Soft Matter

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Cite this: *Soft Matter*, 2013, **9**, 4297

Interpolyelectrolyte complexes based on hyaluronic acid-*block*-poly(ethylene glycol) and poly-L-lysine†

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Received 5th November 2012
Accepted 1st February 2013

DOI: 10.1039/c3sm27549c

www.rsc.org/softmatter

The preparation of spherical, nanosized interpolyelectrolyte complexes by the interaction of hyaluronic acid-*block*-poly(ethylene glycol) (HA-*b*-PEG) with poly-L-lysine (PLL) at a stoichiometric charge-to-charge ratio is described. The complexation was studied by dynamic light scattering and cryogenic transmission electron microscopy for HA-*b*-PEG with different lengths of the HA and PEG blocks. A minimal molecular weight of the HA block (*ca.* 9 kDa) is necessary for efficient complexation, while a minimal molecular weight of the PEG block (*ca.* 5 kDa) is needed to prevent macroscopic aggregation. The formed nanoassemblies have hydrodynamic radii ranging from 45 to 150 nm with very low dispersity indices (D.I. = 0.02–0.05). They appear as soft objects with a nanogel structure and pronouncedly swell on addition of NaCl. The increasing ionic strength leads to disruption of the complexes above *ca.* 120–160 mM of NaCl. Decreasing the ionic strength by dialysis reforms the nanogels, demonstrating the reversibility of nanogel formation. Crosslinking with carbodiimide leads to nanoassemblies with a very well defined structure and high stability against ionic strength.

Introduction

Polysaccharides have extraordinary properties as micro- and nanocarriers for drug delivery as they are renewable, inexpensive, biocompatible and biodegradable.[1] The main strategies to form such structures include chemical crosslinking, self-assembly of hydrophobically modified polysaccharides, and formation of interpolyelectrolyte complexes (IPECs) *via* the interaction of polysaccharides (such as chitosan and hyaluronic acid (HA)) with oppositely charged biopharmaceuticals (such as DNA and proteins).[2] To avoid precipitation and provide water solubility (or at least colloidal stability), IPECs need to bear excess charge.[3] Unfortunately, this charge hampers their circulation in the bloodstream, thereby considerably complicating drug delivery applications.

The formation of IPECs from polysaccharide-*block*-poly(ethylene glycol) (polysaccharide-*b*-PEG) is a straightforward and elegant solution to this problem, due to the well-known stealth effect of PEG. Some examples of IPECs of PEG-grafted chitosan and DNA or peptides can be found.[4–7] It is well known that the use of ionic block copolymers allows one to realize a much greater and in many cases an extraordinary control over the structure of the formed macromolecular coassemblies.[8,9] Indeed, interpolyelectrolyte complexation in such systems leads, for example, to micellar IPECs, even to those with a multicompartment structure.[10]

The absence of IPECs based on polysaccharide-*b*-PEG in the literature is only explained by the difficulties in the synthesis of this family of block copolymers.[11] At the same time, a few examples can be found on interpolyelectrolyte complexation of chitosan with oppositely charged PEG-containing diblock copolymers, such as PEG-*b*-poly(acrylic acid),[12] PEG-*b*-poly(methacrylic acid),[13] or PEG-*b*-poly[sodium 2-(acrylamido)-2-methylpropanesulfonate].[14]

In this paper, we describe, for the first time, the opposite situation: the PEG block is attached to the polysaccharide, *viz.*, to HA. A combination of this bis-hydrophilic diblock copolymer with an oppositely charged polyion is analogous to systems comprising polypeptide-*b*-PEG copolymers, for which the extremely successful formation of micellar IPECs (also referred to as PIC micelles) was found. Furthermore, the application of various polypeptide-*b*-PEG copolymers as drug delivery nanocarriers (DNA, proteins, or charged anticancer drugs) has recently reached phase III clinical trials.[15]

In addition to the biocompatibility and natural origin of polysaccharides, the huge number of reactive groups in the polysaccharide chains (–OH, $–NH_2$, $–CO_2$) opens the opportunity to easily attach drugs, or fluorescent tags, or to crosslink the core of the formed nanoassemblies.

Another idea challenged us to start the present investigation: a polysaccharide-*b*-PEG copolymer is expected to introduce a large number of hydroxyl groups inside the formed IPECs,

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3sm27549c

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thereby imparting them with a rather hydrophilic character. The much stronger hydrophilic character of such IPECs should induce important differences in their properties and structure, when compared to polysaccharide-free IPECs based on polypeptide-*b*-PEG, apparently leading to the formation of highly swollen IPEC nanogels instead of well documented micellar IPECs.[10,16–18]

Nanogels are gels confined to nanometric sized particulate entities. In the case of hydrogels, the particles are porous and contain a rather high content of water. Since the first publications in the late 90s,[19] this relatively novel family of nanoscale materials has shown promising properties for the delivery of drugs, biomacromolecules, and imaging agents. The main preparation methods of nanogels are self-assembly (driven by hydrophobic or electrostatic interactions), polymerization of monomers in a nanoscale environment, crosslinking of preformed polymers, and template-assisted nanofabrication. In general, higher drug-loading capacities can be expected for nanogels than for micelles because the high water content provides a larger cargo space for the incorporation of solutes. This is especially significant when the loaded compound is a biomacromolecule.[20]

The most commonly described methods for the preparation of polysaccharide-based nanogels are chemical crosslinking and self-assembly,[21] but template-assisted nanofabrication has also been described for chitosan.[14] A few examples of HA nanogels prepared either by hydrophobic modification[22–24] or by chemical crosslinking[25] have been described. To the best of our knowledge, no examples of nanogels prepared from polysaccharide-containing diblock copolymers can be found in the literature.

Herein, we prepared and characterized IPECs based on hyaluronic acid-*block*-poly(ethylene glycol) (HA-*b*-PEG). The use of HA is of particular interest because it can complex with positively charged proteins.[3] The resulting nanoassemblies will bear a PEG stealth shell and have a core containing the polypeptide and the polysaccharide. Specifically, we studied the interaction of HA-*b*-PEG with poly-L-lysine (PLL). PLL was selected as a model polypeptide and also because it has been widely applied for interpolyelectrolyte complexation.[15–17,26] Moreover, the formation of layer-by-layer assemblies between PLL and HA has also been described.[27]

Materials and methods

Materials

HA-*b*-PEG diblock copolymers were prepared as described by us previously.[28] The polymers used in this study and their molecular characteristics are included in Table 1.

α-Poly-L-lysine (M_w 9.2 kDa, PDI 1.12) was purchased from Sigma-Aldrich. The molecular weight and PDI of poly-L-lysine (PLL) were measured by GPC-MALS (see below). All other reagents were purchased from Sigma-Aldrich.

Methods

GPC multiangle light scattering (GPC-MALS). GPC multiangle light scattering (GPC-MALS) measurements were performed on a set of three columns kept at 35 °C: PSS suprema precolumn (10 μm, 8 × 50 mm), PL-aquagel–OH mixed (8 μm, 8 × 300 mm) and PL-aquagel–OH-30 (8 μm, 8 × 300 mm), with refractive index detection. GPC was measured at an elution rate of 1 mL min^{-1}. The columns were coupled to a Wyatt HELEOS MALS multiangle light scattering detector (kept at 25 °C) operating at 632 nm to determine the absolute molecular weights. 0.1 M NaN_3–0.01 M NaH_2PO_4 (pH 2.5) was used as an eluent at a flow rate of 1.0 mL min^{-1}. The refractive index increment of the PLL in the same eluent at 25 °C was measured using a PSS DnDc-2010/620 differential refractometer, $dn/dc = 0.18$ mL g^{-1}.

Dynamic light scattering (DLS). Dynamic light scattering (DLS) measurements were performed in sealed cylindrical scattering cells ($d = 10$ mm) at an angle of 90° on ALV DLS/SLS-SP 5022F equipment consisting of an ALV-SP 125 laser goniometer with an ALV 5000/E correlator and a He–Ne laser with a wavelength of 632.8 nm. Prior to the light scattering measurements the sample solutions were filtered using Millipore syringe filters with a pore size of 1.2 (nylon) or 0.2 μm (polyethersulfone). The CONTIN algorithm was applied to obtain the size distribution (intensity weighted). The average hydrodynamic radius and dispersity index were determined by fitting the correlation function using the cumulant method.

Cryogenic transmission electron microscopy (cryo-TEM). A sample droplet of 2 μL was put on a lacey carbon film coated copper grid (Science Services, München), which had been hydrophilized by air plasma glow discharge (Solarus 950, Gatan, München, Germany) for 30 s. Subsequently, most of the liquid was removed with blotting paper leaving a thin film stretched over the lace holes. The specimens were instantly shock vitrified by rapid immersion into liquid ethane cooled to approximately 90 K by liquid nitrogen in a temperature-controlled freezing unit (Zeiss Cryobox, Zeiss NTS GmbH, Oberkochen, Germany). The temperature was monitored and kept constant in the chamber during all the sample preparation steps. After freezing the specimens, the remaining ethane was removed using blotting paper. The specimen was inserted into a cryotransfer holder (CT3500, Gatan, München, Germany) and transferred to a Zeiss EM922 EFTEM (Zeiss NTS GmbH, Oberkochen, Germany). Examinations were carried out at temperatures around 90 K. The TEM was operated at an acceleration voltage of 200 kV. Zero-loss filtered images ($\Delta E = 0$ eV) were taken under reduced dose conditions (100–1000 e nm^{-2}). All images were registered digitally by a bottom mounted CCD camera system (Ultrascan 1000, Gatan, München, Germany) and then combined and processed with a digital imaging processing system (Digital Micrograph GMS 1.8, Gatan, München, Germany).

IPEC formation. Solutions of HA-*b*-PEG and PLL were prepared in a phosphate buffer solution (10 mM $NaHPO_4$, 2.8 mM HCl, pH 7.4, ionic strength (I) = 20 mM). IPEC complexes were prepared by adding a solution of PLL (0.65–0.8 g L^{-1}) to the solution of HA-*b*-PEG (1–1.5 g L^{-1}) under vigorous stirring. The volumes and concentrations were selected to obtain a stoichiometric amount of amino and carboxylic acid groups with a total polymer concentration of *ca.* 1 g L^{-1}. Typically, the batch volume was 3–4 mL of IPEC solution.

Table 1 HA-*b*-PEG prepared by oxime click reaction

HA M_n[a], kDa (DP_n, PDI)[a]	MeO-PEG-ONH_2 M_n[b], kDa	HA-*b*-PEG M_n[c], kDa (PDI)[d]
6.0 (30, 1.23)	2.0	8.0 (1.18)
	5.2	11.2 (1.20)
9.3 (47, 1.28)	2.0	11.3 (1.15)
	5.2	14.5 (1.24)
54.3 (269, 1.22)	2.0	56.3 (1.20)
	5.2	59.5 (1.40)

[a] DP_n (number-average degree of polymerization) and PDI (polydispersity index) determined by GPC-MALS. [b] Determined by MALDI-TOF MS. [c] Determined by NMR. [d] Determined by GPC with dextran standards.

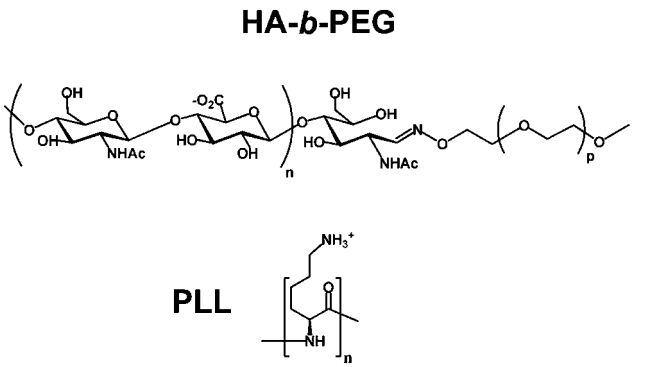

Fig. 1 Chemical structures of HA-*b*-PEG and PLL.

Crosslinking of IPECs. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, 25 equivalents with respect to amino groups or carboxylic acid groups) and *N*-hydroxysuccinimide (NHS, 6 equivalents) were added to the suspension of the IPECs under vigorous stirring. After 24 h the samples were filtered through 0.22 μm membrane filters. Crosslinking was performed 1 week after IPEC formation.

Turbidimetric titration of mixtures of HA and PLL homopolymers. The interpolyelectrolyte interaction between HA and PLL was studied by turbidity measurements using a titrator (Titrando 809, Metrohm, Herisau, Switzerland) equipped with a turbidity sensor ($\lambda = 523$ nm, Spectrosense, Metrohm). HA (6 kDa, 9 kDa and 54 kDa) and PLL were dissolved in the same buffer used for IPEC formation (10 mM $NaHPO_4$, 2.8 mM HCl, pH 7.4, $I = 20$ mM). The solutions were mixed under vigorous stirring and macroscopic precipitation was immediately observed. Polymer concentrations were selected to a stoichiometric ratio of amino and carboxylic acid groups and a total polymer concentration of *ca.* 1 g L^{-1} in the mixture. The suspensions were stirred for 1 hour and then transferred to the titrator stirring cell. The change in turbidity was recorded upon stepwise addition of 1 μL of 4 M NaCl (in the same phosphate buffer) every 30 seconds.

Results and discussion

Formation of the IPECs

IPECs were prepared by adding a solution of α-poly-L-lysine ($M_n = 9.2$ kDa, $DP_n = 65$, PDI = 1.12, as determined by GPC-MALS) to a solution of HA-*b*-PEG in 10 mM phosphate buffer at pH 7.4 at a 1 : 1 ratio between amounts of amino and carboxylic acid groups under vigorous stirring (Fig. 1). The total polymer concentration was kept at *ca.* 1 g L^{-1}. Instant formation of a milky or opalescent solution (depending on the M_n of the HA and PEG blocks) indicated interpolyelectrolyte complexation.

The samples were analysed by dynamic light scattering (DLS). IPECs based on copolymers containing PEG of 2 kDa precipitated or generated particles with very high dispersity indices (D.I. ~ 0.3–0.4) and relatively large hydrodynamic radii (500–600 nm). In addition, an important loss of mass occurred during filtration even through 1.2 μm membrane filters, as observed by the decrease in the count rate. In contrast, IPECs based on copolymers containing PEG of 5 kDa formed nanosized species with very low dispersity indices (Fig. 2 and Table 2). In this case, no mass loss was observed when filtered even through a 0.22 μm membrane filter. In fact, the prepared IPECs remain colloidally stable and do not significantly change their characteristics for at least 4 months at both 1 and 0.1 g L^{-1} (Tables S1 and S2†).

Mixtures of HA (9 and 54 kDa) and PLL homopolymers at a 1 : 1 ratio between amounts of amino and carboxylic acid groups were prepared for comparison purposes. In this case

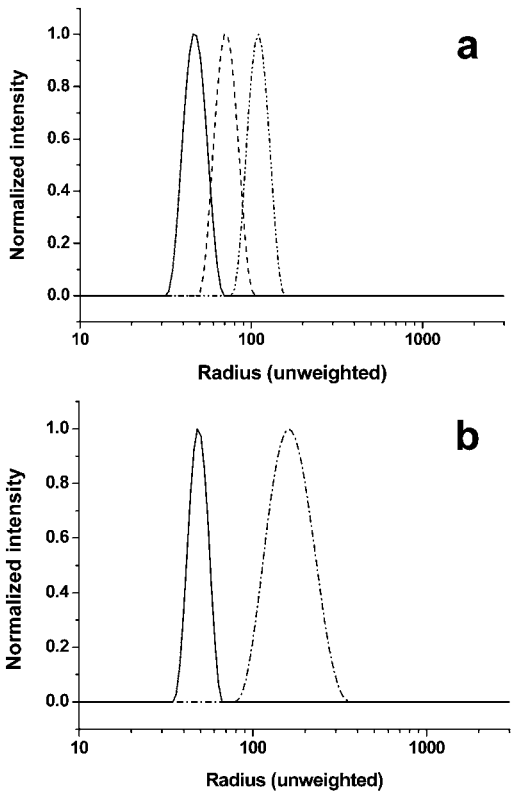

Fig. 2 Hydrodynamic size distributions of the formed IPECs (with PEG of 5 kDa) and PLL obtained by DLS ((a) after mixing and (b) after disruption with NaCl followed by dialysis to remove the salt). Dashed, solid, and dash-dotted lines correspond to HA with 6, 9 and 54 kDa, respectively.

Table 2 Hydrodynamic radii and dispersity indices of the IPECs prepared from HA-*b*-PEG (M_n PEG = 5 kDa) and PLL as determined by DLS

	$\langle R_h \rangle_z^a$(D.I.)		
HA M_n (kDa), DP_n	After direct mixing of HA-*b*-PEG and PLL	After disruption of IPECs with NaCl, followed by dialysis	After crosslinking of IPECs
6.0, 30	85 ± 12 (0.08 ± 0.04)[a]	—	—
9.3, 47	46 ± 1 (0.02 ± 0.006)[a]	48 (0.03)	55 (0.03)
54.3, 269	111 ± 4 (0.04 ± 0.005)[a]	176 (0.08)	145 (0.03)[b]

[a] Mean values and standard deviations derived from 5 different IPEC batches. [b] The radius of the IPECs before crosslinking was 122 nm.

also, the sample became turbid but turbidity steeply decreased after mixing as a consequence of pronounced precipitation. The size of the remaining particles in solution after 24 h was measured. Their hydrodynamic radius (1.5–1.7 μm) was found to be much larger than that in the case of the copolymers.

It is obvious from the obtained results that the presence of PEG of 5 kDa in the copolymer prevents the aggregation of the formed nanoassemblies. Colloidal stability of micellar IPECs with PEG coronas has been shown previously for many synthetic bis-hydrophilic block copolymers.[9,10,18,29,30]

IPECs formed from the copolymers HA_{6k}-*b*-PEG_{5k} present larger hydrodynamic radii and higher dispersity indices than IPECs formed from copolymers HA_{9k}-*b*-PEG_{5k}. In contrast, the particle size increases if HA_{9k} is changed to HA_{54k}. In addition, a much higher standard deviation of the hydrodynamic radius and D.I. is observed for HA_{6k}. In fact, the standard deviation of the hydrodynamic radii for the IPECs comprising HA_{6k} corresponds to 12% of the total radius while only to 2–3% of the total radius in the case of HA_{9k} and HA_{54k}. We believe that this difference comes from the weaker interpolyelectrolyte interaction between HA_{6k} (with only 15 carboxylic acid groups) and PLL when compared to the other two HA. In this way, a minimum molecular weight of HA (*ca.* 9 kDa) is required for efficient complexation.

Cryogenic transmission electron microscopy (cryo-TEM)

To visualize the formed macromolecular co-assemblies, IPECs were examined by cryo-TEM. The electronic contrast was too low for IPECs based on the copolymers comprising HA of 6 and 9 kDa. However, we obtained images for IPECs based on HA_{54k}-*b*-PEG_{5k} (Fig. 3) showing spherical particles. One can also see that some of them merge forming elongated objects and that they seem to be distorted when sticking to the carbon lace (Fig. 3, right). The latter observation strongly suggests that they are soft objects. In addition, the very low electron contrast observed in cryo-TEM agrees with the high water content in such IPECs.

The radius of the IPEC particles observed in the cryo-TEM images is larger than that determined by DLS (111 nm, *cf.* Table 2), which is, in fact, not expected as cryo-TEM yields a number-average estimate of the size while DLS provides a close to *z*-average value. Apparently, this originates from the fact that the formed nanoassemblies can merge and might also be flattened inside the film of less than 100 nm in thickness before vitrification.

Effect of ionic strength

The electrostatic nature of the interaction between HA-*b*-PEG and PLL entails an ionic strength dependence of the properties of the formed IPECs as well as their stability with respect to salt-induced dissociation to the polymeric components. To estimate the interaction between PLL and HA blocks, the mixtures of the oppositely charged homopolymers taken in a 1 : 1 charge-to-charge ratio were mixed leading to a precipitate (an insoluble IPEC) because of the complexation of HA and PLL. The

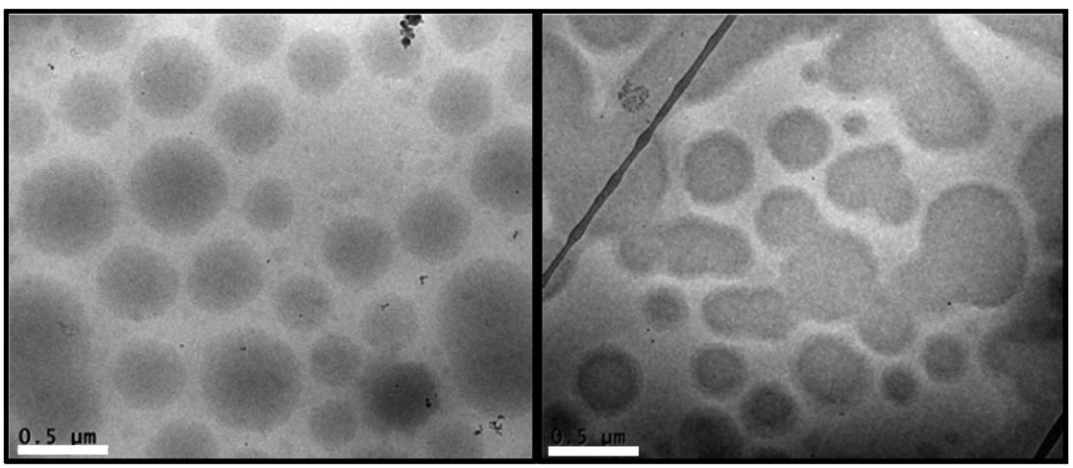

Fig. 3 Cryo-TEM images of IPECs from HA_{54k}-*b*-PEG_{5k} and PLL. The scale bar is 500 nm.

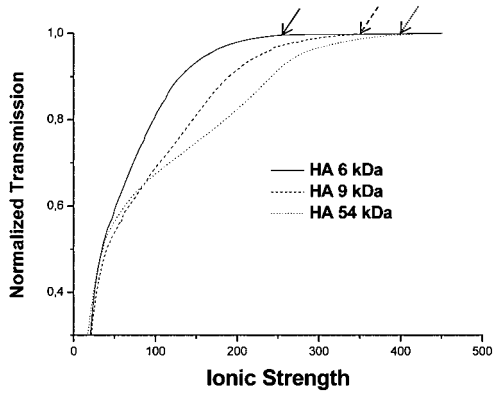

Fig. 4 Turbidimetric titration by addition of NaCl to mixtures of PLL and HA.

suspension was subsequently titrated while the turbidity was measured. The stepwise addition of NaCl results in a gradual decrease in turbidity due to the screening effect of small ions. Finally, turbidity completely disappears when a certain threshold ionic strength is reached (Fig. 4, indicated by arrows), indicating a deep disruption of a system of interpolymer salt bonds in the IPECs.

The titration curves demonstrate that the interaction between HA and PLL strengthens with the increasing DP_n of HA as the threshold ionic strength shifts from 250 to 350 and finally to 400 mM for HA 6, 9 and 54 respectively. The threshold concentration of NaCl can be considered as a measure of stability of IPECs with respect to their salt-induced dissociation to the polymeric components. These results are in good agreement with those previously obtained for IPECs based on oppositely charged linear synthetic polyions.[31]

An examination of solutions of IPECs (HA_{9k}-*b*-PEG_{5k} and HA_{54k}-*b*-PEG_{5k}) at salt concentrations below the threshold value showed that addition of small amounts of 2 M NaCl solution leads to changes in the hydrodynamic size, D.I., and count rate as determined by DLS (Fig. 5).

In the case of IPECs based on HA_{9k}-*b*-PEG_{5k}, the addition of NaCl leads to a gradual increase in the hydrodynamic size (Fig. 5b) and D.I. (Fig. 5c), with an abrupt rise in the ionic strength range between 20 and 100 mM NaCl. Above I = 100 mM, a very low count rate is observed (Fig. 5a). IPECs based on HA_{54k}-*b*-PEG_{5k} behave similarly but the count rate markedly drops above 160 mM (Fig. 5a). In addition, a smaller increase in size and a less abrupt decrease in the count rate (Fig. 5a) and an increase in D.I. (Fig. 5c) were detected for ionic strength between 20 and 100 mM.

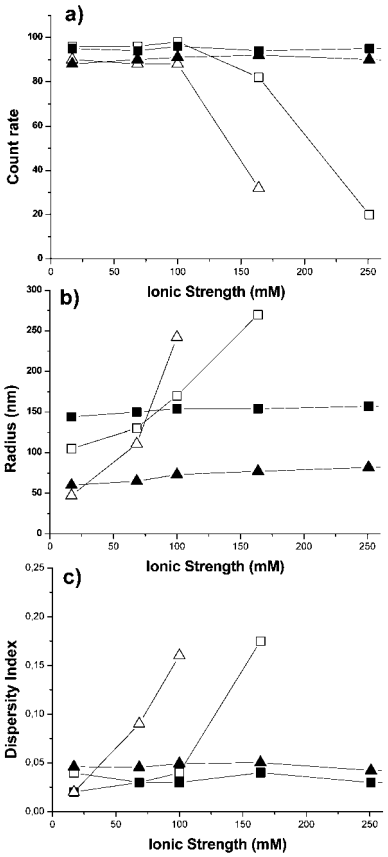

Fig. 5 Count rates (a), hydrodynamic radii (b), and dispersity indices (c) obtained by DLS for IPECs under a stepwise addition of 2 M NaCl. Squares and triangles correspond to HA with 54 and 9 kDa; open and filled symbols correspond to non-crosslinked and crosslinked IPECs, respectively.

Reversibility of the IPEC formation

As a consequence of the preparation procedure, it is unclear whether the nanoassemblies are in equilibrium or are kinetically trapped during their formation. We observed similar DLS results after 2 h, 24 h or even 4 months after mixing of the original solutions of HA-*b*-PEG and PLL (Tables S1 and S2†). However, this cannot completely ensure the formation of equilibrium IPECs. To clarify this point, IPECs (comprising HA blocks of 9 and 54 kDa) were disrupted *via* addition of NaCl up to 1 M, *i.e.*, to much higher ionic strength than needed for a complete disappearance of turbidity in mixtures of the corresponding HA and PLL (Fig. 4). Subsequently, the solution was dialyzed against the same buffer used for the preparation of IPECs, to remove the added NaCl.

IPECs based on HA_{9k}-*b*-PEG_{5k} recover the same hydrodynamic radius (48 nm) and D.I. (0.03). This finding strongly suggests that the interpolyelectrolyte complexation is fully reversible and the formed nanoassemblies are not kinetically entrapped during mixing. In the case of IPECs based on HA_{54k}-*b*-PEG_{5k}, the interpolyelectrolyte complexation is only partially reversible as a considerable increase in the hydrodynamic radius and D.I. is observed (176 nm and D.I. 0.08 compared to 111 nm and D.I. 0.04).

Swollen/nanogel structure of the IPECs

A number of observations point to a very swollen internal organization of the formed IPECs. First of all, a relatively large radius of complexes is observed. In fact, a very rough calculation of the contour length of the HA-*b*-PEG chain, using a structural size of 0.51 nm for the sugar unit and a size of 0.375 nm for the ethylene glycol monomer unit,[32] leads to contour lengths of *ca.* 58, 67 and 172 nm for the HA-*b*-PEG with HA 6 kDa (DP_n = 30), 9 kDa (DP_n = 48) and 54 kDa (DP_n = 260). In spite of the high

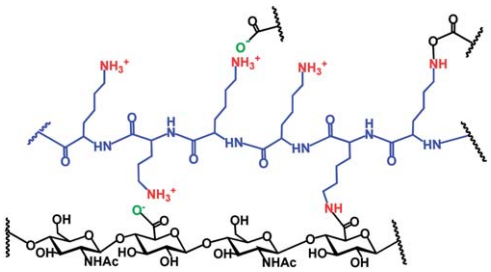

Fig. 6 Crosslinking of IPECs with carbodiimide.

rigidity of HA (persistence length 4.1 nm, ~80 sugar units[33]), the hydrodynamic radii observed by DLS and cryo-TEM appear to be larger than those expected for star-like or crew-cut micelles formed by the copolymers with the above contour lengths. For example, the IPEC micelles obtained *via* the interaction of PLL and poly-(L-glutamic acid)-*b*-PEG exhibited a hydrodynamic radius of *ca.* 24 nm (independently of PLL molecular weight).[17]

A swollen structure also easily explains the observed pronounced salt-induced increase in the size of the IPECs. When considering nanogels, the size should be determined by the molecular weights of the polymeric components, the number of incorporated macromolecules, but also by the swelling degree. It has been reported that the swelling of a nanogel depends importantly on the number of crosslinking points.[20] In the present case, interpolyelectrolyte contacts are considered as crosslinking points. As the NaCl content increases, the number of ionic bonds between PLL chains and HA blocks decreases due to the screening effect of small ions. The consequence is an effective swelling of the nanogel particle that increases its radius (Fig. 5). Over a certain threshold ionic strength, the number of contacts is insufficient to keep PLL chains and HA blocks coupled and the nanogel is disrupted.

Finally, the nanogel structure, formed by many macromolecules and a considerable amount of water molecules, explains the variation of the IPEC radii with the molecular weight. Our hypothesis is that the radius difference between HA_{6k}-*b*-PEG_{5k} and HA_{9k}-*b*-PEG_{5k} comes from the considerably weaker interpolyelectrolyte interaction between HA_{6k} and PLL (Fig. 4). This results in a lower number of interpolyelectrolyte contacts and causes a higher swelling degree for HA_{6k}-*b*-PEG_{5k}, that together with the tiny molecular weight difference between 6 and 9 kDa HA explains the larger hydrodynamic radius of their particles. In contrast, the hydrodynamic radii of the obtained nanogels will increase again if HA_{9k}-*b*-PEG_{5k} is changed to HA_{54k}-*b*-PEG_{5k}. This may be attributed to the formation of a larger complex domain assembled from HA blocks and PLL chains with the increasing DP_n of HA.

Crosslinking of IPECs

A disassembly of IPECs at high ionic strength could be advantageous for some applications, such as template-assisted formation of nanogels[14] or mesoporous materials.[12] For drug delivery applications, however, stability at ionic strength of 100–150 mM is indispensable.

In a genuine drug delivery application, a positively charged protein could be used instead of PLL. In this case, the stability of these complexes will be modulated by hydrophobic interactions as well as H-bonds (mostly due to the hydroxyl groups of HA). Therefore, it is difficult to predict in advance the stability of the formed macromolecular co-assemblies. If IPECs are not stable, a straightforward solution to the problem is the crosslinking of the system.

As proof of concept, IPECs (HA 9k and HA 54k) were crosslinked by the formation of amide bonds between some of the amino and carboxylic acid groups *via* a carbodiimide coupling (Fig. 6).

Successful crosslinking was proven by DLS. In fact, no change was observed in the hydrodynamic radii, count rate, and D.I. when NaCl was added after the addition of carbodiimide, even up to $I = 450$ mM (the data up to 250 mM are presented in Fig. 5). In addition, cryo-TEM images of HA_{54k}-*b*-PEG_{5k} show a very homogeneous structure of the formed nanoassemblies with a more defined and less soft character as observed from the lack of distorted species (Fig. 7). Crosslinked IPECs showed slightly larger hydrodynamic radii while the D.I. values

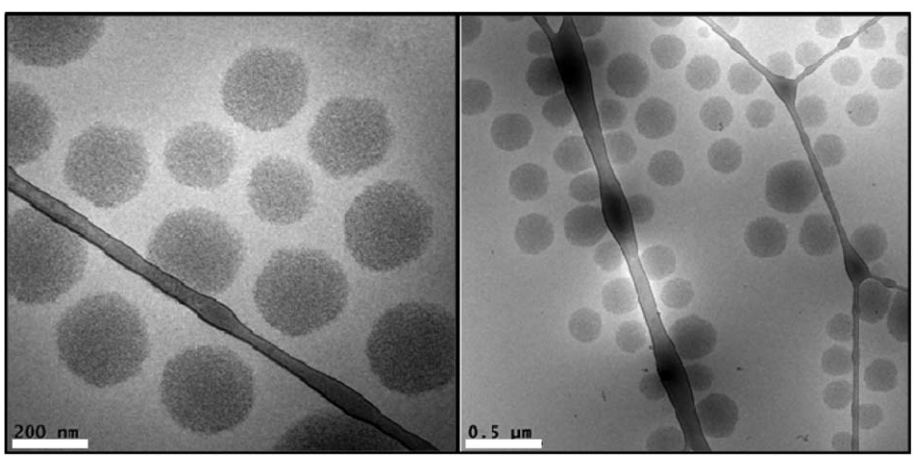

Fig. 7 Cryo-TEM images of IPECs from HA_{54k}-*b*-PEG_{5k} after crosslinking.

remained unchanged (Table 2). The mean radius determined from cryo-TEM is 127 ± 11 nm, which agrees well with the size measured by DLS.

Conclusions

Spherical soft interpolyelectrolyte complexes with a nanogel structure can be easily obtained by mixing HA-*b*-PEG and PLL under mild conditions in aqueous media. A minimal molecular weight of the HA block (*ca.* 9 kDa) is necessary for efficient complexation, while a minimal molecular weight of the PEG block (*ca.* 5 kDa) is needed to prevent macroscopic aggregation. The crosslinking with carbodiimide leads to well-defined nanogels, which are stable against ionic strength. The new concept that we show for the formation of IPEC nanogels uses the charges of the polypeptide and a biocompatible polysaccharide-*b*-PEG to induce macromolecular co-assembly, followed by a subsequent crosslinking. Such a methodology may have an enormous potential in drug delivery because it allows production of well-defined nanogels *via* a very simple method and could be extended to IPECs of a charged polysaccharide-*b*-PEG with other charged biopharmaceuticals (such as proteins or DNA). The higher hydrophilicity and porosity of such nanogels may complement the achievements of other drug delivery systems such as IPEC micelles by enhancing their loading capacity and modifying their accumulation in different tissues as well as the drug release.

Acknowledgements

We thank M. Förtsch for TEM measurements. We also thank Dr M. Drechsler for discussions on cryo-TEM images. R.N.C. acknowledges support by the Barrié de la Maza fellowship.

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