A novel biocompatible polymer derived from D-mannitol used as a vector in the field of genetic engineering of eukaryotic cells

David Pérez [a], María Luisa Moyá [a], María Bautista [b], Rosa León [d], Ana Molina-Márquez [d], Marta Vila [d], Lucía Romero-Azogil [c], Elena Benito [c], María de Gracia García-Martín [c], Paula Moreno-Gordillo [f], Iván V. Rosado [f], Fernando R. Balestra [e], Pablo Huertas [e], Manuel López-López [b,*], Pilar López-Cornejo [a,*]

[a] *Departamento de Química Física, Facultad de Química, Universidad de Sevilla, C/ Profesor García González 1, 41012 Sevilla, Spain*
[b] *Departamento de Ingeniería Química, Química Física y Ciencias de Materiales, Facultad de Ciencias Experimentales, Campus de El Carmen, Avda. de las Fuerzas Armadas s/n, 21071 Huelva, Spain*
[c] *Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, C/ Profesor García González 2, 41012 Sevilla, Spain*
[d] *Laboratorio de Bioquímica, Facultad de Ciencias Experimentales, Campus de Excelencia Internacional del Mar (CEIMAR), Universidad de Huelva, 2110 Huelva, Spain*
[e] *Departamento de Genética, Universidad de Sevilla y Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), Universidad de Sevilla-CSIC-Universidad Pablo de Olavide, 41092 Sevilla, Spain*
[f] *Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Spain*

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ABSTRACT

The design and preparation of new vectors to transport genetic material and increase the transfection efficiency continue being an important research line. Here, a novel biocompatible sugar-based polymer derived from D-mannitol has been synthesized to be used as a gene material nanocarrier in human (gene transfection) and microalga cells (transformation process). Its low toxicity allows its use in processes with both medical and industrial applications. A multidisciplinary study about the formation of polymer/p-DNA polyplexes has been carried out using techniques such as gel electrophoresis, zeta potential, dynamic light scattering, atomic force microscopy, and circular dichroism spectroscopy. The nucleic acids used were the eukaryotic expression plasmid pEGFP-C1 and the microalgal expression plasmid Phyco69, which showed different behaviors. The importance of DNA supercoiling in both transfection and transformation processes was demonstrated. Better results were obtained in microalga cells nuclear transformation than in human cells gene transfection. This was related to the plasmid's conformational changes, in particular to their superhelical structure. It is noteworthy that the same nanocarrier has been used with eukaryotic cells from both human and microalga.

1. Introduction

Genetic engineering involves a group of techniques related to the manipulation, modification and/or recombination of nucleic acids. The manipulation of genetic material is usually carried out *in vitro* and, subsequently, is introduced into an organism to change any of its characteristics or improve its phenotype. This procedure can produce benefits in the clinical and/or biotechnological fields. In the clinical area, gene therapy, which consists of a set of techniques aimed at modulating the expression of certain proteins, is widely used in the treatment of various types of cancers, including pediatric,

gastrointestinal, gynecological, or neurological [1–5]. In the biotechnological field, genetic engineering of plants is an application with the enormous economic impact.

Genetic transformation of plant cells is an important process that allows the deletion, overexpression or modulation of certain genes to improve nutritional qualities, to increase the resistance to pathogens and to improve tolerance to pollutants [6–8]. Unicellular microalgae have been widely used as plant model organisms and have received increasing attention to yield high-added value products including pigments, vitamins, polyunsaturated fatty acids or biofuels [9–12]. Although genetic engineering is a promising strategy for the

improvement of microalgae, their routine genetic manipulation still faces significant challenges, such as increasing the efficiency of genetic transformation and the stability of transgenes.

The microalga *Chlamydomonas reinhardtii*, the first genetically modified eukaryotic alga, has been used as a model system in molecular biology in the last decade due to both its high division rate and its high adaptability to diverse environmental conditions [13]. The expression of recombinant proteins, as well as the complete characterization of the mitochondrial, chloroplastic and nuclear genomes in this organism, makes it an ideal platform for genetic transformation studies.

The normal extended structure of DNA is not conducive to either *transfection* (in animal or human cells) or *transformation* (in plant cells) processes. In this respect, different nanosystems were used to condense DNA molecules and help genetic material cross the cell membranes or cell walls in animal or plant systems, respectively. Nanoparticles, liposomes, micelles, nanotubes, and cationic polymers are some of these structures [14–16]. However, no nanocarriers have been designed to be used in both types of eukaryotic cells (animal and plant) for therapeutic and biotechnological purposes. In addition, transfection and/or transformation of animal or plant cells, respectively, is far from being fully optimized and increased efficiency is highly desirable. Consequently, the design and preparation of new vectors to carry genetic material for various purposes remain an important line of research.

Synthetic polymers are macromolecules consisting of repeating units, called mers, linked by covalent bonds through condensation or addition polymerization process [17]. The polymer conformation in solution depends on different properties such as the chain flexibility, the fraction of charged repeated units, the polymer concentration, the ionic strength of the medium and so on. According to the bibliography [18], polyplexes with small sizes and high zeta potential values show a better cellular uptake. Also, an enhance in the branching of polymers increases their gene transfection efficiency.

In the present work, a sugar-based cationic polyurethane derived from D-mannitol, PUMan, has been synthesized to be used as a nanocarrier of genetic material. The presence of disulfide bonds in its structure ensures its biodegradability as they are stable in extracellular conditions but cleaves inside cells [19] as the authors have demonstrated in previous works [20–22].

In this multidisciplinary study we have investigated the effect of PUMan on calf thymus DNA (ctDNA), the animal expression plasmid pEGFP-C1 and the microalga expression plasmid Phyco69. The ctDNA was used as a gene model to obtain preliminary data on the effect of the vector on the polynucleotide structure. Since ctDNA is not active in transfection processes, the plasmids pEGFP-C1 and Phyco69 were used to test the efficiency of this new cationic polymer, PUMan. The bacterial plasmid pEGFP-C1 was used for transfection experiments performed on the human cell line U2OS and the plasmid Phyco69 for transformation studies performed on the microalga Chlamydomonas reinhardtii [13].

There are many research studies regarding plasmid encapsulation efficiency. However, no mention of how the structural characteristics of the transfected plasmid may affect the encapsulation process. The results obtained here demonstrate the importance of a good structural knowledge of the plasmid to be transfected to achieve a polyplex with better condensation efficiency. The cellular strategy of DNA supercoiling is known to pack the gene information into a reduced nuclear space and, in addition, allows controlled regulation of gene expression [23]. Our results allowed us to establish a relationship between the supercoiling state of the gene material and its condensation process. The secondary structure of DNA and the state of condensation that the polynucleotide must acquire to cross the cell membrane and reach the cell nucleus, which in turn depends in some way on both the nucleotide sequence and the degree of supercoiling, appear to be related. The effect of this relationship was compared in the gene transfection process (in human cells) and in the transformation process (in plant cells).

2. Experimental section

2.1. Materials

Calf thymus DNA (ctDNA) and ethidium bromide were purchased from Sigma-Aldrich. The plasmids Phyco69 and pEGFP-C1 were supplied by PhycoGenetics S.L. and Clontech Biocentifica S.A., respectively. *Chlamydomonas reinhardtii* 704 strain (Cw15, Arg7, mt +) was kindly donated by Dr. Roland Loppes and cultured photomixotrophically in liquid or agar solidified tris-acetate phosphate (TAP) medium under continuous white light irradiation (50 μE m^{-2} s^{-1} PAR) at 25 °C in a culture chamber [24].

The concentrations of the polynucleotides were given by phosphate groups (molar mass of DNA = 618 g mol^{-1} per base pairs) and determined spectrophotometrically by using the known molar absorption data of 6600 mol^{-1}dm^{3}cm^{-1} at 260 nm [25]. The ratios in absorbance at 260 and 280 nm of the solutions were in the range 1.7–1.8, suggesting the absence of proteins [26]. Agarose gel electrophoresis test using ethidium bromide gave an average number of base pairs per DNA molecule of 10000 bp for ctDNA, 4731 bp for pEGFP-C1 and 4846 bp for Phyco69.

The solutions were prepared with distilled and deionized water obtained from a Millipore Milli-Q system, with a conductivity lower than 10^{-6} S m^{-1}. All measurements were performed at pH = 7.4 with a HEPES buffer (I=0.01 mol dm^{-3}) and temperature of 30.0 ± 0.1 °C was maintained.

Information on the experimental section corresponding to the methods and materials for the synthesis and characterization of the monomers and polyurethanes, preparation of samples, *in vitro* assays, as well as the working conditions for all the techniques used here, were included in the Supplementary Material.

2.2. Preparation of polyplexes

A polyplex is a complex formed by the mixture of a polymer and DNA molecules. A polynucleotide concentration of 3 × 10^{-5} mol dm^{-3} was used, while the concentration of polymer was varied depending on the N/P value required. The N/P parameter represents the ratio between the number of positively chargeable amine groups in the polymer (N, nitrogen) and the number of negatively charged phosphate groups (P) in the nucleic acid [27]. DNA [28] and PUMan (Scheme 2) charges are − 2 and + 4, respectively, at physiological pH.

Once the nucleic acid and polymer solutions were mixed, the solutions were incubated at 30 °C for 30 min

3. Results and discussion

3.1. Synthesis and characterization of polymer PUMan

Polyurethane PUMan was synthesized from D-mannitol as described in the Supplementary Material, following Schemes S1 (Synthesis of the monomers) and S2 (Synthesis of the polymers). The physical characterization by the usual techniques of all the synthesized organic compounds is included in the Supplementary Material (Sections S1.1 and S2.1; Figs. S1-S8, and Table 1).
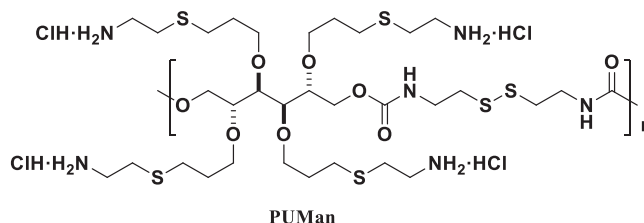
PUMan

3.2. Cytotoxicity and phytotoxicity

Cytotoxicity and phytotoxicity of PUMan were tested in different mammalian cell lines and in the microalga *C. reinhardtii*, respectively. Results are shown in Fig. 1.

PUMan shows a low toxicity at polyurethane concentrations lower than 9 μM for various mammalian cell lines (Fig. 1A). Interestingly, the polymer appears to be slightly less cytotoxic for the normal human cell line (RPE-1) than for the cancer lines in the concentration range 0–9 μM; and it continues being less toxic up to a [PUMan]= 75 μM, except the HepG2 line. Phytotoxicity assays (Fig. 1B) also showed a certain toxicity of PUMan at high concentrations, but it is practically innocuous at concentrations lower than 14.7 mg mL^{-1}. All measurements with cells and microalga were performed at sufficiently low concentrations of the polymer to avoid toxicity.

The characterization of the PUMan/DNA polyplexes (DNA: ctDNA, Phyco69 and pEGFP-C1) was carried out through a multidisciplinary study using techniques such as fluorescence spectroscopy, circular dichroism, zeta potential, and dynamic light scattering.

The attractive electrostatic interaction between the anionic phosphate groups of the DNA and the cationic polymers provokes the folding of the latter on the helix double surface [29]. This folding, and the neutralization of the DNA charges, results in the formation of complexes, called polyplexes, in which the nucleic acid is condensed in diverse structures such as toroidal and rod-like [30].

Next, results obtained for the different types of gene material (ctDNA, Phyco69 and pEGFP-C1) will be presented.

3.3. Formation of the polyplexes PUMan/ctDNA

ctDNA was used as a polynucleotide model to obtain preliminary information about the interaction between the polymer and the nucleic acids. Results demonstrated a conformational change in the structure of ct-DNA caused by the formation of polyplexes. However, the condensation of the polynucleotide is only partial, i.e. the polymer PUMan is not able to completely compact the ctDNA, probably as a consequence of the high pair base number it contains (approx. 10000 bp) [31].

A detailed discussion of the results obtained with ctDNA is given in the Supplementary Material.

3.4. Formation of the PUMan/pEGFP-C1 and PUMan/Phyco69 polyplexes and condensation process

Taking into account that ctDNA is not active for gene therapy processes, a similar multidisciplinary study was carried out with the polymer PUMan and the plasmids pEGFP-C1 and Phyco69.

The fluorescence intensity of EB molecules was measured in solutions containing PUMan/pEGFP or PUMan/Phyco69 polyplexes at different N/P values. The data collected in Fig. 2 indicate that approximately 70%, for pEGFP, and 100%, for Phyco69, of EB molecules are displaced from their intercalative binding mode into solution in the presence of PUMan. This dissimilar behavior of EB indicates that the polymer interacts with the two plasmids differently. Bearing in mind that these displacements of the EB molecules from their intercalative position between the plasmid base pairs have previously been related to conformational changes in the polynucleotide [26,31], the same deliberation can be considered here. That is, the lower variation of EB emission intensity observed in pEGFP-C1 is due to only partial conformational changes in this plasmid caused by the presence of PUMan, probably without affecting its helical state; whereas the higher fluorescence quenching obtained with Phyco69 is related to the higher conformational change undergone by this plasmid in the presence of the polymer.

Conformational changes in nucleic acids can be studied by circular dichroism, CD, spectra. The CD spectra of both plasmids were registered in the absence and presence of the polymer PUMan at different N/P values (Fig. 3). The spectra of pEGFP-C1 showed a shift of the bands towards higher wavelengths in the presence of the polymer, as well as a decrease in the positive band and an increase in the negative band (Fig. 3A). The bathochromic shift and the decrease in the positive band is due to a change in the secondary structure of the plasmid. As happened with ctDNA, the PUMan/plasmid polyplexes provoke a greater change in the base stacking than in the helicity of the double strand, although the behavior observed with respect to the helicity of the double-stranded DNA is different.

A greater modification in the circular dichroism spectra was observed for Phyco69 than for pEGFP (Fig. 3B). In fact, a complete loss of the secondary structure, related to a total condensation of the plasmid, is observed for the former. These results agree with those obtained from fluorescence measurements, which consider that PUMan causes a partial condensation of the human pEGFP-C1 plasmid and a total conformational change (compaction) in the structure of the Phyco69 plasmid.

The charge of the polyplexes is a key property that influences both its morphology and its structure. In fact, the neutralization of the polynucleotide charge favors its condensation. It is accepted that the condensation of a polynucleotide happens when 90% of the charge is neutralized [32]. In this regard, the zeta potential of the plasmid/PUMan polyplexes was measured (Fig. 4). As can be seen, the neutralization of pEGFP-C1 and Phyco69 takes place at N/P values around 8 and 12, respectively, being higher for Phyco69 than for pEGFP-C1. The similarity in both the base pairs (4731 bp in pEGFP-C1 and 4846 bp in Phyco69, obtained from electrophoresis gel with the linearized plasmids, Fig. S10) and the (G+C) percentage (52% bp in pEGFP-C1 and
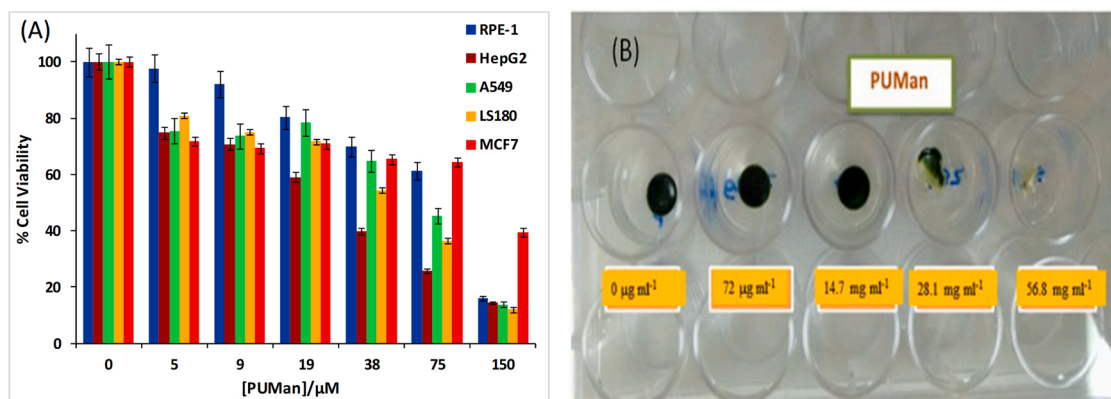

Fig. 1. (A) Viability of different cell lines at different polymer concentrations. (B) Degree of toxicity effect (phytotoxicity) produced by PUMan on the growth of *C. reinhardtii* at different polymer concentrations.
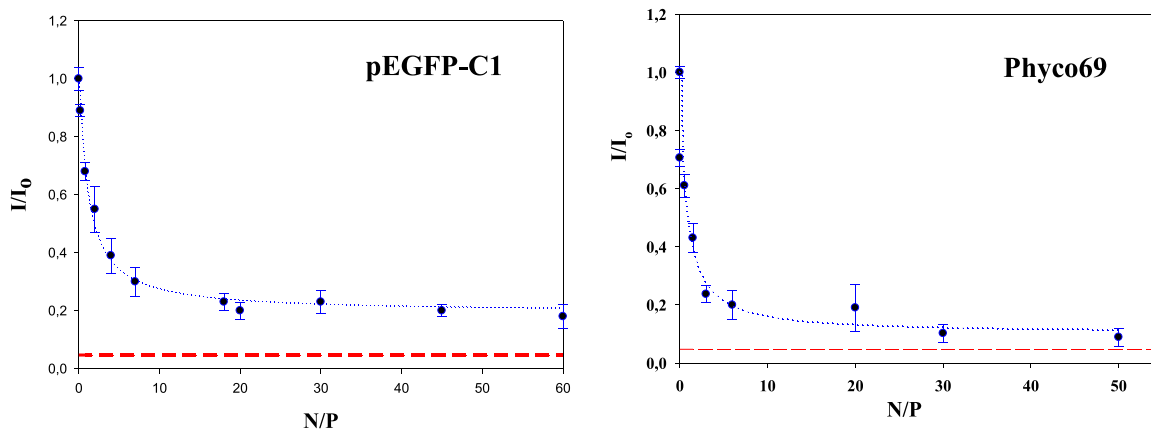
Fig. 2. Plots of the relative emission intensity of EB versus the N/P ratio for pEGFP-C1 and Phyco69. [DNA]$= 3 \times 10^{-5}$ mol dm^{-3} [EB]$= 2.4 \times 10^{-6}$ mol dm^{-3} HEPES 10 mmol dm^{-3}. The red line shows the emission intensity of EB in solution in the absence of plasmid.
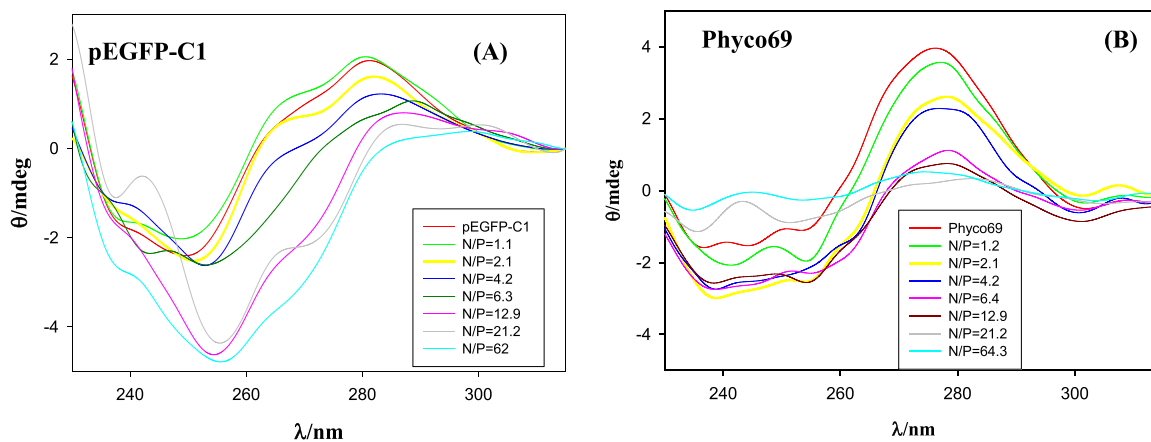

Fig. 3. Circular dichroism spectra of plasmid (pEGFP-C1 (A) and Phyco69 (B)) at different N/P ratio. [DNA]$= 3 \times 10^{-5}$ mol dm^{-3} HEPES 10 mmol dm^{-3}.
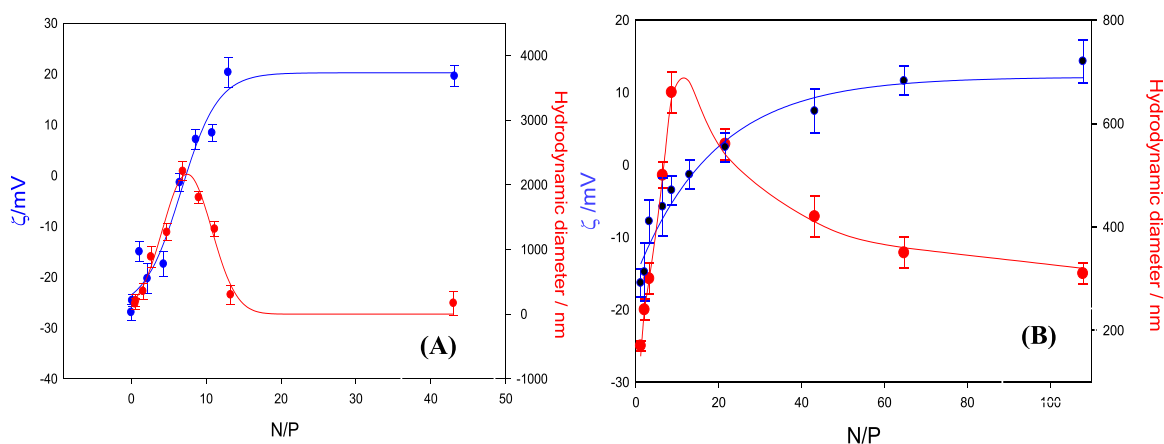

Fig. 4. Zeta potential and hydrodynamic diameters of the polyplexes PUMan/pEGFP-C1 (A) and PUMan/Phyco69 (B) at different N/P ratio. [DNA]$= 3 \times 10^{-5}$ mol dm^{-3} HEPES 10 mmol dm^{-3}.

53% in Phyco69) of the two plasmids does not explain this behavior.

Zeta potential in colloidal chemistry is defined as the electrical potential at the called slipping plane, that is, the interface which separates mobile fluid from fluid that remains attached to the surface. That is, zeta potential values could depend on the surface of the nanosystems. Bearing this in mind, the variation in the N/P value required to reach $\zeta = 0$ could be related to the presence of several supercoiled structures of the plasmids, as well as to the equilibrium between their relaxed and

supercoiled states. Indeed, a red shift was observed in the pEGFP-C1 CD spectrum (Fig. 3A), which was not seen for Phyco69. According to these results, polyplex formation occurs for the two plasmids investigated, but the interaction of PUMan with pEGFP-C1 and Phyco69 plasmids is different.

To demonstrate this hypothesis, electrophoresis assays were performed with pEGFP-C1 and Phyco69 plasmids in the absence and presence of PUMan (Fig. 5). As was previously mentioned, the plasmids
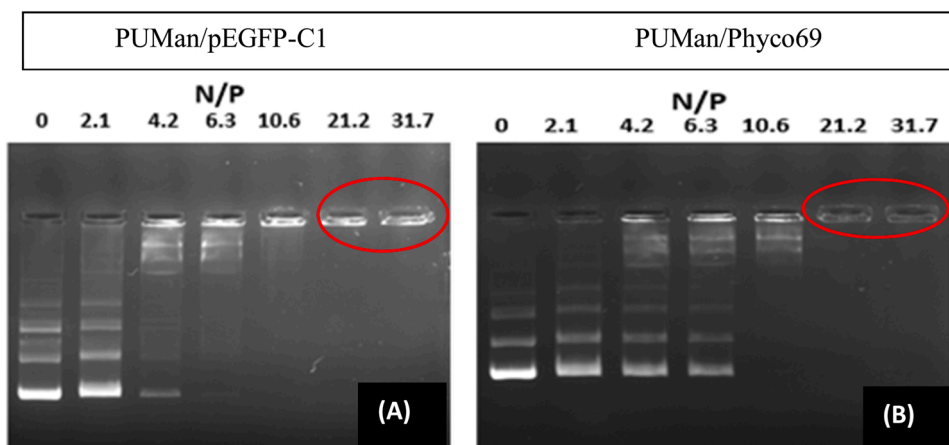
Fig. 5. Electrophoretic mobility of polyplexes PUMan/pEGFP-C1 and PUMan/Phyco69 polyplexes at different N/P ratios. All wells were loaded with the same quantity of plasmid, [Plasmid]= 60 ng/μL.

pEGFP-C1 and Phyco69 are circular double-stranded DNA molecules with 4731 and 4846 nucleotide pairs, respectively. Undigested plasmids can adopt different conformations Besides, a balance of supercoiled, open circular and linear forms with different apparent electrophoretic mobility is usually found in solution, appearing various bands in the agarose gel electrophoresis [33]. Electrophoretic mobility was found to decrease in the presence of the polymer for the two plasmids (Fig. 5). This effect starts to be visible at low N/P ratios. At high N/P values, the entire plasmid is inside the well and exhibits no mobility in the presence of an electric field. This observation is related to the neutralization and/or the charge inversion of the polyplex. The absence of movement in the band, and thus the neutralization of both plasmids, occurs at different N/P values for the two polyplexes: a clear movement of the band happens with the PUMan/Phyco69 polyplex at N/P = 10.6 (Fig. 5B), while no movement takes place for the PUMan/pEGFP-C1 polyplex at the same N/P value (Fig. 5A). Therefore, a higher amount of polymer (i.e., a higher N/P value) is needed to achieve neutralization of Phyco69, compared to pEGFP-C1. This behavior seems to indicate that the supercoiled structures of the two plasmids are different and that the structures of the various polyplexes formed may also be distinct.

It is worth remembering the decrease in the EB fluorescence emission observed in the presence of PUMan (Fig. 2). In fact, the degree of condensation acquired by the plasmids, due to the presence of the PUMan in the solution, causes the release of EB from the hydrophobic environment of the plasmid base pairs and a decrease in the fluorescence of these molecules. This effect, more acute in the case of the plasmid Phyco69 (Fig. 2), can also be related to the condensation degree of the native plasmid.

As can be seen in Fig. 5, no movement of the bands happens at N/P values of 21.2 and 31.7 for the two plasmids. However, comparing the wells of the electrophoretic assays for N/P > 20, fluorescence emission is observed for pEGFP-C1 plasmid (Fig. 5A), and no emission is obtained for the Phyco69 plasmid (Fig. 5B) for the same N/P values (see red circles). Considering that this fluorescence emission is due to EB molecules intercalated between the base pairs of the nucleic acid, this result confirms that some EB molecules remain inserted between the base pairs of the partial condensation of pEGFP-C1 plasmid. In contrast, all EB molecules are displaced from their intercalative position into the solution caused by the total condensation of Phyco69.

A similar behavior was observed for digested plasmids (see blue circles in Fig. S10). No movement of the bands was observed at N/P > 20. However, emission intensity is observed in the wells for pEGFP-C1 (Fig. S10A) and no emission is observed for Phyco69 (Fig. S10B). This demonstrates the partial condensation of pEGFP-C1 and the total condensation of Phyco69 in the presence of the PUMan.

Hydrodynamic diameters obtained and showed in Fig. 4 also

demonstrate the formation of PUMan/pEGFP-C1 and PUMan/Phyco69 polyplexes. The observed increase in size is due to the electrostatic interactions between the positively charged groups of the polymer and the negatively charged phosphate groups of the plasmids, that decreases when the N/P value augments. A maximum value in the diameter is obtained when the charge of the polynucleotide is neutralized, that is, when the zeta potential of the polyplexes is approximately zero and aggregation occurs. A further increase in N/P leads to a further increase in the polyplex charge, which prevents their aggregation and thus decreases the size of the complex. In general, the polyplexes formed are larger for pEGFP-C1 than for Phyco69 for similar N/P values, which may be related to the partial condensation of the former.

The technique of atomic force microscopy tapping mode is used to study biological samples through an intermittent tip-sample contact, building their topography [34,35]. AFM images were obtained to gain more precise information about the condensation of the pEGFP-C1 and Phyco69 plasmids in the presence of PUMan, visualizing the structures of the formed polyplexes. Fig. 6 collects such images at different N/P values. The images show the conformational change that the plasmids undergo in the presence of the polymer as the N/P ratio increases. The structures observed for the two plasmids appear to be different. In fact, the aggregates seem to be more compact for the Phyco69 plasmid than for the p-PEGFP-C1 plasmid (see blue arrows for a N/P value of 64.3 for both polyplexes in Fig. 6D and H). This confirms the total loss of the double strand helicity for Phyco69, previously observed from the CD spectra and, in contrast, the maintenance of helicity for the pEGFP-C1 plasmid in the presence of PUMan.

3.5. Gene transfection and transformation processes

There is a direct relation between the secondary structure of a nucleic acid and the condensation process it undergoes in the presence of some vectors. It depends, to some extent, on both the sequence of bases and the supercoiling of the DNA double strand.

Following the multidisciplinary study on the effect of the cationic sugar-based polyurethane PUMan on the pEGFP-C1 and Phyco69 plasmids performed herein, gene transfection assays with the mammalian expression plasmid pEGFP-C1 and transformation assays with the microalga expression plasmid Phyco69 were carried out.

Regarding gene transfection assays with pEGFP-C1, a very low percentage of the green fluorescent protein (GFP) was observed in the U2OS cell line for different N/P values (Fig.S11). Transfection was lower than 1% for all the N/P values studied (12 and 43), compared to the 32% obtained for the FuGENE 6 transfection reagent used as a positive control. To improve better these results, some assays were carried out in the presence of helpers such as DOPE or FuGENE (the mixtures PUMan +
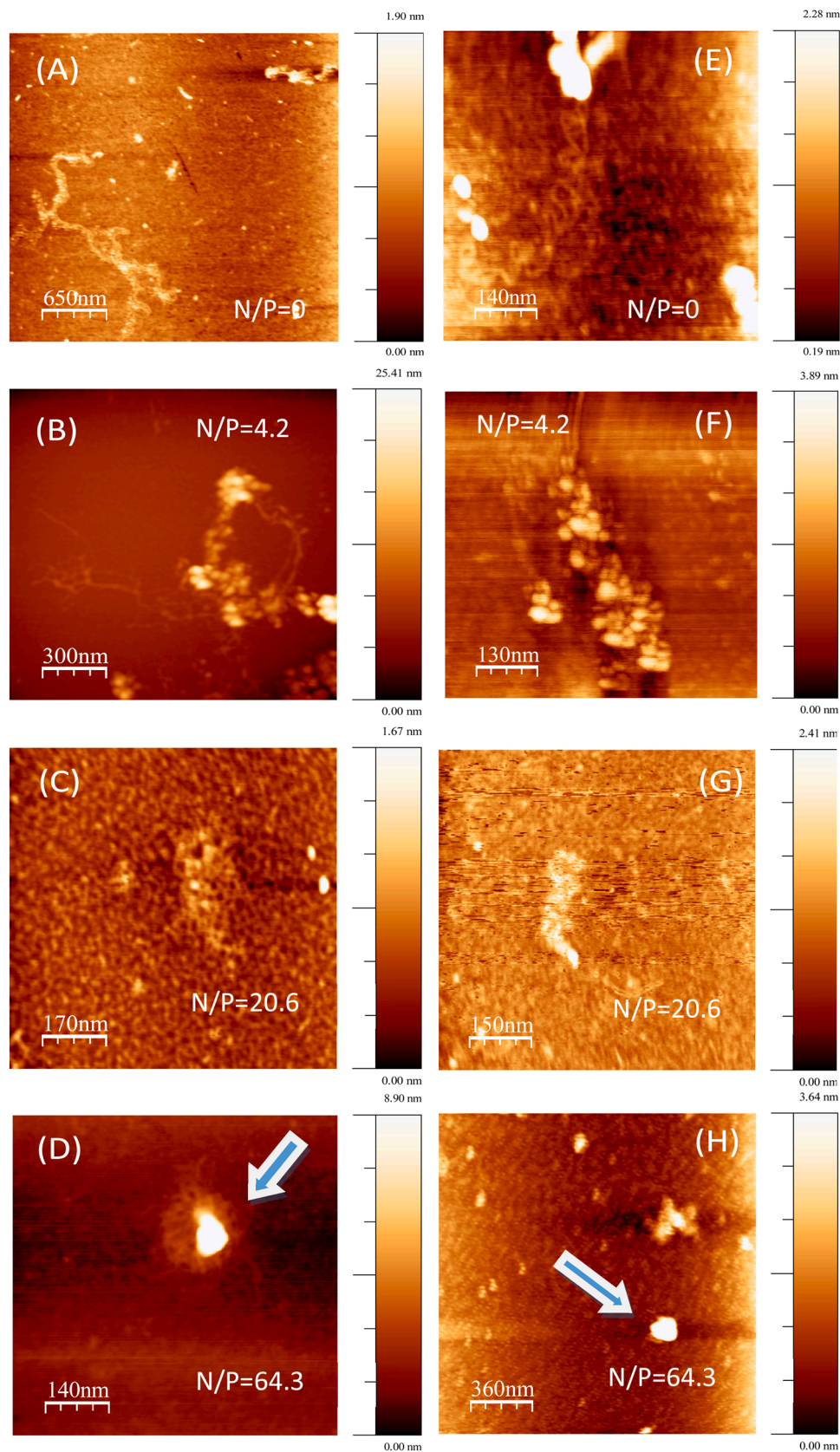
Fig. 6. AFM images of plasmids and PUMan/plasmid complexes at different N/P values. (A) Pure pEGFP-C1; (B) PUMan/pEGFP-C1 at N/P = 4.2; (C) PUMan/pEGFP-C1 at N/P = 20.6; (D) PUMan/pEGFP-C1 at N/P = 64.3; (E) Pure Phyco69; (F) PUMan/Phyco69 at N/P = 1.2; (G) PUMan/Phyco69 at N/P = 20.6; (H) PUMan/Phyco69 at N/P = 64.3. Blue arrows show the condensed polyplexes formed.

FuGENE + pEGFP-C1 and PUMan + DOPE + pEGFP-C1 were studied for PUMan:FuGENE and PUMan:DOPE molar ratios of 1:1). Results indicated that DOPE does not improve the transfection efficiency.

Although the mixture PUMan + FuGENE + pEGFP-C1 showed a transfection efficiency about 23%, this was lower than that obtained with FuGENE + pEGFP-C1 in the absence of the polymer. The formation of PUMan/plasmid complexes diminishes the efficiency achieved by FuGENE 6 as a transfection reagent.

The low transfection efficiency obtained with the plasmid pEGFP-C1 could be related to the partial condensation of the plasmid in the presence of PUMan. Even if the sizes of the PUMan/pEGFP-C1 polyplexes seem adequate to be used in transfection processes, the configuration of the plasmid may also play an important role in such assays.

It is interesting to compare these results with others obtained previously. A low transfection efficiency ($\sim$ 1%) was obtained with polyplexes consisting of a cationic polymer derived from L-arabinitol (PUArab) and the plasmid pEGFP-C1 [36]. In this case, the characterization study of these polyplexes showed only a partial condensation of the plasmid. In fact, the CD spectra of the plasmid showed a shift of the bands to longer wavelengths, without observing a complete loss of the secondary structure which corresponds to a total condensation of the plasmid. Furthermore, the emission intensity of EB molecules decreased when this polymer was added to the solution, but it did not reach the intensity value that the dye molecules emit in the solution; that is, only a partial movement of the EB molecules from their intercalative position between the plasmid base pairs to the solution happened in the presence of the polymer. This similarity in results confirms the influence of nucleic acid architecture on the transfection process. This is also agreed with results showed by Zhou et al. [18], who indicated that successful gene transfection critically depends on the DNA condensation.

A protocol of gene transformation was also designed and executed in the microalga *Chlamydomonas reinhardtii* at the N/P ratios of 10 and 17. These values correspond to polyplexes PUMan/Phyco69 with charges slightly negative and positive, respectively, according to the zeta potential values obtained. Results demonstrated a successful genetic transformation that increases with the N/P value, i.e. with the PUMan concentration. Therefore, the polymer PUMan can be used as a vector to form polyplexes that favors the transformation process (Fig. 7). The small number of transformed clones of *C. reinhardtii* are observed after transformation with the glass beads method in absence of PUMan, at N/P = 0. The number of transformants increases for higher N/P ratio, reaching around 58% of transformants at a N/P = 17 value. Images of the plates with the transforming colonies of *C. reinhardtii* after six days of incubation with light at 25 ºC are showed in Fig. 8.

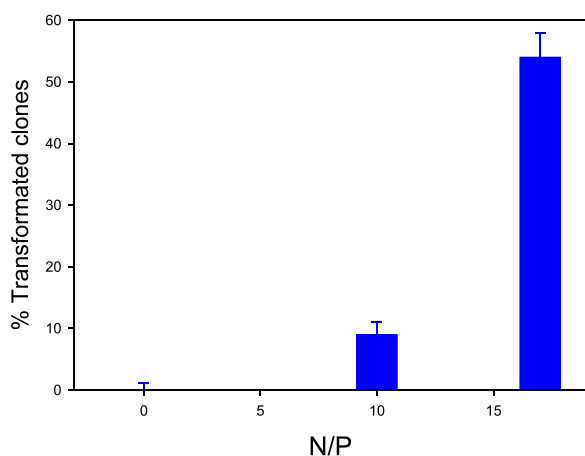as nanocarrier of Phyco69. Error bars represent standard deviations (n = 3).

Fig. 7. Number of transforming colonies of *C. reinhardtii* obtained using PUMan.

The different results obtained for the transfection and the transformation processes may be related to the condensation of the plasmids. pEGFP-C1 is partially condensed by the formation of polyplexes with the polyurethane polymer PUMan and a poor transfection efficiency was observed in U2OS cell line. On the contrary, the condensation of Phyco69 is total and good nuclear transformation efficiency was obtained in the microalgae *C. reinhardtii*. Accordingly, there is a relationship between the conformational change generated in the plasmid by a non-viral vector and the gene transfection (or transformation) efficiency generated by such a nanocarrier in gene delivery assays. Condensation of the polynucleotide must be complete for transfection (or transformation) to be successful and thus for gene delivery to be fruitful.

It should be note that the characteristics of each cell type can also influence the transfection/transformation processes. There are many similarities between animal cells and microalgae, but several differences are also found. For example, the size and shape of the cells are different; the presence of flagella, lysosomes, plastids or glyoxysomes in plant cells are absent in microalga cells; and so on. In addition, *C. reinhardtii* possesses a cell wall of carbohydrates and polypeptides [37] than is not found in animal cells. The latter could prevent the transformation process; however, this process is more favorable than the transfection process in animal cells with the use of the PUMan, as is demonstrated in this work. Therefore, although the cellular characteristics of animal cells and microalgae are different and may influence the tranfection/-transformation processes, plasmid condensation is an important factor to take into account.

4. Conclusions

Gene therapy is a treatment used to deliver genes into host cells, which is being applied in most branches of Medicine [38,39]. Naked DNA is not able to cross the cellular and nuclear barriers so that several methods have been used to facilitate such a transfection process [40]. To date, how to achieve maximum transfection efficiency remains a challenge. When non-viral vectors are used in this therapy, both the size and the superficial charge of the vector/plasmid complexes are important parameters influencing the transfection (or transformation) process. However, other factors such as plasmid bending and kinking or even plasmid composition must also be taken into account in their optimization.

A biocompatible polyurethane was synthesized to be used as a vector with therapeutic and biotechnological purposes. PUMan/pEGFP-C1 and PUMan/Phyco69 polyplexes were prepared and characterized thorough a multidisciplinary study. Results obtained showed a very low transfection efficiency with pEGFP-C1 and a successful transformation with Phyco69. Comparing the characterization results obtained for the polyplexes and their efficiency as vectors, a relationship between the conformational structure of the plasmids and the delivery of the plasmids into the cells was found. It was demonstrated that the conformation of the plasmids in the polyplexes plays an important role in the mechanism of gene delivery into cells.

To the authors' knowledge, this is the first time that the same polymeric vector has been used for transfection/transformation of both human and plant eukaryotic cells. No relationship has been previously established between transfection (or transformation) efficiency and the conformation of the nucleic acid in the polyplex. It has been demonstrated that a DNA total condensation is essential to get better transfection/transformation efficiencies. Further approaches to obtain new polyplexes derived from carbohydrate-based biodegradable polymers are under course.

CRediT authorship contribution statement

Conceptualization, supervision and project administration M.L.-L.; P. L.-C. and M.L.M.; methodology, D.P.; M.G. G.-M.; E.B.; M.L.-L-; P.H.; L. R.-A.; R. L.; P.L.-C. and M.L.M.; software, D.A.; and P.L.-C.; formal
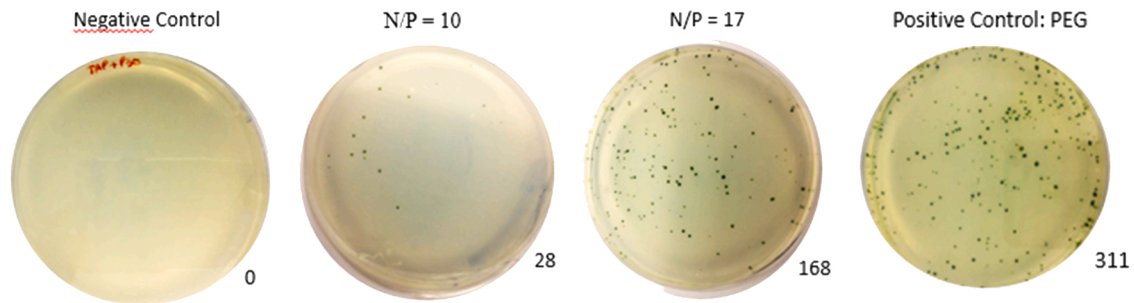
Fig. 8. Plates with the transforming colonies of *C. reinhardtii.* The number of colonies is equal to 0 for the negative control, equal to 28–corresponding to 9% of transformation clones- for a N/P value of 10, equal to 168 - corresponding to 54% of transformation clones- for a N/P value of 17, and equal to 311 for the positive control. PEG: poly(ethylene glycol) 8000 20%.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfb.2023.113219.

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