ISSN-2249-5746

International Journal Of Ayurvedic And Herbal Medicine 2:1 (2012) 180:186

Journal Homepage http://interscience.org.uk/index.php/ijahm

The microbial pullulan as therapeutic tool in Medicine

Bishwambhar Mishra, Suneetha Vuppu

School of Bio Sciences and Technology, VIT University,
Vellore-632 014, Tamilnadu, India
***Correspondence Author :-** Bishwambhar Mishra School of Bio Sciences and Technology, VIT University,Vellore-632 014, Tamilnadu, India
Email:-vsuneetha@vit.ac.in, bishwambharmishra@vit.ac.in

In general, the body tissue is composed of cells with the local environment that plays an essential role in the attachment, proliferation, migration or differentiation of cells in the 3-dimensional organization. The cell behaviour is influenced by the surface and bulk properties of substrates like bio compatible inert material. Pullulan is one of the bio polymer which is frequently used in pharmaceutical, food and cosmetics industries. This polymer helps in fast dissolving drug delivery system which is suited for the drugs that undergoes highly first pass metabolic process and is used for enhancing the bioavailability with the reduction of dosage frequency. A glucose-based polysaccharide basically pullulan, has been shown to have a slow rate of hydrolysis in both the raw and cooked state at in vitro and in vivo conditions. The various types of inert bio-conjugates having anti-cancer and anti-inflammatory properties were synthesised by pullulan derivatisation and surface modifications. In the present review, recent advancements and literature regarding health sciences, medicine and the pharmaceutical approaches has been discussed.

KEY WORDS: Pullulan, Bio-Polymer, Drug delivery system, inert material, Bioconjugates

INTRODUCTION

Pullulan, which is a linear α-D-glucan made up of maltotriose and maltotetrose repeating units which are interconnected by α(1→6) linkages, is a water-soluble homopolysaccharide produced extra-cellularly by *Aureobasidium pullulans*, which is a polymorphic micromycete .[1] The regular alternation of α (1→4) and α (1→6) linkages results in two distinctive properties like structural flexibility and enhanced solubility . This biopolymer having economic importance with increased patented applications in food, pharmaceutical, agricultural and chemical industries.[2,3,4]Materials scientists are currently paying more attention for the crystallization of inorganic compounds within a largely organic matrix of naturally occurring bio-compatible and inert material like Pullulan. They are used for a medical application, comprises totally or partially of a living structure or biomedical device which can able to augment and replace the natural function. [4]

The daily intake of pullulan would be up to 10 gm per day for a person depending upon the food categories as estimated by FDA . [5] Pullulan is highly water soluble hence it is used as a carrier for drug and helps in controlled release of drug in plasma. Mostly hydrophobized pullulan is used as a carrier for drug delivery studies . It is also possible to Pullulan can be chemically modified to produce low solubility or a modified polymer that is completely insoluble in water. [6] Pullulan derivatives are developed and their applications towards the above mentioned aspects were also studied by various groups. [6,7] Pullulan derivatives were also obtained with targeting agents to produce self-organized drug loaded nano-particles for the development of receptor mediated cancer cells targeting . [8,9]

Currently, the various applications of pullulan in biomedical field is increasing contemporarily due to its non-toxic, non-immunogenic, biocompatible and inert nature. In comparison to dextran, the utilization rate of pullulan in serum is much more faster than that of dextran with degradation index is 0.7 after 48 hours of incubation while for dextran it is 0.05. [10]

The present paper describes the various aspect of Pullulan in the field of health sciences, medicine and the pharmaceutical approaches.

Pullulan as fast dissolving Coating film in medicinal formulation

So many polymers are present for preparation and formulation of fast dissolving buccal films. These polymers can be used alone or in combination with other groups to obtain the desired film properties. The physical properties of these film should be tough enough so that there won't be any damage while handling . In this context Pullulan is a natural polymer obtained from non-animal origin and does not require chemical modification. About 80% (w/w) of pullulan can be replaced by starch in the preparation of fast dissolving films without affecting the required properties of Pullulan. Different polymers like PVP, PVA, Gelatin, Eudragit RL100, HPMC E15, HPMC K4M, HPMC E5 and Pullalan were used to formulate fast dissolving buccal films by solvent casting method out of which it was confirmed that pullalan is best polymer for oral fast dissolving strips. [11]Water soluble hydrocolloids used to prepare films are hydroxyl-propylmethylcellulose, hydroxypropylcellulose, pullulan, sodium alginate, pectin and carboxy-methylcellulose. [12]

Nanoparticles of Pullulan acetate as an anti-tumour agent

It has been proved that pH-induced anticancer drug released from pH-sensitive liposomes made up of carboxylic group, which is stable at neutral pH but leaky at mildly acidic condition could be a new mode of cancer treatments. However due to the lack of responsive property to the range of tumour acidity, these carriers limit their utility as tumour targeting. More recently, pH sensitive nano-carriers have been more justified as cytosolic drug delivery carriers, instead of targeting to tumor pH, via endocytosis mechanism to improve drug bioavailability because endosomes and lysosomes are more acidified by proton- translocating ATPase to an average pH of approximately 5.0. Thus it is necessary to develop a nanocarriers which are truly responsive to tumor pH for direct tumor targeting. To acquire the appropriate pH-sensitivity, self-assembled hydrogel nanoparticles were prepared by introducing sulfadimethoxine (SDM, pKa; 6.1) into pullulan acetate (PA) as in Fig. 1.

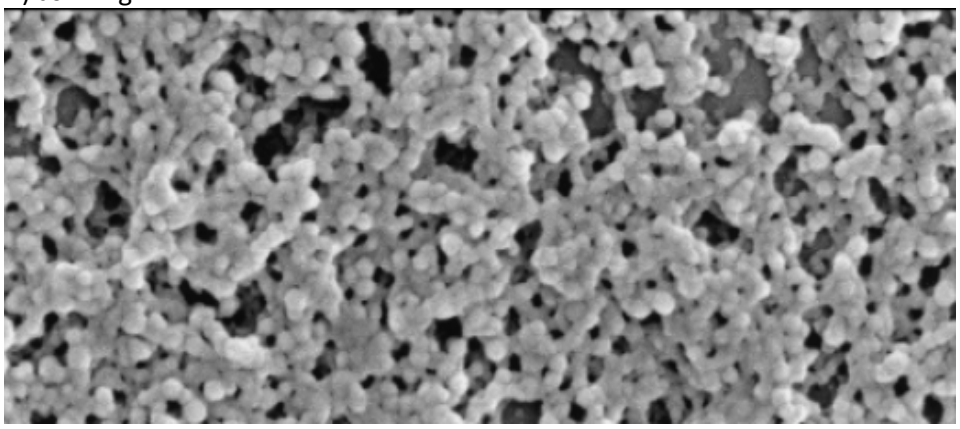

Fig.1: Field-emission scanning electron microscopic (FE-SEM) photographs of PA/SDM

Hepatic uptake of pullulan by receptor-mediated mechanism

A variety of macromolecular drug carrier systems have been developed in many types of animal models for clinical experiment. Among these, pullulan is one of the most promising carriers for delivery of both drugs and enzymes. Fluorescein-labeled pullulan (FP-60; MW 58,200) was prepared and the hepatic distribution of FP-60 was examined taking rat as a living system at *in vivo* condition using a high-performance size-exclusion chromatography. Intravenously administered FP-60 was found to be rapidly eliminated from the blood circulation followed with an appreciable distribution in the hepatic cells of rat. The distribution of FP-60 in the parenchymal liver cell fractions of rat was as shown in Fig.2. [14]

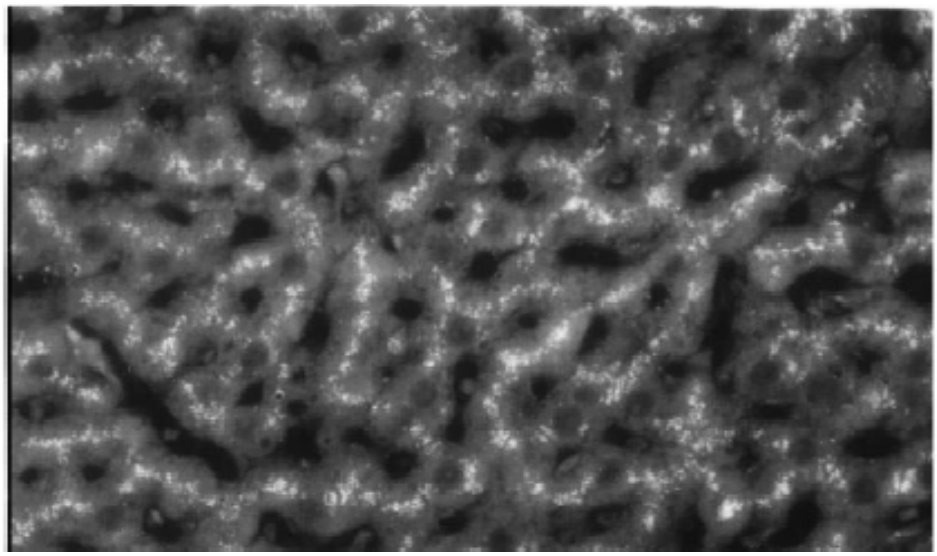

Fig.2 Fluorescence microscopic examination of the liver cross sectioned, revealed that FP-60 was effectively incorporated to the liver parenchymal cells

Cholesteryl pullulan for the formulation of stable liposomes

The methods for the preparation of cholesteryl pullulan has been discovered.In this method 1,4-diazabicyclo(2,2,2)octane was used as a catalyst which can able to reduce the reaction time. The synthesized cholesteryl pullulan is mainly used for the coating of the vincristine liposomes. Phosphatidylcholine liposomes with and without vincristine sulfate were made by sonication. The % of vincristine which has been encapsulated within the liposomes was determined by its solubilization with the bilayers using Triton-X 100. The liposomes were coated with different concentrations of cholesteryl pullulan solution which has been shown in Fig.3. [15]

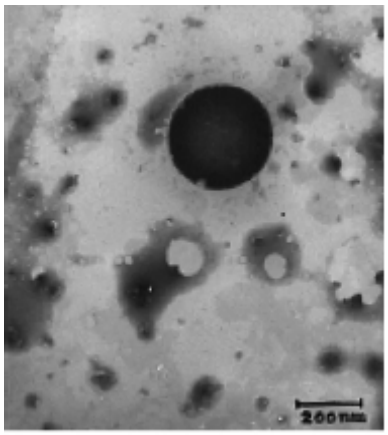

Fig.3: Transmission electron micrograph of cholesteryl pullulan liposomes.(Source: Sivakumar *et al.*2003)

Pullulan–cyclodextrin microspheres for the drug delivery

Pullulan microspheres with cyclodextrin were prepared chemically by crosslinking with epichlorohydrin of an alkaline solution of pullulan.In order to find the release profile of the microspheres, they were packed in a glass column and the liquid chromatographic behaviour was performed by isocratic elution method using different drugs and typical organic compounds , taken as model drugs, were examined . The suitable method here developed can predict appropriate drugs to be included in Pullulan–cyclodextrin microspheres as a potential biodegradable drug delivery system in fig.4 and 5. [16]

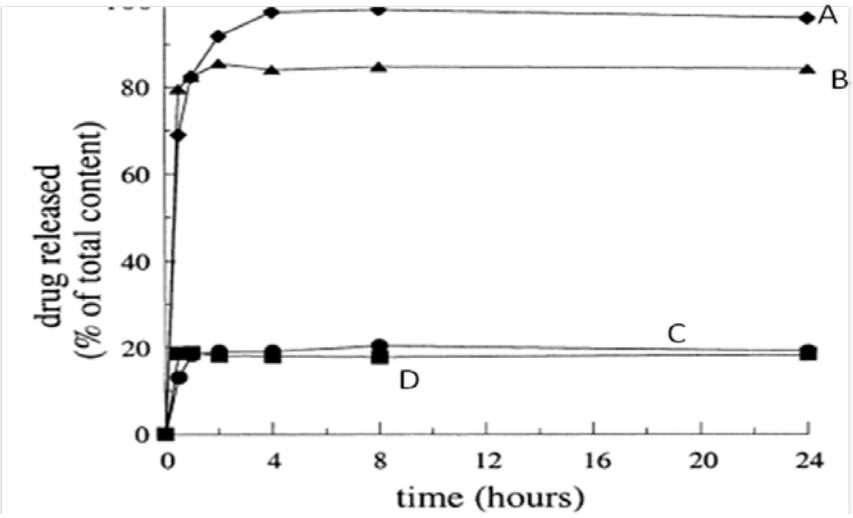

Fig.4: Release profiles of different drugs from Pullulan-Cyclodextrin micro- spheres: indol (A), 3-aminobenzoic acid (B), propranolol (C), and diclofenac (D) released from microspheres. (Source: G. Fundueanu *et al*.,2003)

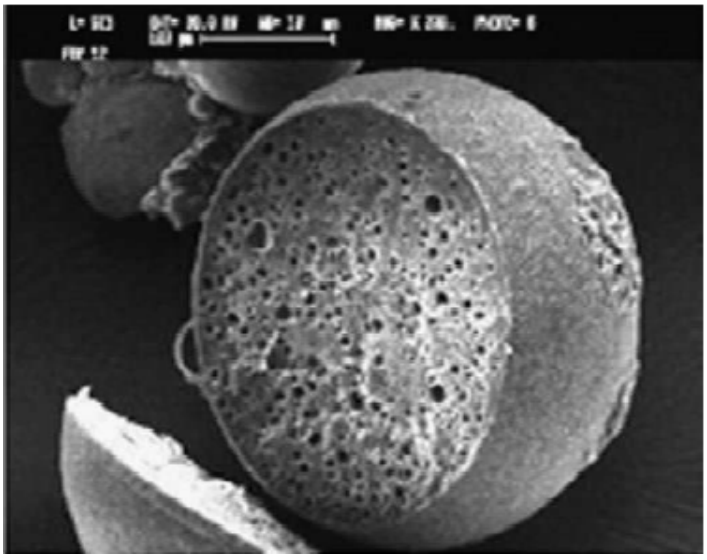

Fig.5: SEM photograph of Pullulan-Cyclodextrin microspheres.

(Source: G. Fundueanu *et al*.,2003)

Glycemic, insulinemic and breath hydrogen responses to pullulan in healthy humans

Most carbohydrate-containing ingredients that are compatible with liquids are rapidly digested.The polymeric derivatised pullulan has slow rate of *in vitro* hydrolysis in both the raw and cooked state . Reduction of the molecular weight for pullulan, the stability of a liquid nutritional formula is increased . Low–molecular weight pullulan can be prepared by the hydrolysis of high–molecular weight pullulan using a food grade enzyme, pullulanase. Low–molecular weight pullulan can be digested rapidly and subjecting an increased glycemic response in healthy humans similar to that of maltodextrin. Pullulan with a molecular weight of 1,00,000 (high–molecular weight) has been shown to have a glycemic attenuating response, but this form of pullulan is not easily incorporated into enteric drug formulation because it makes protein precipitation but on the other hand low molecular weight pullulan appears to make it more available for rapid enzymatic digestion in the small intestine easily . [17]

Pullulan gels and their interaction with biological active substances

Great attention has been paid in the last years for the development of polymeric hydrophilic gels by crosslinked macromolecular networks by chemical or physical methods. It is a well known fact that the peptides and the proteins administered in oral route possess a low bioavailability, due to their instability in the gastrointestinal tract and to their low permeability through intestinal mucous. The new method like synthesis and formulation of carboxymethyl pullulan microparticles which has been substituted with long chain of alkyl-amido groups and to their interaction with enzymes. The lysozyme immobilized on the supports preserves its enzymatic activity; the enzymatic process rate is controlled by the diffusion of the substrate to the reactive sites of the enzyme. Thus, the immobilized lysozyme on these supports can be used in processes which need a controlled rate of the enzymatic activity. The lysozyme with hydrophilic support complexes made up of pullulan can be used also for healing of the infected wounds, where it is to be expected to act both as a fluid adsorbent and as a topical anti bacterial agent. [18]

CONCLUSION

Basic applications of Pullulan are not only for materials to design and prepare medical devices and drug delivery system for drug therapy, but also substantially important and necessary to further develop the basic research of stem cell biology. However, such the concept of these wonderful biomaterial applications is still in its infancy. However, one of the main problems for creating the regeneration environment presently is absolutely due to shortage of biomaterial researchers who can investigate the cell scaffold, drug delivery system and cell culture for the biological substitution of organ functions. Such researchers must have knowledge in the interdisciplinary field like medicine, biology, dentistry, tissue engineering and pharmacology along with the material sciences.

REFERENCES

1. Deshpande MS, Rale VB, Lynch JM . *Aureobasidium pullulans* in applied microbiology: A status report. Enzyme Microb. Technol.1992; 14(3): 514-527.

2. Shingel KI . Current knowledge on biosynthesis, biological activity, and chemical modification of the exopolysaccharide, pullulan. Carbohydr. Res.2004 ; 33(2): 447-460.

3. Singh RS, Saini GK, Kennedy JF (2008). Pullulan: microbial sources, production and applications. Carbohydr. Polym.2008; 73(3): 515-531.

4. Bishwambhar Mishra, Suneetha Vuppu and Kalyani Rath. The role of microbial pullulan, a biopolymer in pharmaceutical approaches: A review. Journal of Applied Pharmaceutical Science.2011;1(06): 45-50.

5. Tsujisaka, Y., Mitsuhashi, M. (1993).Polysaccharides and their derivatives Pullulan. In: Whistler RL. BeMiller IN (ed.) Industrial gums (pp. 447-460) 3rd edn. Academic Press, San Diego.

6. Akiyoshi K., Kobayashi S., Schibe S., Mix D., Baudys M., Kim S. W.: Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulan as a carrier of protein drugs: complexation and stabilization of insulin. Journal of Controlled Release.1998; 54(3): 313-320.

7. Park, K.H., Kang D., Na K. : Physicochemical characterization and carcinoma cell interaction of self-organized nanogels prepared from polysaccharide-biotin conjugates for development of anticancer drug carrier. Journal of Microbiology and Biotechnology.2006;16(1):1369–1376.

8. Kim E.J, Cho S.H., Yuk S.H.: Polymeric microspheres composed of pH/temperature- sensitive polymer complex. Biomaterials. 2001;22 (3):2495–2499.

9. Bruneel D., Schacht E.: Chemical modification of pullulan: 3. Succinoylation .Polymer. 1993; 34 (2):2656-2658.

10. AS Kulkarni, HA Deokule, MS Mane, DM Ghadge. *J current Pharm. Research*. 2010; 2(1), 33-35.

11. M. Repka; J Swarbrick; J Boylan. In Encyclopedia of Pharmaceutical Technology, 2nd Edition, 2002, vol 2, p. 1488–1504.

12. Kun Na1 and You Han Bae1,2Self-Assembled Hydrogel Nanoparticles Responsive to Tumor Extracellular pH from Pullulan Derivative/Sulfonamide Conjugate: Characterization, Aggregation, and Adriamycin Release *in Vitro.Pharmaceutical Research. 2002;(5):55-60.*

13. Yoshiharu Kaneoa , Tetsuro Tanakaa, Takayuki Nakanoa, Yasunori YamaguchibEvidence for receptor-mediated hepatic uptake of pullulan in Rats Journal of Controlled Release.2001; 70 (1) 365–373.

14. P.A. Sivakumar, K. Panduranga Rao The use of cholesteryl pullulan for the preparation of stable vincristine liposomes Carbohydrate Polymers. 2003; 51(3):327–332.

15. Gheorghe Fundueanu , Marieta Constantin , Doina Mihai , Fabrizio Bortolotti ,Rita Cortesia ,Paolo Ascenzic, Enea Menegattia Pullulan–cyclodextrin microspheres A chromatographic approach for the evaluation of the drug–cyclodextrin interactions and the determination of the drug release profiles Journal of Chromatography B. 2003;79(1) :407–419.

16. Julie K. Spearsa, Lisa K. Karr-Lilienthala, Christine M. Grieshopa, Elizabeth A. Flickingera, Bryan W. Wolf b, George C. Fahey Jr.a,T Glycemic, insulinemic, and breath hydrogen responses to pullulan in healthy humans Nutrition Research. 2005;25(3): 1029–1041

17. G. Mocanua , D. Mihaia, L. Pictonb, D. LeCerfb, G. Mullerb A ssociative pullulan gels and their interaction with biological active substances Journal of Controlled Release.2002; 83 (2) :41–51.