have anti-adhesion properties and help alleviate various problems such as postoperative pain, infertility, and intestinal obstruction (Bang et al., 2016). Also, the innovative pullulan bioconjugate can

be used to selectively treat bone metastases in breast cancer (Bonzi et al., 2015). On the other hand hydrogels have also received considerable attention as wound dressings due to the fact that they have the same physical properties as natural soft tissues and can absorb large amounts of aqueous liquid. Therefore, hydrogels provide a moist wound environment and can protect against bacterial infections (LayFlurrie, 2004; Loke et al., 2000). Pullulan-collagen hydrogels have consistent porosity, replicate dermal structure and successfully integrate stem cells for early wound healing (Galvez et al., 2009), and present numerous features, such as: antioxidant properties, enhance cell recruitment and activation, accelerate wound healing, enhance the expression of monocyte chemoattractant protein 1 at both transcriptional and protein levels through addition of adipose-derived mesenchymal stem cells, etc. (Wong et al., 2011). Phosphorylated pullulan (PPL) provides good adhesion to hard tissue via ionic bonds and helps repair bone defects (Shiozaki et al., 2011) and can act as a carrier for antibacterial agents and promote the regeneration of femoral defects. Carboxylated pullulan combined with human-like collagen and 1,4-butanediol diglycidyl ether can serve as a promising soft filler for tissue engineering (Li et al., 2015). 3D Pullulan Cellulose Acetate Scaffold has cell compatibility to promote cell attachment, diffusion and proliferation for skin tissue engineering (Atila et al., 2015). The cholesterol-bearing pullulan (CHP)-nanogels can encapsulate hydrophobic active substances for tissue engineering, and can create a moist environment for full-thickness wounds and promote controlled release of some prostaglandins (e.g. E1 prostaglandin) for angiogenesis, neoepithelialization, and damage regeneration (Kobayashi et al., 2009).

2.3. Medical imaging

Medical imaging uses fluorescent probes for labelling living cells, like quantum dots. Although quantum dots possesssuitable properties for biomedical imaging (nanometric saize, bright fluorescence, narrow and symmetricemission spectra, broad excitation, good photo-stability), their delivery into body cells is still challenging (Probst et al, 2003). Hasegawa et al. (2005) designed pullulan-bearing cholesterol and amino group-modified cholesterol nanaparticles for the delivery of quantum dots into body cells. Compared with conventional cationic liposomes, the cholesterol bearing pullulan nanoparticles showed better cellular uptake of the quantum dot therefore these nanoparticles could be promising fluorescent probes for imaging. Wu et al. (2010) described hydroxypropyl cellulose-poly(acrylic acid)-pullulan based hybrid nanogels loaded withcadmium selenide QDs with applications inbiomedical imaging.

CONCLUSIONS

This review highlighted the applications of pullulan in biomedical field (drug delivery, gene delivery, tissue engineering, medical imaging). Pullulan, microbial exo-polysaccharide produced by yeast like fungus *Aureobasidium pullulans*, presents biocompatibility, biodegradability, non-mutagenic, non-toxic, non-carcinogenic, non-immunogenic properties, as well as other functional properties. Furthermore, pullulan can be easily derivatized by several chemical reactions such as etherification, amidification, esterification, oxidation and co-polymerization in order to widen its applications. These unique properties of pullulan and its derivatives make them excellent candidates for various biomedical applications.

Due to the interesting activity and effectiveness shown from pullulan and its derivatives especially in the biomedical field, more research need to be done to explore intensive the use of this polymer in applications related to personal care and cosmetics.

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