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Self-Assembly of Guanosine and Deoxy-Guanosine into Hydrogels: Monovalent Cation Guided Modulation of Gelation, Morphology and Self-Healing Properties

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In this study, we report the effect of monovalent cations including Na^+, K^+, Rb^+, Ag^+, Au^+, Tl^+, Hg^+ and NH_4^+ on the stimulation of purine nucleosides guanosine (G) and deoxy-guanosine (dG) to self-assemble into hydrogels. The gelation properties in terms of gel crystallization, lifetime stability, thermo-reversibility, minimum gelation concentration, gel melting temperature, thixotropic property and others were thoroughly investigated and compared not only between two nucleosides but also among different metal ions. A few metal ions were found to induce G/dG to form gels with much improved lifetime stability. The results revealed that dG is a much better gelator than G for introduction of thixotropic property. Interestingly, morphological, fluorescence and rheological (thixotropic) properties of the gels were found to modulate significantly by changing the metal ions. In the presence of K^+ ion, G produces self supporting tight gel but it has poor lifetime stability. In contrast, the presence of K^+ ion stimulates dG to form a very loose gel but associated with excellent lifetime stability and thixotropic property. In this context, we have successfully engineered a self-supporting stable co-gel using simple co-gelation method by mixing equimolar amount of K^+ induced G and dG gel and this co-gel shows long lifetime stability, self-healing and injectable properties which may allow for a broad range of biological applications. Furthermore, Ag^+ induced G gel was exploited for the light triggered in-situ fabrication of uniform AgNPs within gel to make a nano-bio hybrid material.

Introduction

Bio-molecular self-assembly is an inspirational approach to the design and fabrication of functional soft materials.[1-12] Among the five nucleosides, guanosine-based self-assembly into G-quadruplex has been exploited in areas as diverse as supramolecular chemistry and nanobiotechnology.[12-25] Although metal ion induced guanosine-based G-quadruplex and hydrogel formation has been first reported in the early 1960s, yet only a small number of metal ions (Na^+, K^+ and Ag^+) are known to contribute to gel formation with guanosine (or guanosine analogues) via G-quadruplex formation.[25-27] However, to the best of our knowledge, metal ion induced hydrogelation of deoxy-guanosine has not been explored yet. Despite this lengthy history, there is a limitation for the potential applications of guanosine gels (Na^+ and K^+ induced G gels) because of their poor lifetime stability. However, researchers have succeeded in developing a few guanosine derivative based hydrogels with enhanced lifetime stability.[28-37] Lehn and coworkers reported guanosine-5′-hydrazide based hydrogelation in the presence of monovalent ions including Na^+, K^+ and NH_4^+.[29, 30] An acetylated guanosine derivative, 8-methoxy-2′,3′,5′-tri-O-acetylguanosine forms hydrogel in the presence of Na^+ and K^+ ions.[31] In a recent report, disodium 5′-deoxy-5′-thioguanosine-5′-monophosphate self-assembles into a helical G-quadruplex structure in aqueous solution to form hydrogel.[32] Here, we report that the two purine nucleosides [guanosine (G) and deoxy-guanosine (dG)] self-assemble in water in the presence of a range of monovalent cations including Na^+, K^+, Rb^+, Ag^+, Au^+, Tl^+, Hg^+ and NH_4^+ to form hydrogels. The gelation properties have been thoroughly investigated and compared not only among different metal ions but also between two purine nucleosides. Interestingly, it was found that in the presence of a few metal ions, dG produces gels with much improved gelation properties in terms of lifetime stability and mechanical reversibility.

Self-healing, an ability of systems to spontaneously repair their damage and improve the life-time of materials, is one of the

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most fascinating properties of materials.[38-43] Self-healing is an important function in living organisms. The development of self-healable supramolecular gel is one of the emerging fields in material research. However, the reported self-healing gels are mainly polymeric gels.[38-43] Low molecular weight gelator (LMWG) based self-healing organogels[44-50] and hydrogels[51-58] have been recently reported. However, the modulation of the self-healing property of gels is a demanding task.[52, 54] In this study, we report nucleoside based hydrogels showing metal ion dependent self-healing properties which is unprecedented. The use of metal ions in making supramolecular gels[50, 55, 59-70] is a potent approach with unusual properties and most of the reported metallogels lack of a deeper insight towards the fine-tuning of gel properties by using different metal ions. Interestingly, in our study, most of the dG gels show excellent thixotropic property whereas G gels lack such a property. Moreover, in the presence of K^+ ion, G forms a self supporting gel but of poor lifetime stability, whereas dG produces a very loose gel associated with excellent lifetime stability. In this context, we have successfully engineered a self-supporting stable co-gel by mixing an equimolar amount of G-KCl and dG-KCl gel and this co-gel has long lifetime stability and excellent self-healing property.

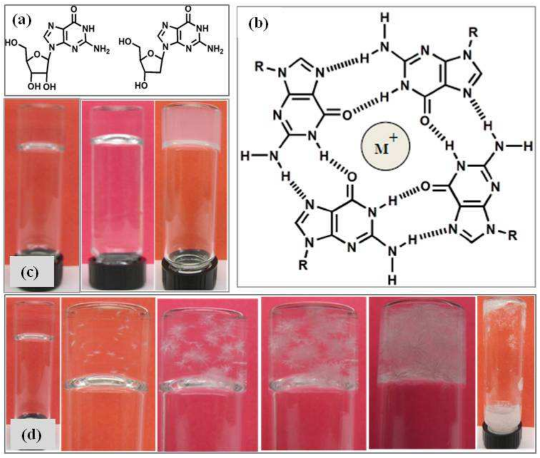

Fig. 1 (a) Chemical structures of guanosine (left) and deoxy-guanosine (right); (b) Metal ion templated G-quartet formation; (c) Photographs of self-supporting hydrogels obtained from G triggered by Rb^+ (left), Ag^+ (middle) and Tl^+ (right); (d) Photographs of potassium ion induced G gels showing the crystallization/decomposition over a period of hours: left to right with increasing time.

RESULTS AND DISCUSSION

Guanosine self-associates into G-quartets that are comprised of a planar arrangement of four guanosine molecules linked together by eight hydrogen-bonding interactions and stack on top of one another to form stable helices/G-quadruplex (Fig. 1b). Generally, these complexes are stabilized by only certain alkali metal ions, particularly K^+ and Na^+ through a size-selective coordination mechanism and this sometimes results in hydrogelation. Here, it was found that the monovalent metal ions having ionic radii in the range 1–1.61 Å (for coordination number eight) can help G/dG to form hydrogels presumably via G-quadruple formation. Li^+ and Cs^+ do not support hydrogelation as their ionic radii are either too small (0.92 Å) or too large (1.74 Å) respectively. G/dG hydrogels were prepared by mixing G/dG and metal salts with varying stoichiometries, starting from G/dG:M molar ratios of 1 : 1 to 1 : 8. The photographs of some gels have been depicted in Fig. 1c,d and images show they are self-supporting gels ranging from very transparent to translucent.

Table 1. Gelation properties of hydrogels obtained from guanosine (G) and deoxy-guanosine (dG) at different conditions as mentioned below at foot note.

metal ion	gelation	minimum gelation concentration (mgc)[a] (%, w/v)	crystallization	lifetime stability[b] (at 22°C)	thermo-reversibility	T_{gel} (°C)[c]
Guanosine (G)						
Na^+	gel	0.94 0.78	Y	35 min 15 min	Y	49 38
K^+	gel	0.70 0.57	Y	12 hr 15 min	Y	56 35
Rb^+	gel	0.75 0.67	Y	1 hr 10 min	Y	54 37
NH_4^+	gel	1.14 0.81	Y	28 min 30 min	Y	43 40
Tl^+	gel	0.4 0.4	Y	14 hr 30 min	Y	66 46
Hg^+	gel	1.54 –	N	>1 month	N	–
Ag^+	gel	0.57 0.40	N	>4 month	N	–
Au^+	LG	–	Y	>2 days	N	–
Deoxy-guanosine (dG)						
Na^+	VS	–	Y	–	Y	–
K^+	LG	1.19 0.89	N	>4 month	Y	37 36
Rb^+	LG	2.78 –	Y	– 3 hr	Y	– 35
NH_4^+	LG	2.68 –	Y	– 2 days	Y	– 36
Tl^+	gel	0.47 0.28	N	>4 month	Y	54 33
Hg^+	gel	1.34 –	N	>1 month	N	–
Ag^+	gel	0.47 0.23	Y	>2 days >4 hr	N	–
Au^+	LG	–	N	>2 days	N	–

Foot Note: [a] minimum gelation concentration (mgc) of a gelator was calculated only in terms of the amount of G/dG present in a gel, mgc values at two different ratio of metal ions are given: 1:2 (above) and 1:4 (below); [b] lifetime stability of a gel at a concentration 1.42 % w/v, 1:2 (above) and at mgc, 1:2 (below); [c] T_{gel} value at 1.42 % w/v, 1:2 (above) and at mgc 1:2 (below); Y indicates yes (gel crystallizes) and N indicates no (gel does not crystallize). VS and LG indicate viscous solution and loose gel respectively.

The gel forming ability and gelation properties (lifetime stability of gel, crystallization/decomposition of gel, thermo-

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reversibility, minimum gelation concentration and gel melting temperature, T_{gel}) of G and dG gels with different metal ions are summarized in Table 1. It is noted that guanosine forms metastable gels with most of the aforementioned metal ions except Ag^+, Au^+ and Hg^+. The metastable gels slowly crystallize within the hydrogel matrix upon standing over a period of time at room temperature, leading to the collapse of the gels. In contrast, most of the deoxy-guanosine based gels are stable and show no sign of crystallization except Ag^+, Rb^+ and NH_4^+. For example, K^+ induced G gel (1% w,v, 1:2) slowly crystallizes within the gel matrix upon standing at room temperature and gel breaks completely within 2 hours (Fig. 1d). This happens as the gel-forming assemblies are kinetically trapped and they are not thermodynamically stable assemblies obtained from the G gelator for most of the metal ions. The lifetime stability of G gels (at 1.42%,w/v, 1:2) has the following order $Ag^+ > Hg^+ > Au^+ > Tl^+ > K^+ > Rb^+ > Na^+ > NH_4^+$. dG gels have long time stability for K^+, Tl^+ and Hg^+ and a lower stability for Rb^+ and NH_4^+. Ag^+ induced dG gel has a few hours lifetime for the ratio of 1:1 or 1:2 and longer lifetime for composition ≥1:4. It is noted that Rb^+ and NH_4^+ induced both G and dG gels are metastable where as Hg^+ induced both G and dG gels have long time stability and never experience crystallization. It should be mentioned that rate of crystallization of a metastable gel decreases (i.e. the lifetime stability of a gel increases) with increasing concentration of gelator (G/dG), lowering the environmental temperature and increasing concentration of (mole ratio) of metal ions having other parameters constant. G forms self-supporting tight gels with all metal ions whereas dG produces self-supporting tight gels with Ag^+, Tl^+ and Hg^+ and loose gels in the presence of K^+, Rb^+ and NH_4^+.

The mgc values of G gels (at 1:2 ratio) vary in the sequence $Tl^+ < Ag^+ < K^+ < Rb^+ < Na^+ < NH_4^+ < Hg^+$ and this suggests that gel inducing ability of metal ions towards G follow the reverse order. The mgc values of dG gels (at 1:2 ratio) vary in the order $Tl^+ \sim Ag^+ < K^+ < Hg^+ < NH_4^+ < Rb^+$. However, for most of the gels, mgc values decrease with an increasing mole ratio of metal ions (from 1:2 to 1:8). All G and dG gels are fully thermo-reversible except Ag^+, Au^+ and Hg^+. These three metal ions induced G gels are partially thermo-reversible and those of dG gels are thermo-irreversible. This suggests that complex formation is very rigid for these metal ions. The T_{gel} values of thermo-reversible G gels for different metal ions (at a fixed gelator concentration of 1.42 %, w/v and a fixed ratio of 1:2) follow the order $Tl^+ > K^+ > Rb^+ > Na^+ > NH_4^+$. T_{gel} values of all thermo-reversible gels increase with increasing concentration of gelators (G/dG) and with increasing mole ratio of metal ions (Fig. S1, Supplementary Information).

To probe the templating effect of different metal ions on the assembly of G, circular dichroism measurements were performed using 1.42 wt% G gels (1:2). In case of K^+ ion induced G gel, an intense negative band was observed at 300 nm with a positive band at 260 nm (Fig. 2). The obtained oppositely signed bands (negative–positive) can be related to a left-handed helical stacking of G-quartets.[21] The CD spectra are visibly quite different for different metal ions suggesting the different templating effect and assembly formation (Fig. 2).[31] In the case of temperature dependent CD study of K^+ ion induced G gel (Fig. S2), the signal at 300 nm was gradually decreased with an increase in temperature and surprisingly the signal approached almost the baseline at a temperature higher than the T_{gel} value, where gelator molecules remain in the solution state and not in the assembled state. The temperature dependency of the CD signals clearly suggests that the CD response is a consequence of the assembly of chiral nucleosides to supramolecular chirality, rather than the inherent molecular chirality of the individual nucleoside.

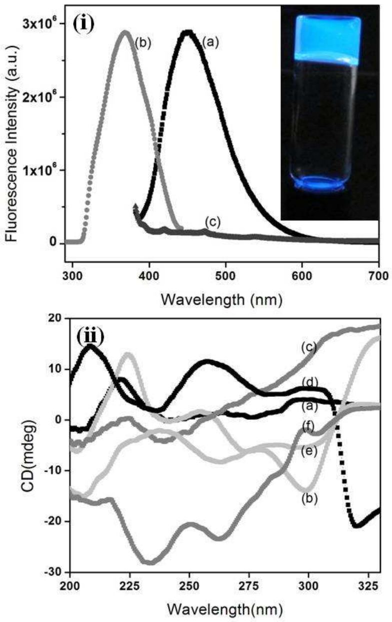

Fig. 2 Spectroscopic studies of G-gels. (i) Fluorescence emission (a) and excitation spectra (b) of K^+ ion induced G gel and emission of Ag^+ ion induced G gel (c); Inset: photograph of K^+ induced G gel under UV light (excitation at 365 nm); (ii) CD spectra of G gels (concentration 1.42%,w/v; 1:2 metal ion ratio) for different metal ions at identical conditions: (a) Na^+, (b) K^+, (c) Rb^+, (d) NH_4^+, (e) Tl^+ and (f) Ag^+.

Interestingly, fluorescence studies of G gels shows metal ions guided emission property. Most of the metal ions (Na^+, K^+, Rb^+, NH_4^+ and Tl^+) induced G gels exhibit an emission maximum at 450 nm upon excitation at 367 nm and these gels show bright blue fluorescence under the exposure of UV light using excitation at 365 nm (Fig. 2i including inset). In contrast, Ag^+, Au^+ and Hg^+ ions induced G gels do not show blue fluorescence under UV light and fluorescence spectroscopic experiment exhibits much lower intensity (about 20 times lower) compared to other metals. This suggests that the thermo-irreversible G gels (Ag^+, Au^+ and Hg^+) are almost non fluorescent in nature as a result of the quenching effect of these metal ions. On the other hand, none of the dG gels exhibit blue fluorescence under UV light and spectroscopic analyses show that their fluorescence intensies are much lower (about 10 times) than G gels (Fig. S3). Though there are reports of fluorescence study on the effect of metal ions (Na^+, K^+) on the

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excited states of guanine nanostructures, energy transfer in DNA quadruplexes,[71, 72] yet to the best of our knowledge no report is available on the metal ion regulated blue fluorescence of guanosine gels.

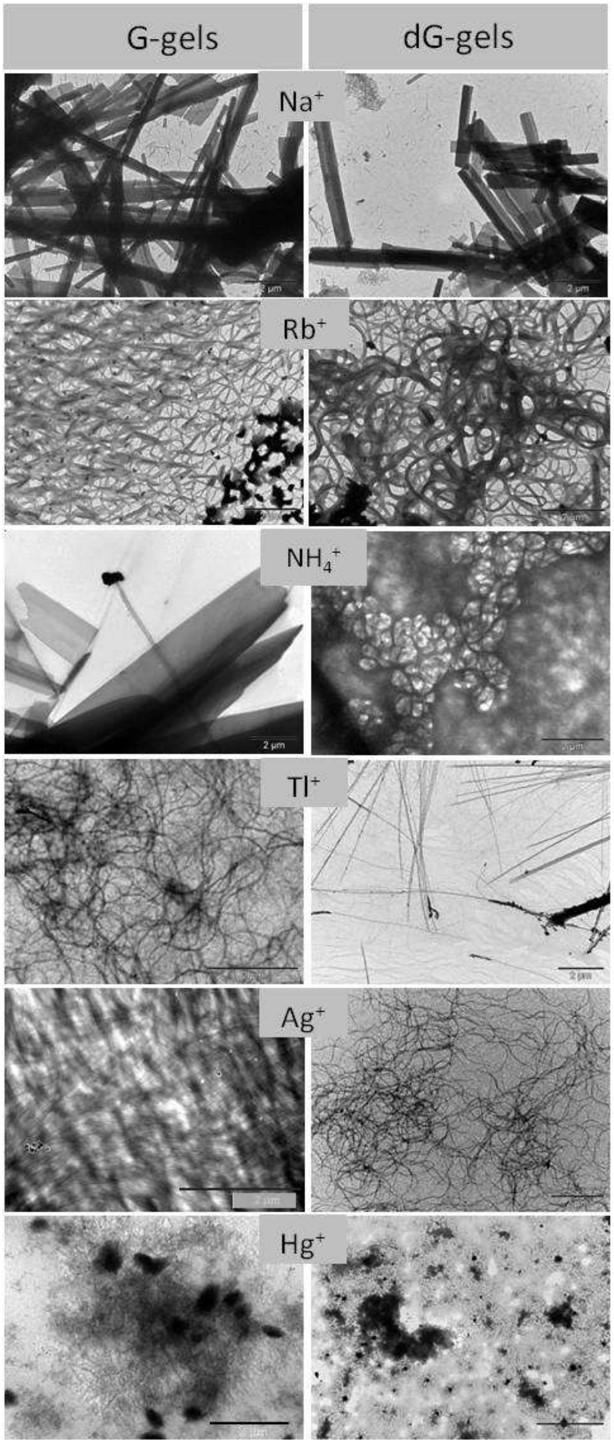

Fig. 3 TEM images of different metal ion templated G gels (left row) and dG gels (right). Scale bar is 2 μm for all images.

The morphological insight of G and dG gels was characterized using transmission electron microscopy (TEM). The TEM images of the gels in the presence of different metal ions are shown in Fig. 3. Na$^+$ ion induced G and dG gels exhibit nanosheet like morphology. The average width of these sheets is 500 nm and each nanosheet remains 5-6 μm in length. Rb$^+$ ion induced G gel shows interconnected small fibers having average width 70 nm. In comparison, Rb$^+$ ion induced dG gel shows a network of curling fibers having width 40-120 nm. Interestingly, NH$_4^+$ ion induced G gel forms flat nanosheet and dG gel exhibits fibrous network. Tl$^+$ ion induced G gel forms uniform network of fibers having average diameter 40 nm. In contrast, Tl$^+$ ion induced dG gel comprises of mostly rod-like morphology with some thinner fibers as well. The average diameter of these rods is 75 nm. Ag$^+$ induced G gel shows a dense network structure of thin fibers having an average width of 15 nm and each fiber is a few micrometers in length (Fig. 3 and Fig. S4) indicating a high aspect ratio; whereas Ag$^+$ induced dG gel consists of fibers with relatively wider diameter (average 45 nm). Hg$^+$ ion induced G and dG gels exhibit unstructured morphology. The TEM studies highlight the dramatically different templating behaviour of the metal ions with the gelator and this reflects their morphology. Therefore, the tuning of morphology is possible just by altering metal ions. It was found from TEM studies that the width of the gel fibres is much higher than that of the dimension or G-quartet of the gelator. This suggests that several G-quartets are self assembled to form the supramolecular aggregated fibrillar structure.

Viscoelastic properties of the hydrogels were examined by measuring their rheological properties using gels at a fixed concentration 1.42 %,w/v and a constant metal ion concentration (1:2). The linear viscoelastic frequency sweep response is shown in Fig. S5a in the supplementary information for Tl$^+$ ion induced G and dG gels. This exhibits a storage modulus (G') value of G gel>dG gel that suggests G gel is stronger than dG gel. This result is consistent with the T_{gel} value of G gel> dG gel for Tl$^+$ ion. All gels exhibit G' greater than G'' suggesting that they are effective physical hydrogels. G' values of G gels follow the order Tl$^+$>Ag$^+$>K$^+$> Rb$^+$>Na$^+$>Hg$^+$>NH$_4^+$>Au$^+$ and this matches well with T_{gel} order (Table S1a, Supplementary Information). Morphological study also agrees with the strength of the gels as Tl$^+$, Ag$^+$ and K$^+$ ion templated G gels comprised of relatively tight fibrous network structures. The G' value of a gel increases gradually with increasing concentration of metal ion ratio from 1:1 to 1:8 (Table S1b, Supplementary Information) and this matches well with T_{gel} studies. Therefore, tuning of the mechanical properties of a gel is possible by simply changing the metal ion, metal ion concentration and of course by changing gelator concentration.

We carried out rheological studies in order to investigate and compare the thixotropic property among different metal ion induced G and dG gels. The thixotropic behaviour of the gel, which becomes less viscous under stress and return to their original state when stress is removed, was monitored by two independent experiments that utilized both the flow and the oscillation method. For first thixotropic experiment, gels were

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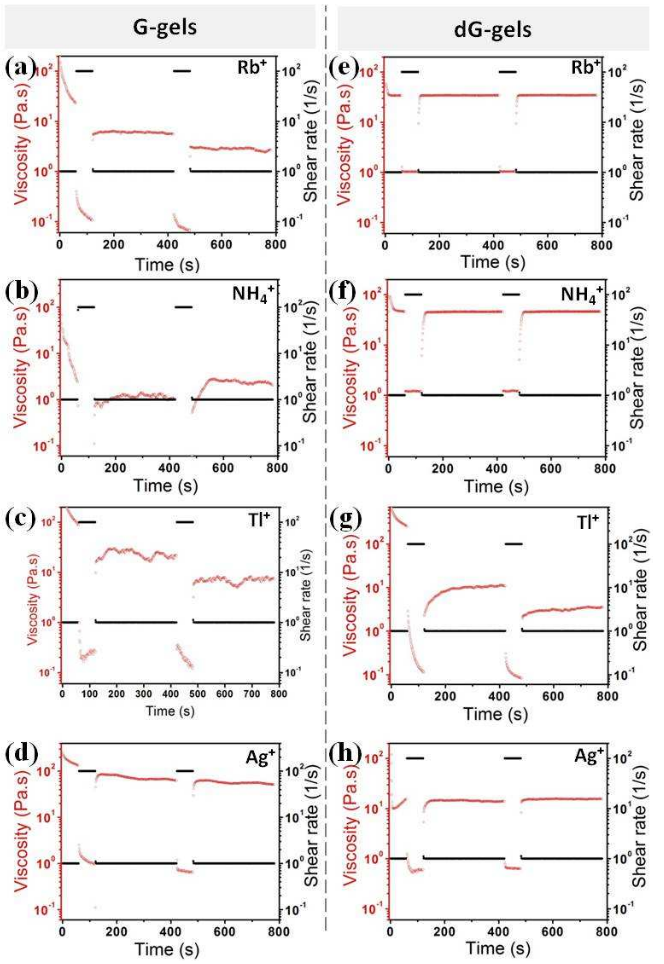

Fig. 4 Thixotropic experiments using flow method. Breaking and recovery of gel based on the viscosity of gel by applying an alternative shear rate of 100 and 1 s^{-1}. (a-d) G-Rb^+ gel, G-NH_4^+ gel, G-Tl^+ gel and G-Ag^+ gel respectively; (e-h) dG-Rb^+ gel, dG-NH_4^+ gel, dG-Tl^+ gel and dG-Ag^+ gel respectively. Red and black indicate corresponding viscosity and shear rate respectively. Experiments were performed with gels having a concentration 1.42 % w/v, (1:2 metal ratio) and environmental temperature at 15 °C. The concentration was 3% w/v for Rb^+ and NH_4^+ ion induced dG gels as their mgc values are 2.78% w/v and 2.68% w/v respectively.

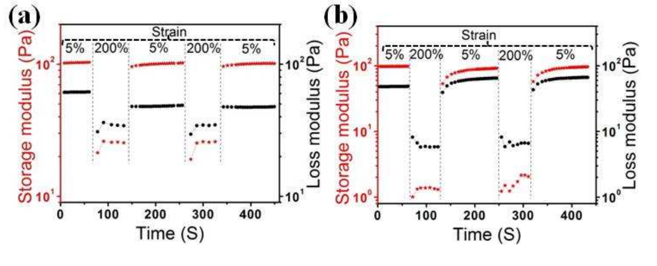

Fig. 5 Thixotropic experiments by continuous step strain measurement of (a) dG-Rb^+ gel and (b) dG-NH_4^+ gel. Breaking and recovery of gel by applying an alternative strain of 200% and 5% respectively with progress of time at a constant angular frequency of 10 rad/s. Red and black indicate corresponding storage modulus (G′) and loss modulus (G″) respectively. Experiments were performed with gels having a concentration 3 % w/v, (1:2 metal ratio) and environmental temperature at 15ºC.

subjected to breaking and recovery of gel based on viscosity of gel by applying alternative shear rate of 100 and 1 s^{-1} (Fig. 4). This experiment shows that the G gels do not have any thixotropic property except Ag^+ ion induced G gel, which can only recover approximately 50% of its original viscosity. In contrast, most of the dG gels including K^+, Rb^+, NH_4^+ and Ag^+ have excellent thixotropic property. However, Tl^+ and Hg^+ ions templated dG gels do not show thixotropic property. The thixotropic property of dG gels were also supported by continuous step strain measurements where gel was subjected to alternative 200% and 5% strain (at a constant angular frequency of 10 rad/s) with time and three cycles were performed (Fig. 5). From this study, it can be concluded that dG is better gelator than G for introduction of thixotropic property. Here, Tl^+ is the worst for making of thixotropic gel.

Co-gel

Co-gels, where the two gelators interact with each other, is an approach to access a wide range of gel properties which are not achievable by the use of only one of the two gelators.[73-76] It has already been mentioned that G produces self supporting tight gel in the presence of KCl but it has poor lifetime stability (15 min at the mgc and see Fig. 1d, Table 1). In contrast, dG forms very loose gel in the presence of KCl but this loose gel has very good lifetime stability (>4 months) as well as excellent thixotropic property. Therefore, neither G-KCl gel nor dG-KCl gel is suitable for application where gel should have enough tightness as well as longer lifetime stability. Here, we have engineered to develop a self-supporting co-gel by simple mixing equimolar amount of G-KCl and dG-KCl gel. This co-gel has very good lifetime stability (without sign of crystallization), enough tightness and self-healing property and these may allow it for a broad range of applications. The tightness of these gels was measured in terms of storage modulus (G′). The frequency sweep study at 1% strain (at a constant concentration of gels 1.42% w/v) shows that the G′ values follow the order G gel (4000 Pa)>co-gel (500 Pa)>dG (53 Pa) gel (Fig. 6a).

The self-healing propensities of KCl induced G, dG and co-gels were studied by rheological data (Fig. 6b,c,d). The gels were subjected to mechanical loading (shear rate 100) and the recovery (shear rate 1) of the viscosity was monitored for each of the samples. This experiment shows that G gel can hardly recover its original viscosity (about 10%) after damage and the viscosity is gradually decreased with increasing time at a constant shear rate 1 s^{-1}. It is quite expected as G gel experiences crystallization with increasing time and the rate of crystallization increases dramatically in the presence of higher strain or shear rate. In contrast, dG gel can fully (100%) recover its original viscosity in less than 7.5 s after damage suggesting its excellent self-healing property (Fig. 6c). It is interesting to examine whether the co-gel has self-healing property and Fig. 6d exhibits that it can fully (100%) recover its original viscosity in less than 15 s after damage. Therefore, we have successfully transferred the self-healing property of dG gel into co-gel. The

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magnitude of viscosity follows the order G gel>co-gel>dG gel. Furthermore, the self-healing property of co-gel was supported by continuous step strain measurements (Fig. S5b in Supplementary Information). Interestingly, when a block of gel was cut into a few pieces and then joined together, the pieces merged into a continuous block suggesting remarkable self-healing properties (Fig. 6e-g). The shear-thinning behavior and rapid healing properties of the co-gel make it a preferable choice for injectable scaffolds. The co-gel has injectability as demonstrated in Fig. 6h. The coexistence of injectability and self-healing properties is desirable for potential clinical gel materials.

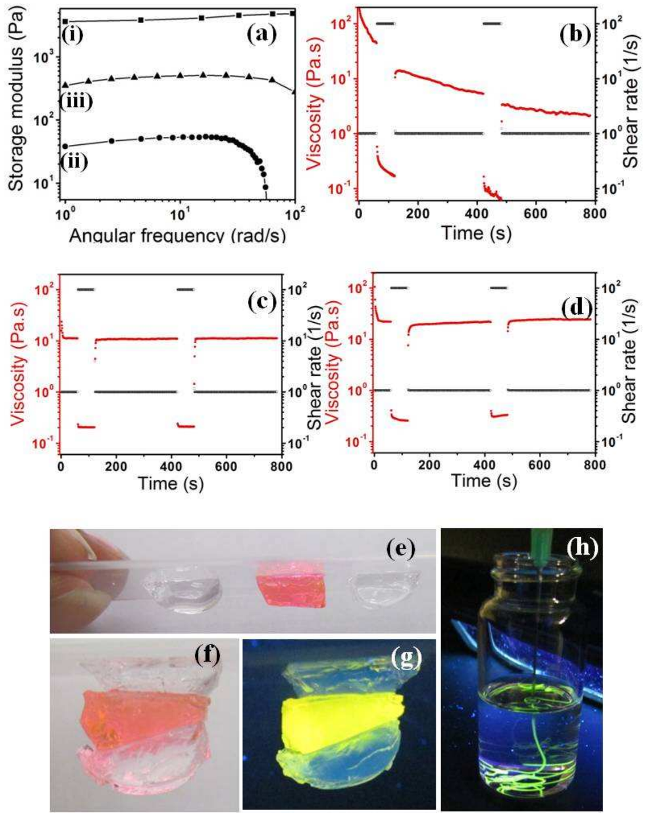

Fig. 6 (a) Frequency sweep experiments to measure storage modulus value for K$^+$ ion induced G gel (i), dG gel (ii), and co-gel (iii). Thixotropic experiments of K$^+$ ion induced G gel (b), dG gel (c), and co-gel (d). Red and black lines indicate corresponding viscosity and shear rate respectively. (e) co-gel was cut into a few pieces (middle one is Rhodamine 6G doped co-gel) showing self-supporting behavior (f) three pieces merged into a continuous block suggesting remarkable self-healing properties (g) image f under UV light (h) Injectability test of co-gel doped with Rhodamine 6G under UV light.

There are two possibilities in a two component mixed gel: (a) co-gel, where the two gelators (G and dG) assemble with each other to make architecture containing both components and (b) self-sorting gel, where the gelators self-assemble independently to form distinct structures.[73] It is expected that the co-gelators G and dG having similar binding motifs, will show a preference for mixed assemblies (mixed G-quadruplex). To probe this, we have performed CD, fluorescence and TEM experiments. CD shows an intense band at 300 nm for both G and dG gels but they are opposite in sign (Fig. 7a). The nature of CD spectra for co-gel is quite different with both G and dG gels and this is not the consequence of only mixture (self-sorting) of two components. This presumably suggests that the two components namely G and dG co-assemble with each other to form a new architecture, probably supporting a real co-gel formation. Fluorescence study of co-gel shows an emission maximum at 450 nm like G gel but intensity is almost half compared to G gel (Fig. S3). TEM images for K$^+$ ion induced G, dG and co-gel are shown in Fig. 7b-d and these exhibit average widths of 16, 20 and 33 nm for G gel, dG gel and co-gel respectively. The T$_{gel}$ value for co-gel is 52 ºC (1.42 % w/v, 1:2), which is not the average of two individual gels (56 ºC for G gel and 37 ºC for dG gel).

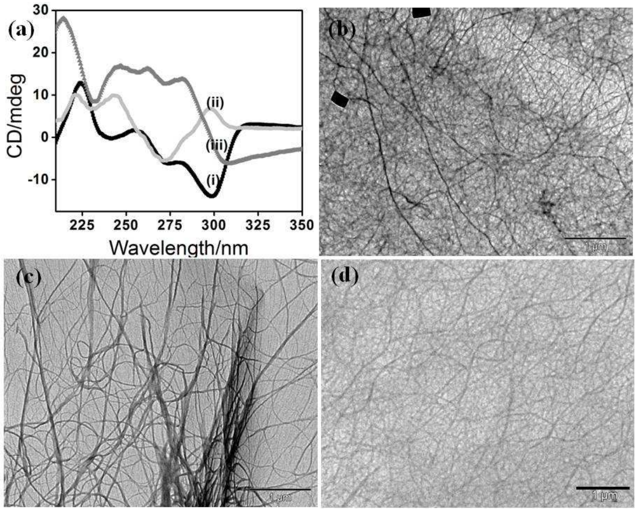

Fig. 7 (a) CD spectra of K$^+$ ion induced (i) G gel, (ii) dG gel and (iii) Co-gel having identical conditions (concentration 1.42%,w/v; 1:2 metal ion ratio, temperature 10°C). TEM images of K$^+$ ion induced (b) G gel, (c) dG gel and (d) Co-gel for comparison. Scale bar is 1 μm for all images.

Functional gels

Nanoparticles (NPs) in gels are very interesting as they constitute a mix of soft and hard matter nanosystems.[77, 78] Gels in the swollen stage offer a large free space within the 3D cross-linked networks that acts as a nanoreactor for the nucleation and growth of NPs. There are several examples of the *in situ* formation of metal NPs in gel-phase which act as a reaction medium.[26, 59, 64, 66, 79-87] The pre-entrapment of corresponding metal salts within the gel phase is common for making NPs in situ.[79-86] However, examples of the synthesis of silver NPs within silver ion coordinated gel are limited.[59, 64, 66, 87] In the present study, the Ag$^+$ ion induced G gel offers an opportunity to investigate the feasibility of *in situ* fabrication of Ag nanoparticle in the supramolecular hydrogel system. This

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can be done via an environmentally friendly method of photoreduction, without adding any external reducing or stabilizing agents or even corresponding silver salts.

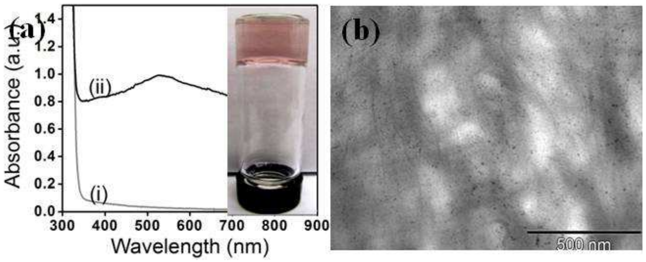

Fig. 8 (a) UV-Vis spectra of Ag^+ induced G gel before (i) and after (ii) illumination of light. Inset: Photograph of the hydrogel after illumination of light. (b) TEM image of AgNPs containing hybrid G gel.

Interestingly, the silver ion induced G gel can spontaneously produce hybrid gel containing silver NP under illumination of light. We investigated the spontaneous reduction of silver ions by using UV/Vis absorption spectroscopy. At the beginning, the Ag^+-induced G gel (1:2) was colourless and very transparent and the UV/Vis spectrum showed no absorption within the range of λ=350 to 800 nm. Subsequent exposure to light (120V, 60Hz. 0.17 A) resulted in a gradual colour change from colourless to light pink (Fig. 8a) suggesting the reduction of silver ions to silver (Ag^0).[83] The colour started to generate after 1 hour and 45 minutes of the exposure to light and it was saturated after 2 hours and 30 minutes. This type of colour change was not observed in the absence of light or in the absence of silver ions or gelator. This indicates that both the gelator (G) molecule and light have a role in silver-ion reduction. A broad surface plasmon resonance band appeared around λ=530 nm suggesting the formation of small silver nanoparticle within the gel (Fig. 8a).[83] Here, we believe that some Ag^+ ions (surface cations[23] not within the channel cavity between the two G-quartets) are photo-reduced in situ. TEM study showed the development of homogeneous small AgNPs having an average diameter of 7 nm within the gel network (Fig. 8b and Fig. S6 in the Supplementary Information).

Next, we tried to exploit (a biologically relevant K^+ ion induced) metastable G gel for the controlled release of therapeutics using vitamin B2 as a model. In a very preliminary study it was found that, almost 100 % of vitamin molecules were released from the hydrogel matrix into PBS buffer in case of the G gel in potassium phosphate buffer at pH 7.4 within 50 hours at 37 °C, whereas release was up to ~75% for dG gel within 70 hours. A detailed discussion can be found in the supplementary information (Fig. S7).

Conclusions

Monovalent cations (Na^+, K^+, Rb^+, Ag^+, Au^+, Tl^+, Hg+ and NH_4^+) induced hydrogelation of guanosine and deoxy-guanosine were thoroughly investigated. The gelation properties in terms of gel crystallization, lifetime stability, mgc, thixotropic property and others were methodically investigated and compared. It was found that G forms more stable gels with Ag^+ and Hg^+ than other ions. Some of the dG gels showed much improved gelation properties in terms of lifetime stability and thixotropic properties as compared to G gels. Interestingly, morphological, fluorescence and thixotropic properties of the gels were found to modulate significantly by changing the metal ions, suggesting metallogelation as a powerful strategy for the metal dependent efficient tuning of the self-assembly. In addition, a self-supporting co-gel of K^+ ion induced G and dG gels was developed which showed very good lifetime stability, enough tightness and self-healing property that may allow it for a broad range of applications including tissue engineering. Silver ion induced G gel offered an opportunity to investigate the *in situ* fabrication of uniform AgNPs within gel-network to make a nano-bio hybrid material via an environmentally friendly method of photoreduction, without adding any external reducing or stabilizing agents or even corresponding silver salts. In a very preliminary study, a biologically relevant K^+ ion induced, metastable hydrogel was exploited for a controlled and virtually quantitative delivery of vitamin B12 at 37°C and pH 7.4 (see supplementary information). Further investigation regarding drug release studies is under process.

Materials and methods

Materials. Guanosine and deoxy-guanosine were purchased from Aldrich and Berry & Associates respectively. $AgNO_3$, AuCl and RbCl were purchased from Aldrich. HgCl and Tl_2SO_4 were bought from BDH. NaCl, KCl and NH_4Cl were obtained from Caledon. The water used in all experiments was of Millipore Milli-Q grade.

Instrumentation.

UV/Vis spectroscopy. UV/Vis absorption spectra of the Ag^+ induced G-gel and silver nanoparticles containing G-gel were recorded by using a UV/Vis spectrophotometer (Agilent 8453).

Fluorescence spectroscopy. All photoluminescence spectra were recorded by using a steady state spectrofluorometer QuantaMaster (PTi) instrument with a 1 cm path length quartz cell. The slit width for the excitation and emission was set at 5 nm.

Transmission electron microscopy (TEM). TEM images were recorded by using a Hitachi 7500 transmission EM. Hydrogels and the Ag nanoparticle containing hybrid gel were dried on formver-coated copper grids (300 mesh) by slow evaporation in air, then allowed to dry separately in a vacuum. The samples were prepared with gels having their respective mgc concentrations.

Rheology. Rheological experiments were performed using a rheometer Discovery HR2 (TA Instruments) by using the cone-plate geometry in a Peltier plate (cone angle: 48). Thixotropic experiments of a gel were performed by breaking and recovery of gel based on viscosity. Thixotropic property of a gel was also supported by continuous step strain experiment. This was carried out first by breaking the gel upon the application of a

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strain of 100% (for co-gel). After complete rupture of the gel (as denoted by G″> G′), its recovery was observed at a constant strain of 5%. This breakage and recovery process was repeated for few times to ensure the ability of gel to restore. The entire study was carried out at a constant angular frequency of 10 rad/s.

Circular Dichroism (CD). CD spectra were recorded between 200 and 350 nm using a JASCO J-810 spectrometer. Experiments were carried out by placing the gel material into a quartz plate having 0.1 mm path length. The experiments were performed with gels having concentrations 1.42%,w/v and metal ion ratio G/dG:M 1:2.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Drug release study, additional figures and tables.

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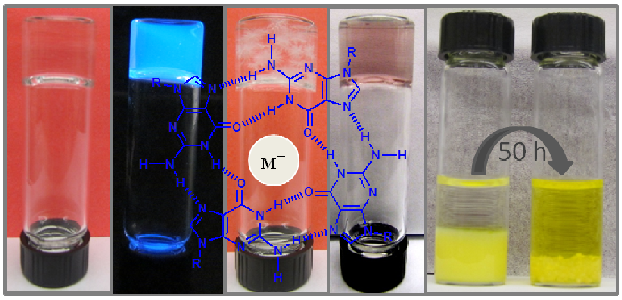
Graphical Abstract

The gelation, morphological, fluorescence and thixotropic properties of the purine nucleosides based hydrogels were found to modulate with metal ions.



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