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Assessment of Pullulan, a Microbial Polysaccharide, as a Matrix for Senotherapeutics Delivery †

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Abstract: This study's objective was to assess pullulan, in the form of pullulan acetate, as a matrix for senotherapeutics delivery. Polymeric nanoparticles loaded with various senotherapeutics (metformin, quercitin, kaempferol, curcumin, and luteolin) were prepared via nanoprecipitation or double emulsion methods using pullulan acetate as a biodegradable polymeric matrix. Quercitin, kaempferol, curcumin, and luteolin nanoparticles showed good yield (<70%), satisfactory values of entrapment efficiency (<60%), and nanometric sizes ranging between 205 and 270 nm, with narrow dispersity and good stability at 4 °C. The formulations demonstrated that pullulan showed great potential for producing nanoparticles with application in senotherapeutics delivery.

Keywords: nanoparticles; pullulan; senotherapeutics; senotherapy

1. Introduction

In the aging process, senescent cells accumulate in various organs or tissues, most likely due to inefficient clearance of the immune system, and thus contribute to the onset and progression of diseases characteristic of senescence (cardiac dysfunction, hyporeactivity, vascular calcification, type 2 diabetes, osteoporosis, degeneration of vertebral discs, pulmonary fibrosis, etc.). These diseases associated with senescence often lead to the development of additional health issues or co-morbidities; therefore, they increase mortality rates and place a substantial burden on public healthcare systems. Various scientific publications suggest that one potential way to mitigate the negative effects of aging is by removing/killing senescent cells from organs and tissues [1,2].

Senotherapy (the administration of senotherapeutic substances) is an emerging field of research for developing possible treatments and strategies that specifically target cellular senescence. Several Food and Drug Administration (FDA)-approved substances effective as senotherapeutics are reported, such as quercetin (a flavonoid with antioxidant properties), dasatinib (chemotherapeutic, a tyrosine kinase inhibitor), piperlongumin (a natural product isolated from a variety of pepper species), navitoclax (also known as ABT-263), fisetin, and luteolin [3,4]. Apart from these, other promising substances have been identified for use as senotherapeutics, such as epigallocatechin gallate, phloretin, silybin, resveratrol, genistein, sulforaphane, allicin, berberine, triptolide, metformin, apigenin, kaempferol, ouabain, digoxin, rapamycin, ruxolitinib, 4,4′-dimethoxychalcone, tocotrienol analogs, curcumin, etc., [5–8]. Some of them have already been validated through preclinical studies and are in the process of being accepted for clinical trial investigation in senotherapy [2].

Researchers are currently exploring innovative systems for the delivery of senotherapeutics, like polymeric nanoparticles [9,10]. Nanoparticles provide a versatile platform for the targeted delivery of drugs related to senescence. Their ability to target specific cells, control drug release, protect labile drugs, enable combination therapy, and facilitate

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diagnostic imaging makes them invaluable in developing effective and safe treatments for age-related diseases [9,10].

Pullulan, a microbial polysaccharide obtained by *Aureobasidium pullulans* strains, possesses a range of desirable properties for drug-delivery applications, including non-mutagenicity, non-toxicity, non-carcinogenicity, non-immunogenicity, biocompatibility, and biodegradability [11,12]. Additionally, pullulan can be modified to expand its potential applications as a drug delivery carrier because it exhibits water solubility and cannot self-associate in aqueous solutions. Pullulan acetate, a pullulan derivative, offers versatility in drug-delivery applications due to its ability to form various drug carriers, control drug release rates, and maintain biocompatibility. Its potential to protect drugs from degradation and target specific sites makes it a valuable material in developing innovative drug-delivery systems [13,14].

This study's objective was to assess pullulan, in the form of pullulan acetate, as a matrix for senotherapeutics delivery. To this end, metformin, quercitin, kaempferol, curcumin, and luteolin were used as model senotherapeutic agents, and senotherapeutic-loaded pullulan-based nanoparticles were prepared and characterized in terms of entrapment efficiency, size, and their polydispersity index using spectrophotometric and dynamic light-scattering techniques.

2. Materials and Methods

2.1. Materials

Pluronic F127 (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), magnesium sulfate heptahydrate ($MgSO_4$*$7H_2O$), diammonium sulfate ($(NH_4)_2SO_4$), and sodium nitrate ($NaNO_3$) were purchased from Sigma–Aldrich (Burlington, MA, USA). Acetone (analytical grade) was obtained from AdraChim SRL (Bucharest, Romania) and used without further purification. The water used for all experiments was distilled.

2.2. Pullulan Production and Pullulan Acetate Synthesis

Pullulan was obtained via the process of microbial biosynthesis (72 h) using the microorganism *Aureobasidium pullulans* ICCF 36 from the Collection of Microorganisms of Industrial Importance of the INCDCF-ICCF using a bioprocess medium consisting of glucose 8%, $NaNO_3$ 0.2%, $(NH_4)_2SO_4$ 0.2%, KH_2PO_4 0.5%, NaCl 0.2%, and $MgSO_4 \times 7H_2O$ 0.08%. The biosynthesis was conducted at 28 °C, 220 rpm, for 72 h. Following the microbial biosynthesis process, the pullulan obtained was further chemically functionalized using the Motozato method with dimethyl formamide, pyridine, and acetic anhydride, obtaining pullulan acetate.

2.3. Preparation of Polymeric Nanoparticles Loaded with Various Senotherapeutics

Polymeric nanoparticles loaded with metformin, quercitin, kaempferol, curcumin, and luteolin were prepared via nanoprecipitation using pullulan acetate as a biodegradable polymeric matrix and Pluronic F127 as a stabilizer. Polymeric nanoparticles loaded with metformin were prepared using the double emulsion method. All formulations were centrifuged at 10,000 rpm for 30 min to separate free substances from nanoparticles loaded with senotherapeutics (Table 1).

Table 1. Formulation of polymeric nanoparticles loaded with various senotherapeutics.

Formulation Code	Senotherapeutic	Preparation Method	Polymer–Senotherapeutic Ratio
NP@Metmorfin_met1	Metmorfin	nanoprecipitaion	10:2
NP@Metmorfin_met2	Metmorfin	double emulsion	10:2
NP@Quercitin_met1	Quercitin	nanoprecipitaion	10:2
NP@Kaempferol_met1	Kaempferol	nanoprecipitaion	10:2
NP@Luteolin_met1	Luteolin	nanoprecipitaion	10:2
NP@Curcumin_met1	Curcumin	nanoprecipitaion	10:2

2.4. Characterisation of Polymeric Nanoparticles Loaded with Senotherapeutics

Nanoparticles were assessed regarding entrapment efficiency, yield, polydispersity index (PDI), average diameter, and stability. Entrapment efficiency was calculated as the percentage ratio between the amount of senotherapeutic loaded in nanoparticles and the amount of senotherapeutic added during nanoparticle preparation. The amount of senotherapeutic loaded in nanoparticles was assessed via a spectrophotometric technique using a UV/VIS spectrophotometer (Helios, ThermoFisher Scientific, Waltham, MA, USA). The yield was calculated as a percentage ratio of the mass of the produced nanoparticles and the total mass of the starting materials that were introduced into the process. The polydispersity index and the average diameter of nanoparticles containing senotherapeutics were determined using dynamic light scattering with a particle size analyzer (Beckman-Coulter-N4-PCS-Submicron, Paris, France). To mitigate the impact of multiple scattering, distilled water was used to dilute the nanoparticles at a dilution ratio 1:10. These measurements were conducted at room temperature, with ten runs for each measurement. The stability of senotherapeutics-loaded nanoparticles was assessed after keeping the nanoparticles in amber glass vials at 4 °C for three months, and the entrapment efficiency was determined after different storage periods (initial, 1, 2, and 3 months).

2.5. Statistical Analysis

Experiments were carried out in triplicate, and the obtained experimental data were presented as mean values ± standard deviation. Differences were regarded as statistically significant when the *p*-value was less than 0.05.

3. Results and Discussions

This study aimed to evaluate the potential of pullulan, in the form of pullulan acetate, as a carrier matrix for senotherapeutic compounds. In pursuit of this goal, senotherapeutic-loaded nanoparticles were obtained using pullulan acetate as a biodegradable polymeric matrix, Pluronic F127 as a stabilizer, and the senotherapeutic agents were metformin, quercetin, kaempferol, curcumin, and luteolin. For the preparation of polymeric nanoparticles, a series of methods were considered, such as nanoprecipitation, ionic gelation, solvent–emulsion evaporation, and double emulsion. Of these, nanoprecipitation and double emulsion–solvent evaporation were applied to obtain polymeric nanoparticles loaded with metformin, and only nanoprecipitation was used to produce nanoparticles containing quercetin, kaempferol, curcumin, and luteolin. In general, both methods are fast, easy to apply in the laboratory, and spontaneously lead to the formation of nanoparticles with a satisfactory degree of entrapment and good yield. The methods to produce polymeric nanoparticles loaded with metformin, quercetin, kaempferol, curcumin, and luteolin were evaluated by determining the entrapment efficiency and preparation yield; characteristics are displayed in Table 2.

Table 2. Entrapment efficiency and preparation yield of polymeric nanoparticles loaded with senotherapeutic substances.

Code Formulation	Entrapment Efficiency (%)	Preparation Yield (%)
NP@Metmorfin_met1	60.21 ± 0.25%	65.89 ± 0.33%
NP@Metmorfin_met2	71.21 ± 0.25%	70.01 ± 0.15%
NP@Quercitina_met1	77.54 ± 0.33%	71.20 ± 0.20%
NP@Kaempferol_met1	77.30 ± 0.13%	75.80 ± 0.12%
NP@Luteolina_met1	78.27 ± 0.29%	75.29 ± 0.15%
NP@Curcurmin_met1	68.55 ± 0.32%	71.09 ± 0.29%

Metformin-loaded nanoparticles prepared using the double emulsion–solvent evaporation method had higher entrapment efficiency (EE = 71.21 ± 0.25%) values than those prepared via nanoprecipitation (EE = 60.21 ± 0.25%). The methods used to obtain metformin-loaded polymeric nanoparticles, namely nanoprecipitation and double

emulsion, successfully generated metformin-loaded nanoparticles. The entrapment efficiency was determined by the properties of the polymer, drug, surfactant, etc. The affinities of the therapeutic agent and the polymer towards different solvents explain the moderate entrapment efficiency values of the formulations prepared via nanoprecipitation.

For quercitin, kaempferol, curcumin, and luteolin, the nanoprecipitation method successfully generated nanoparticles with yield values in the range of 71.09 ± 0.29–75.80 ± 0.12% and entrapment efficiency values in the range of 68.55 ± 0.32–78.27 ± 0.29%. The method used to obtain polymeric nanoparticles loaded with quercetin, kaempferol, curcumin, or luteolin, namely nanoprecipitation, successfully generated nanoparticles with a fairly good yield (<70%) and satisfactory entrapment efficiency values (<60%).

The nanoparticles showed good stability at 4 °C with almost the same senotherapeutic content entrapped after one month (drug loss less than 0.5%) and after three months (drug loss less than 2%), and no aggregation was observed during storage.

The features of the nanoparticles, like size and the polydispersity index, are crucial in drug-delivery applications due to the influence on their behavior in vitro and in vivo. The polymeric nanoparticles loaded with senotherapeutic substances showed satisfactory nanometric sizes, with the average diameter of nanoparticles ranging from 205 ± 0.51% to 270 ± 0.39% nm. A low PDI indicates monodispersity and uniform particle sizes, while a high PDI suggests polydispersity, indicating a broader range of particle sizes within the sample. The polydispersity index of nanoparticles containing senotherapeutics was below 0.3, indicating a narrow dispersity.

According to these results, pullulan derivatives (e.g., pullulan acetate) show good potential to produce nanoparticles that could be used to deliver senotherapeutic substances.

4. Conclusions

Polymeric nanoparticles loaded with various senotherapeutics (metformin, quercitin, kaempferol, curcumin, and luteolin) were prepared via nanoprecipitation or double emulsion methods using pullulan acetate as a biodegradable polymeric matrix. Metformin, quercitin, kaempferol, curcumin, and luteolin nanoparticles showed good yield (<70%), satisfactory values of entrapment efficiency (<60%), and nanometric sizes ranging between 205 and 270 nm, with narrow dispersity and good stability at 4 °C. The formulations demonstrated that pullulan showed great potential for producing nanoparticles with application in senotherapeutics delivery. More studies (in vitro and in vivo) are needed to evaluate the nanoparticles' safety profile and the efficiency of senescent cell lines.

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References

1. Lagoumtzi, S.M.; Chondrogianni, N. Senolytics and senomorphics: Natural and synthetic therapeutics in the treatment of aging and chronic diseases. *Free Radic. Biol. Med.* **2021**, *171*, 169–190. [CrossRef] [PubMed]
2. Tchkonia, T.; Palmer, A.K.; Kirkland, J.L. New Horizons: Novel Approaches to Enhance Healthspan Through Targeting Cellular Senescence and Related Aging Mechanisms. *J. Clin. Endocrinol. Metab.* **2021**, *106*, e1481–e1487. [CrossRef] [PubMed]
3. Chaib, S.; Tchkonia, T.; Kirkland, J.L. Cellular senescence and senolytics: The path to the clinic. *Nat. Med.* **2022**, *28*, 1556–1568. [CrossRef] [PubMed]

4. Wang, Y.; Chang, J.; Liu, X.; Zhang, X.; Zhang, S.; Zhang, X.; Zhou, D.; Zheng, G. Discovery of piperlongumine as a potential novel lead for the development of senolytic agents. *Aging* **2016**, *8*, 2915–2926. [CrossRef] [PubMed]
5. Soukas, A.A.; Hao, H.; Wu, L. Metformin as Anti-Aging Therapy: Is It for Everyone? *Trends Endocrinol. Metab.* **2019**, *30*, 745–755. [CrossRef] [PubMed]
6. Zia, A.; Farkhondeh, T.; Pourbagher-Shahri, A.M.; Samarghandian, S. The role of curcumin in aging and senescence: Molecular mechanisms. *Biomed. Pharmacother.* **2021**, *134*, 111119. [CrossRef] [PubMed]
7. Domaszewska-Szostek, A.; Puzianowska-Kuźnicka, M.; Kuryłowicz, A. Flavonoids in Skin Senescence Prevention and Treatment. *Int. J. Mol. Sci.* **2021**, *22*, 6814. [CrossRef] [PubMed]
8. Guerrero, A.; Herranz, N.; Sun, B.; Wagner, V.; Gallage, S.; Guiho, R.; Wolter, K.; Pombo, J.; Irvine, E.E.; Innes, A.J.; et al. Cardiac glycosides are broad-spectrum senolytics. *Nat. Metab.* **2019**, *1*, 1074–1088. [CrossRef] [PubMed]
9. Zhang, Q.; Li, S.; Chen, F.; Zeng, R.; Tong, R. Targeted delivery strategy: A beneficial partner for emerging senotherapy. *Biomed. Pharmacother.* **2022**, *55*, 113737. [CrossRef] [PubMed]
10. Adamczyk-Grochala, J.; Lewinska, A. Nano-Based Theranostic Tools for the Detection and Elimination of Senescent Cells. *Cells* **2020**, *9*, 2659. [CrossRef] [PubMed]
11. Moscovici, M. Present and future medical applications of microbial exopolysaccharides. *Front. Microbiol.* **2015**, *6*, 1012. [CrossRef] [PubMed]
12. Singh, R.; Saini, G.; Kennedy, J. Pullulan: Microbial sources, production and applications. *Carbohydr. Polym.* **2008**, *73*, 515–531. [CrossRef] [PubMed]
13. Singh, R.S.; Kaur, N.; Kennedy, J.F. Pullulan and pullulan derivatives as promising biomolecules for drug and gene targeting. *Carbohydr. Polym.* **2015**, *123*, 190–207. [CrossRef] [PubMed]
14. Tang, H.B.; Li, L.; Chen, H.; Zhou, Z.M.; Chen, H.L.; Li, X.M.; Liu, L.R.; Wang, Y.S.; Zhang, Q.Q. Stability and in vivo evaluation of pullulan acetate as a drug nanocarrier. *Drug Deliv.* **2010**, *17*, 552–558. [CrossRef] [PubMed]