Pullulan-based nanoparticles: future therapeutic applications in transmucosal protein delivery

"*The analysis of all the data available in the literature suggests the adequacy of the biomaterial pullulan for drug-delivery strategies. Particularly, although counting on a reduced number of works, pullulan-based nanoparticles reveal potential regarding transmucosal protein delivery.*"

Keywords: nanoparticles ■ proteins ■ pullulan ■ transmucosal delivery

Current advances in the field of drug delivery are aimed at finding adequate strategies for the administration of different drugs. Transmucosal delivery is the first-line option for the systemic delivery of many drugs, including proteins, a group of molecules with recognized therapeutic potential and wide range of applications (drug and genetic therapy, immunization, among others). This strategy, however, demands developing adequate carriers, as proteins are very sensitive molecules, easily undergoing degradation in physiological media, mainly due to pH and enzymatic occurrences. Polymeric nanoparticles have taken the forefront of this carrier-design challenge, offering appealing properties that include a reduced size providing intimate contact with mucosal surfaces, which potentiates any active action of the carrier regarding transmucosal delivery. Nanoparticles have been generally demonstrated to improve protein pharmacokinetic profiles, not only by providing increased stabilization but, in some cases, also permitting controlled release and enhancing drug absorption [1]. Moreover, the high surface-to-volume ratio displayed by these carriers increases drug loading capacity [2]. Polysaccharides have been indicated for many years as the most promising materials in drug carrier development, namely regarding nanoparticles. In this regard, a particular emphasis is placed on chitosan. Other polysaccharides, however, have been paving their way, also demonstrating great ability for protein delivery. Pullulan is one of those polysaccharides; the first works reporting the application of pullulan-based nanoparticles for drug delivery dating back to 1998, when a cholesteryl group bearing pullulan was used to produce nanoparticles for the delivery of both an oncoprotein [3] and insulin [4].

Pullulan is a neutral, water-soluble polysaccharide produced from starch by the fungus *Aureobasidium pullulans*, by means of a process of fermentation [5]. Chemically, this polymer consists of α-(1,6)-linked maltotriose residues, which in turn are composed of three glucose molecules connected to each other by α-(1,4) glycosidic bonds [6,7]. Several properties have been reported for this material that make it attractive for drug-delivery purposes, including its adhesive ability, as well as the capacity to form fibers and thin biodegradable films [7]. The high content of hydroxyl groups on pullulan chains further endows the polymer with inherent physiological activity and also provides chemical flexibility.

Biodegradability is a mandatory characteristic of any material being considered for an application in drug delivery, and pullulan is expected to comply with it. Actually, once administered it will be exposed to the hydrolysis of glycoside bonds and the subsequent metabolism of glucose [8]. The ability to adhere to cell surfaces might also be advantageous within the application, improving the carriers' residence time and, consequently, the potential for timely absorption of the encapsulated protein. In this regard, apart from the demonstrated role of pullulan on the adhesion of *A. pullulans* to biological surfaces, such as leaves [9], cell adhesion to pullulan-based surfaces was reported on a number of occasions [10–12]. Pullulan-based nanoparticles were actually reported to adhere to the nasal epithelium in a study regarding nasal vaccination [13], and the authors' group has also verified the adherence of pullulan nanoparticles to respiratory epithelial cells (Calu-3 and A549), although to a limited extent [Rodrigues S, Unpublished Data].

Ana Grenha
Author for correspondence:
University of Algarve, Centre for Molecular & Structural Biomedicine/Institute for Biotechnology & Bioengineering, Faculty of Sciences & Technology, Campus de Gambelas, 8005–139 Faro, Portugal
Tel.: +351 289800100
Fax: +351 289818419
E-mail: amgrenha@ualg.pt

Susana Rodrigues
University of Algarve, Centre for Molecular & Structural Biomedicine/Institute for Biotechnology & Bioengineering, Faculty of Sciences & Technology, Campus de Gambelas, Portugal

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Pullulan has been increasingly described as matrix-forming material of drug and gene nanocarriers [5,7,13–17]. The most common characteristic of these carriers is the use of hydrophobic derivatives of pullulan, namely cholesteryl-pullulan, because this provides an amphiphilic polymer with the capacity to form nanoparticles by self-aggregation. These nanoparticles have been demonstrated to form stable complexes with both hydrophobic and hydrophilic drugs, such as proteins, reinforcing their flexibility. In a remarkable detail, apart from the capacity to form nanocarriers with suitable properties for transmucosal protein delivery, it has also been reported that pullulan-based nanoparticles are able to preserve the structure of encapsulated proteins, thus ensuring their biological activity [4]. Nevertheless, although the potential application in protein delivery was mentioned frequently, the demonstration of results in the area has been scarce. The delivery of insulin, bovine serum albumin, DNA and IL-12 was effectively proposed [4,15–18]. The authors' group has recently given a contribution to the field. Instead of using a hydrophobic derivative of pullulan, our strategy involved the synthesis of charged hydrophilic derivatives of the polymer that provided the possibility of establishing electrostatic interactions among them, or with other charged materials of interest. From the synthesized derivatives (carboxylated, phosphorylated, aminated and sulfated) the latter two were used to produce polysaccharide-based nanoparticles, upon complexation with either chitosan or carrageenan, or even with each other. The carriers were produced using a mild technique of polyelectrolyte complexation, which only involves hydrophilic conditions and a simple mixture of solutions, while permitting a very rapid formation of the carriers (less than 10 min). These nanoparticles exhibited great ability for the association of proteins with distinct properties (insulin and bovine serum albumin, MW of 5.7 and 67 kDa, respectively) and were proposed for nasal and pulmonary transmucosal delivery. Having demonstrated a clear non-cytotoxic behavior in model respiratory cell lines (Calu-3 and A549) [17], the nanoparticles also revealed to not induce an inflammatory response upon contact with the same cell lines [Rodrigues S, Unpublished Data]. Finally, preliminary *in vivo* tests in rats demonstrated an increased therapeutic response of insulin-loaded pullulan-based nanoparticles administered intranasally as compared with an insulin solution of similar dose [Grenha A, Unpublished Data].

The analysis of all the data available in the literature suggests the adequacy of the biomaterial pullulan for drug-delivery strategies. Particularly, although counting on a reduced number of works, pullulan-based nanoparticles reveal potential regarding transmucosal protein delivery. The ability to associate and release biomacromolecules is demonstrated, good indications are available concerning biocompatibility, and biodegradability is also ensured. However, the real possibilities of success should be considered on a rational and cautious basis. Looking at chitosan nanoparticles, for instance, which are incomparably more explored and for a longer time, after more than two decades of research and really good, diverse and important demonstrations of effectiveness and safety, no formulation has been made commercially available so far. Nanomedicine is still a controversial matter, mainly because of safety issues. The regulatory agencies demand uncountable studies and demonstrations to consider any approval, which is highly desirable if considering that global human health is the addressed subject. However, it is natural that nanomedicine, as a recent subject in the ambit of regulation, faces even more difficult challenges.

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As a demonstration that nanomedicine is still in its infancy regarding the regulatory challenges to be faced, it is interesting to consider a work from 2012 that revealed the significant accumulation of nanoparticles (40–350 nm) in the ovaries [19]. When other organs like the lung, liver, spleen and kidneys are usually examined to determine the biodistribution of nanoparticles and, thus, evaluate potential toxicity, the ovaries seem to have been neglected. The observation of that work raises an extremely important question: although *in vitro* cell studies give very relevant indications on the biocompatibility behavior of nanocarriers, it is

becoming undeniable that a complete characterization of potential toxicity is only possible with early *in vivo* studies. For this reason, keeping track of the whole biological portrait and the complex interactions between the nanocarrier and the biological structures, which might induce toxicological issues, is essential when designing a nanodelivery system.

It seems, then, that the potential of pullulan-based nanoparticles for transmucosal protein delivery is there, but a very long way is still ahead to cover.

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