On the influence of polyethyleneimine modification in nanogel-driven drug delivery

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GRAPHICAL ABSTRACT

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ABSTRACT

Nanogels are nanometric three-dimensional structures formed by chemically or physically cross-linked polymers with hydrophilic or amphiphilic macromolecular chains. Biocompatibility, biodegradability, water solubility and nontoxicity are the properties of these colloids that make them very interesting to be used in biomedical fields and especially in drug delivery applications. Several strategies have been proposed for the preparation of nanogels capable to uptake and release a great variety of drug compounds; however, the encapsulation of hydrophobic compounds inside the structure of nanogels has been proven difficult. The goal of this work is to study how drug delivery performances can be influenced by the chemical modification of linear polyethyleneimine (PEI) structure and if the ability to load and release hydrophobic drugs from nanogels can be improved in this way. In vitro drug release tests were performed by using rhodamine B and pyrene as model hydrophilic and hydrophobic drug mimetics, respectively. The maintenance of an efficient drug release ability was confirmed for hydrophilic compounds (cumulative release up to 80 % in 120 h) like in our previous formulations, but an optimal loading and encapsulation efficiency, with a time-controlled release, emerged also for hydrophobic molecules, due to the introduction of the modifications in the nanogel framework.

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1. Introduction

Drug delivery is the term commonly used to refer to all the approaches, the formulations and the technologies employed to transport pharmaceutical active compounds to specific target site, achieving a therapeutic effect in the body tissues [1,2]. Various principles are related to the development of these devices, including biocompatibility, stability, safety and obviously delivery efficiency. Moreover, drug delivery systems have to deal with typical features of the drugs such as pharmacokinetics, with the final aim to improve them [3]. The field of drug delivery is strongly related to the concept of the dosage of the drug and its therapeutic range. In fact, conventional drug delivery systems exhibit many times rapid or uncontrolled release of drugs with subsequent under dosing or overdosing problems and therefore therapeutic inefficiency [4,5]. Because of this, in the last decades great efforts have been made in the investigation and in the development of novel formulations, like targeted controlled drug delivery systems able to localize the delivery of the drugs in their target site and avoid uncontrolled release [6]. These features are essential to improve the efficiency of these devices, limiting the side effects on other body districts.

Different approaches are described in literature to meet the needs of this therapeutic strategy; the nano systems are for sure among the most interesting devices because of their dimensions, that promote cellular internalization, and the breadth of solutions available [7–9]. In this context, nanogels (NGs) emerge as versatile biomaterials extremely effective in the field of targeted drug delivery [10,11]. They are commonly defined as three-dimensional colloidal hydrogel nanoparticles obtained through physical or chemical crosslinking between polymeric chains. Their formulation is carbon-based, characterized by high water content, great stability, biocompatibility, porosity, swelling behavior and obviously great ability in the loading and the release of drugs [12,13]. Moreover, nanogels attracted great interest in the cellular targeting field because of the possibility to surface functionalize their structure to modify their biological behavior [14,15].

This approach has been already discussed in previous works, in which the possibility to modify both the physical properties and the drug release profile of nanogels has been demonstrated working with polymers, but also cell membranes, proteins and antibodies [16–18]. Hydrophobic drugs impose the necessity to develop proper formulated drug carriers, to increase their solubility in aqueous media improving their pharmacokinetics and therefore their efficiency [19]. In the last two decades great efforts have been made in this direction, since great interest has arisen around this kind of molecules. In 2002 Couvreur et al. proposed the design of micro/nano capsules composed of aqueous pockets surrounded by a hydrophobic membrane [20]. These structures, categorized as liposomes or polymersomes depending on the nature of their shell, can encapsulate small hydrophobic molecules inside their internal pocket to achieve high loading efficiency [21,22]. Another valuable strategy that is used to perform the delivery of these molecules is the covalent conjugation that guarantees the formation of a stable bond, with enhanced stability and controllable drug loading, but with issues related to the toxicity of the solvents used and of the coupling agents [23,24]. Finally, another important strategy that plays a central role in the drug delivery of this kind of molecules is the physical adsorption, highly used with nanoparticles exploiting inter-molecules interactions, such as ionic interactions, H-bonds or Van der Waals forces [25,26]. The key point in this kind of procedure is to properly design the drug delivery systems, including all the acting factors that ensure good encapsulation of the active hydrophobic molecules. The high biocompatibility and the ease of use of this strategy made this approach one of the most advantageous for the design of hydrophobic drugs delivery systems [19].

In this work, in order to address this issue, we chemically modified the polymeric chains constituting our nanogels to enhance the physical adsorption of hydrophobic molecules to be loaded and released in a controlled system. In details, polyethylene glycol (PEG) - linear polyethyleneimine (PEI) nanogels were synthesized [27–29] introducing hydrophobic moieties on the PEI chains before the formation of the nanogel framework, through nucleophilic substitution reactions.

PEG-PEI nanogels were developed for the selective treatment of astrocytes in the central nervous system [30]. Indeed it is well known that different nanovectors are internalized into astrocytes, but none in a selective way for treating the astroglial pro-inflammatory response. Previous study demonstrated the selective efficacy of drug (rolipram) delivered by PEG-PEI NGs in limiting the pro-inflammatory response mediated by astrocyte activation in a mouse model of spinal cord injury [30]. The goal of this work is to study how drug delivery performances can be influenced by the chemical modification of PEI structure. In this direction, firstly we verified the successful chemical functionalization of the polymers and then the possibility to use these products in the synthesis of nanogels with PEG. Three different molecules, benzyl bromide, phenylacetyl chloride and 3-bromopropionic acid, have been selected as examples of both aromatic and aliphatic hydrophobic moieties, and in all cases, we were able to perform the functionalization of polyethyleneimine chains and the subsequent syntheses of nanogels. The physical characteristics of the final colloidal systems showed significant differences among the formulated devices in dimensions, drug loading and release ability, confirming the influence of the hydrophobic moieties introduced in the polymers. Similarly, in vitro drug delivery tests exhibit significant variations induced by the chemical functionalization [31]. Visible effects have been obtained working with pyrene as model hydrophobic drug, where improvement in the loading capacity and modulated release ability of the nanogels have been proved as expected, due to the introduction of the aromatic or aliphatic hydrophobic moieties in the system.

2. Materials and methods

2.1. Materials

Polymers: linear polyethyleneimine 2500 (PEI, \( M_w = 2.5 \text{ kDa} \)) by Polysciences Inc., (Warrington, USA) and polyethylene glycol 8000 (PEG, \( M_w = 8 \text{ kDa} \)), by Merck KGaA, (Darmstadt, Germany). All other chemicals were purchased from Merck (Merck KGaA, Darmstadt, Germany) and used as received, without any further purification. Solvents were of analytically grade purity.

2.2. Synthesis of PEI functionalized with benzyl bromide

The synthesis of PEI-benzyl bromide has been performed with the following procedure. First, linear PEI (300 mg, 0.12 mmol) was dissolved in MeOH (4 mL) and then benzyl bromide and Na\(_2\text{CO}_3\) were added to this solution while it was cooled with a bath of ice. Three different molar ratios between PEI and benzyl bromide have been independently tested (1:1, 1:5 and 1:12.5) to investigate the influence of this parameter on the final degree of functionalization of the polymers. The Na\(_2\text{CO}_3\) was added in each case with a ratio 2:1 respect to benzyl bromide to neutralize the system. The system was left under stirring in dark for 24 h at room temperature. The solution was then vacuum filtered to remove Na\(_2\text{CO}_3\) and MeOH was evaporated under reduced pressure. The obtained product was redissolved in water, purified through dialysis against water (\( M_w \) cut-off = 3.5 kDa) and lyophilized. The \(^1\text{H} \) NMR analysis was used to investigate the degree of functionalization of the modified PEI. This polymer from now on will be indicated with PEI-benz.

2.3. Synthesis of PEI functionalized with phenylacetyl chloride

The synthesis of PEI-phenylacetyl chloride has been performed with the following procedure. First, linear PEI (300 mg, 0.12 mmol) was dissolved in MeOH (4 mL) and then phenylacetyl chloride and Na\(_2\text{CO}_3\) were added to this solution while it was cooled with a bath of ice. Given
the results obtained in the PEI functionalization with benzyl bromide with the three different molar ratios, in this case we directly worked with 1:5 as ratio between PEI and phenylacetyl chloride.

The Na₂CO₃ was added with a ratio 2:1 respect to phenylacetyl chloride to neutralize the system. The system was left under stirring in dark for 24 h at room temperature. The solution was then vacuum filtered to remove Na₂CO₃ and MeOH was evaporated under reduced pressure. The obtained product was redissolved in water, purified through dialysis against water (Mₚ cut-off = 3.5 kDa) and lyophilized. The ¹H NMR analysis was used to investigate the degree of functionalization of the modified PEI. This polymer from now on will be indicated with PEI-phen.

2.4. Synthesis of PEI functionalized with 3-bromopropionic acid

The synthesis of PEI-3-bromopropionic acid has been performed with the following procedure. First, linear PEI (300 mg, 0.12 mmol) was dissolved in MeOH (4 mL) and then 3-bromopropionic acid and Na₂CO₃ were added to this solution while it was cooled with a bath of ice. Given the results obtained in the PEI functionalization with benzyl bromide with the three different molar ratios, in this case we directly worked with 1:5 as ratio between PEI and 3-bromopropionic acid. The Na₂CO₃ was added with a ratio 2:1 respect to 3-bromopropionic acid to neutralize the system. The system was left under stirring in dark for 24 h at room temperature. The solution was then vacuum filtered to remove Na₂CO₃ and MeOH was evaporated under reduced pressure. The obtained product was redissolved in water, purified through dialysis against water (Mₚ cut-off = 3.5 kDa) and lyophilized. The ¹H NMR analysis was used to investigate the degree of functionalization of the modified PEI. This polymer from now on will be indicated with PEI-prop.

2.5. Nanogels PEG-PEI synthesis

The synthesis of PEG–PEI nanogels (NGs) has been performed as described in our previous works. Briefly, the first step is the chemical functionalization of PEG hydroxyl group (2 g, 0.025 mmol) with carbonyldiimidazole (600 mg, 3.7 mmol) in acetonitrile, the product has been indicated with PEG-CDI. The synthesis of NGs has been performed with the following methodology.

Two different solutions were prepared: the PEG-CDI (200 mg, 0.025 mmol) was dissolved in CH₂Cl₂ (3 mL), while in another flask, the PEI-solution under stirring at room temperature and the final system was then sonicated for 30 min. The system was allowed to stir for 17 h at r.t. to promote the evaporation of CH₂Cl₂. The aqueous solution was then purified through dialysis against slight acid water (Mₚ cut-off = 3.5 kDa) and lyophilized. These nanogels, in accordance with the functionalized PEI employed, PEI-benz, PEI-phen and PEI-prop, will be respectively indicated with NGs-benz, NGs-phen and NGs-prop. The obtained nanogels were characterized with ¹H NMR and DLS analyses.

2.7. Characterization techniques

The chemical characterization of functionalized polymers and synthesized nanogels was performed using NMR analyses, carried out on a Bruker AC (400 MHz). The solvents employed were deuterium oxide (D₂O) or acenotirile-d₃ for the polymers, according to their solubility, and deuterium oxide for nanogels formulations. The physical characteristics of nanogels, hydrodynamic diameter, polydispersity index (PDI) and ζ-potential were evaluated using the Dynamic Light Scattering (DLS) technique, dissolving samples in phosphate-buffered saline solution (PBS). Morphological evaluations were performed with atomic force microscopy (AFM).

2.8. Cytocompatibility evaluation of nanogels

Mouse fibroblasts (L929) were cultured in complete medium (Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10 % fetal bovine serum, 1 % penicillin/streptomycin, 1 % L-glutamine 200 mM. L929 were seeded in 24-well plates at concentration of 50,000 cells/well in 1 mL complete medium and grown at 37 °C, 5 % CO₂. After 24 h, the medium was changed and NGs (0.05 % weight/volume) were then added to cell cultures for up to 3 days. After 3 days of culturing, the cytotoxicity of macrostructure was evaluated by performing an MTS assay. The absorbance was measured at 570 nm, and the results were compared with that of the control wells to determine relative cell viability.

2.9. Loading of nanogels with drug mimetics

Rhodamine B (RhB) and pyrene (Pyr) were chosen as, respectively, hydrophilic and hydrophobic drug mimetics in the drug loading and release tests [34–36]. These molecules have been selected because of their chemical-physical properties, with structures, LogP and molecular weights similar to many real drugs.

The loading of RhB in nanogels formulations has been performed in accordance with the procedures described in our previous works. Briefly, a RhB solution (1 mg/mL) was prepared dissolving the drug mimetic in distilled water. Then, the lyophilized NGs (NGs-benz, NGs-phen and NGs-prop) were independently suspended in an aqueous solution (20 mg/mL) and 1 mL of the RhB solution was added dropwise (1 mL/min) to 1 mL of each nanogels solutions under stirring. The system was left to stir in the dark at room temperature. Through this procedure the loading of RhB for the three different NGs has been performed. Similarly, in the second case, a drug mimetic solution was prepared dissolving the pyrene in dimethyl sulfoxide (5 mg/mL). The lyophilized NGs (NGs-benz, NGs-phen and NGs-prop) were independently suspended in dimethyl sulfoxide (20 mg/mL) and then the pyrene solution (50 μL) were added dropwise in 1 mL of nanogel solution under stirring and the system was stirred in the dark at room temperature for 17 h to promote the hydrophobic interaction between the molecules and the system.

\[
\text{Drug mimetic loaded} = \left( 1 - \frac{M \text{drug mimetic in cleaning water}}{M \text{drug mimetic added}} \right) \times 100
\]  

The loading efficiency ( % loading) was calculated through Eq. (1):

The aqueous solution was then purified through dialysis against slight acid water (Mₚ cut-off = 3.5 kDa) and lyophilized. These nanogels, in accordance with the functionalized PEI employed, PEI-benz, PEI-phen and PEI-prop, will be respectively indicated with NGs-benz, NGs-phen and NGs-prop. The obtained nanogels were characterized with ¹H NMR and DLS analyses.
2.10. In vitro drug release

The drug release mechanism was investigated using a phosphate-buffered saline solution (PBS) as release environment at pH 7.4 and 37°C. Each nanogel sample was put in large amount of PBS and, at defined time points, aliquots (3 × 100 μL) were collected to estimate the drug release. Each time the sample volume collected was replaced with fresh solution, to avoid mass-transfer equilibrium with the external release environment. In the case of RhB the aliquots were directly analyzed, while for Pyr they were lyophilized, dissolved in acetonitrile, and then analyzed [36].

The UV spectroscopy at fixed wavelength (λRhB = 570 nm, λPyr = 334 nm) was employed to estimate the release amount of drug mimetic and the percentage of released drug was calculated using the following Eq. (2), as the ratio between the total quantity of drug released in the external environment and the total amount initially loaded in the system:

\[
\text{Cumulative Release } \% = \left( \frac{\sum_{t=0}^{t_f} m_{drug} \text{ released}}{m_{drug} \text{ injected}} \right) \times 100
\]  

2.11. Statistical analysis

Experimental data were analyzed using Analysis of Variance (ANOVA). Statistical significance was set to p value < 0.05. The results are presented as mean value ± standard deviation.

3. Results and discussion

3.1. PEI Chemical functionalization with benzyl bromide

The PEI chemical functionalization with aromatic or aliphatic hydrophobic molecules was one of the key points of our work to introduce these moieties in the polymeric chain and therefore to modify the behavior of the synthesized nanogels. Benzyl bromide was the first molecule we analyzed, investigating the efficacy of the procedure to identify the best strategy of the work. The PEI functionalization took place because of a nucleophilic substitution in -NH sites of the polymer. The results of the functionalization were verified with 1H NMR spectroscopy and in Table 1 the degree of functionalization of the different molar ratios between PEI and benzyl bromide (BB) are reported.

The results shown in the table match with what we expected: the greater the ratio between benzyl bromide used and the polymer, the higher the percentage of functionalization obtained. Given these results, we decided to work with the polymer obtained with the ratio 1:5: in fact, the 20% of functionalization of the -NH sites of PEI is a satisfactory result that allowed us to introduce this hydrophobic group in the PEI chain, yet leaving a sufficient number of reactive -NH free sites to drive the crosslinking with PEG in the subsequent synthesis of the nanogel [37,38]. The 1H NMR spectrum for the PEI functionalization with benzyl bromide is reported in the Fig. S1 of the Supporting Information.

3.2. PEI Chemical functionalization with phenylacetyl chloride

As previously mentioned, given the results obtained in the PEI functionalization with benzyl bromide, we directly worked with 1:5 ratio between PEI and phenylacetyl chloride. We successfully functionalized the polymer also with this strategy, and the final grade of grafting was estimated around 18%. The 1H NMR spectrum for the PEI functionalization with phenylacetyl chloride is reported in the Fig. S2 the Supporting Information.

3.3. PEI Chemical functionalization with 3-bromopropionic acid

As explained above, also in this case we decided to directly work with 1:5 ratio between PEI and 3-bromopropionic acid. The functionalization was successful, and we obtained a grade of grafting estimated around 24%. The 1H NMR spectrum for the PEI functionalization with 3-bromopropionic acid is reported in the Fig. S3 Supporting Information.

3.4. Synthesis of PEG-functionalized PEI nanogels

The synthesis of nanogels was performed with the procedure described in the previous section, working with the different functionalized PEI. As we did with polymers, we chemically characterized the final nanogel framework through the 1H NMR spectrum and we verified their formation as reported in the Fig. S4 of the Supporting Information. This confirms again the functionalization of PEI and the successful strategy to design a nanogel framework containing the selected hydrophobic moieties. In the following Fig. 1 the three reaction schemes for the formation of the different nanogels frameworks are reported.

3.5. Nanogels physical characterization

The synthetized nanogels were physically characterized through dynamic light scattering (DLS) analyses. This investigation was performed using phosphate-buffered saline, pH 7.4, to assess the particle size distribution and the ζ-potential. The results are illustrated in Table 2.

The analyses confirm that all the samples have nanometric dimensions that enable potential cellular internalization. As it can be seen in Table 2, the diameters of NGs formed by functionalized PEI with benzyl bromide, phenylacetyl chloride, and 3-bromopropionic acid are different from the PEG-PEI NGs and the variations are related to the interactions between nanoparticles and the external medium.

Indeed, the size of the conjugated molecules and the interactions between molecules and PEI chains can affect the NGs dimensions in aqueous environment. Considering NGs-benz and NGs-phen, their bigger dimensions are probably due to their hydrophobic nature that influences the arrangement of the systems in water and their hydrodynamic diameters. On the other hand, the smaller moieties introduced in the NGs-prop are responsible for its smaller dimensions. This last formulation, due to synthetic procedure and the purification process, at the pH conditions of PBS present -COO- moieties which are exposed to the surface, and this explains the negative ζ-potential value. The positive ζ-potential values of NGs-benz and NGs-phen are instead due to the residual protonation of the system obtained during the functionalization of PEI and the NGs synthesis. A morphological evaluation can be observed with AFM analysis (Fig. 2a): all particles showed a spherical and smooth surface with sizes similar and partially comparable with the ones obtained with DLS. The slight variations are relative to the sample processing between DLS and AFM analyses that is different. In Fig. 2b the trend of the particle size versus time is presented and it is clear that, in all cases, the NGs diameter remains substantially unaltered. Indeed, only small changes are visible underlying the colloidal stability and absence of aggregation.

The same evolution is observable also for coated NGs, finally proving the stability of the added coating strategies. Cytocompatibility of decorated NGs was evaluated with MTS assay culturing L929 fibroblasts in vitro for 3 days. The concentration of NGs used is the same used in pharmacological treatments and in other biomedical studies [39]. The results, in Fig. 2c, clearly showed the absence of potentially toxic components in all the NGs tested with respect to the control group (100% in the graph).

Table 1

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Ratio PEI-BB</th>
<th>Degree of functionalization</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEI benz 12.5</td>
<td>1:12.5</td>
<td>50%</td>
</tr>
<tr>
<td>PEI benz 5</td>
<td>1:5</td>
<td>20%</td>
</tr>
<tr>
<td>PEI benz 1</td>
<td>1:1</td>
<td>5%</td>
</tr>
</tbody>
</table>
3.6. Drug loading and release profiles

The drug loading and release properties of synthesized nanogels were evaluated using two different drug mimetics commonly used in the literature \cite{28,40}. The rhodamine B (RhB) is used as a hydrophilic and pyrene as a hydrophobic drug mimic. In Table 3, the loading percentages in nanogel structures obtained working with RhB and pyrene are reported.

The obtained results showed that the loading percentage of RhB in side PEG-PEI nanogels is slightly higher than the nanogels formed with functionalized PEI. Therefore, we can assess that the functionalization of PEI with these molecules has little effects on the RhB loading capacity of the nanogels probably due to the higher hindrance of the modified PEI chains. Instead, the hydrophobic interactions with the pyrene, expressed as its loading percentage, remains high for all the typologies of modified nanogels, and this can be attributed either to the NGs capacity to establish strong interactions with hydrophobic molecules and to the lower affinity of pyrene to the external water environment respect to the polymeric framework. In particular, the NGs obtained with functionalized PEI with hydrophobic moieties guarantee a higher degree of loading compared with the conventional PEG-PEI NGs. This is in accordance with what we expected: the PEI functionalization with these molecules makes them more hydrophobic and this ensures better encapsulation of hydrophobic drugs such as pyrene inside their framework.

Once the drug loading capacity of nanogels had been evaluated, the drug release of mimetic drugs was investigated through UV-spectroscopy. In Fig. 3, the release profiles of the four different nanogels loaded with rhodamine B as drug mimetics are represented as the ratio between the released amount in the external medium and the amount effectively loaded inside different formulations of NGs. The release profiles of the different nanogels formulations loaded with rhodamine B are reported in the following Fig. 3.

In all cases, the drug release presented a biphasic pattern with an initial burst release followed by a sustained release. As it is shown in Fig. 3 A), it is possible to notice that the NGs-blank present the most sustained release in time with the higher values of cumulative release reached. Similarly, also NGs-phen present very high values of total drug released, and its profile seems not to be affected by the polymer functionalization of the framework. On the other hand, NGs-benz and NGs-prop present a flatter profile probably due to the influence of the molecules used to functionalize the framework. For example, considering the NGs-prop, the afore-mentioned presence of \(-\text{COO}^\cdot\) moieties can have a beneficial role in the encapsulation of rhodamine and so the release is limited.

The influence of the systems in delivering RhB was studied plotting...
the release percentage against the time to the power of 0.43 ($t^{1/2.3}$ in Fig. 3 B)). As reported in literature [41], this mathematical model is representative of Fickian diffusion, and the y-axis intercept value is an indication of the percentage of burst release: an ideal controlled drug delivery system should present linear trend and the y-axis intercept equal to zero. All the trends for the synthetized nanogels are linear and in the case of NGs-benz and NGs-prop a reduced linear interval, with lower y-axis intercept, is observed, confirming the benefits on this feature of this functionalization strategy. On the other hand, in the following Fig. 4 the release profiles of the different nanogels formulations loaded with pyrene are reported.

Considering the plots, it is evident how the pyrene releases in the external aqueous environment exhibit very low rate due to its hydrophobic nature. For this drug mimetic NGs-blank presents the higher release rate, while the nanogels synthetized with the functionalized PEI present lower patterns because of the best encapsulation and interactions with pyrene that is guaranteed by the frameworks of the nanogels. This is a pivotal point: the selected functionalization for PEI are able to ensure to the whole final framework higher hydrophobicity thanks to the hydrophobic moieties introduced.

This results in best encapsulation of hydrophobic drugs and controlled and reduced release in water environment that, for functionalized nanogels, reaches an initial plateau in the first 36–48 h. Also, in this case the influence of the systems in delivering pyrene was studied plotting the release percentage against the time to the power of 0.43 ($t^{1/2.3}$ in Fig. 4 B). The trends of the plots are linear, and they all present very flat profiles related to very reduced burst release. This is an additional validation of the beneficial effects of those functionalizations in the drug release rate from nanogels.

**Table 3**

<table>
<thead>
<tr>
<th>Nanogel</th>
<th>RhB Loading (%)</th>
<th>Pyrene Loading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGs-blank</td>
<td>88</td>
<td>90</td>
</tr>
<tr>
<td>NGs-benz</td>
<td>83</td>
<td>98</td>
</tr>
<tr>
<td>NGs-phen</td>
<td>72</td>
<td>97</td>
</tr>
<tr>
<td>NGs-prop</td>
<td>77</td>
<td>99</td>
</tr>
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</table>

Fig. 2. A) AFM images of the four different nanogels formulation. B) Particle size ($n = 3$) versus time of NGs blank (blue dots), NGs benz (orange dots), NGs phen (grey dots) and NGs prop (yellow dots). C) Fibroblast viability after incubation for 3 days in the presence of nanogels. The columns represent the mean ± S.D.; $n = 3$.

Fig. 3. A) In vitro drug release profiles of RhB at pH = 7.4 from NGs-blank (blue), NGs-benz (orange), NGs-phen (grey), NGs-prop (yellow). B) The slope of the RhB release against the variable time expressed at $t^{1/2.3}$ is representative of the Fickian diffusion coefficient of the drug in NGs. The values are calculated as a percentage with respect to the total mass loaded (mean value ± standard deviation is plotted).
In this research we proposed the chemical functionalization of polyethylenimine with three different hydrophobic molecules. Those chains were then employed in the synthesis of PEG-PEI NGs structures, the final features of the framework were characterized, and their drug loading and release ability were investigated. We observed the efficacy the final features of the framework were characterized, and their drug loading and release ability of hydrophilic drugs, thanks to the hydrophobic interactions, as well as controlled release compared to the classical PEG-PEI NGs formulations, without affecting the drug loading and release ability of hydrophilic molecules. These aspects can be pivotal in the encapsulation and controlled delivery of hydrophobic drugs which many times exhibit difficulties in their management. Further developments will certainly be focused on the study of new functionalizations to obtain drug carriers with desired behavior.

CRediT authorship contribution statement

Filippo Pinelli: Methodology, Validation, Writing – original draft. Marjan Saadati: Methodology. Arianna Rossetti: Methodology, Validation. Filippo Rossi: Conceptualization, Supervision, Writing – review & editing. Alessandro Sacchetti: Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Filippo rossi reports financial support was provided by Polytechnic of Milan. Filippo rossi reports a relationship with Polytechnic of Milan that includes: employment.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfa.2022.130623.

References


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