A key challenge for the effective treatment of gastrointestinal diseases including inflammatory bowel disease is to develop an orally administered drug delivery system capable of prolonged retention in the gastrointestinal tract. Herein we report a bioadhesive liquid coacervate based on hydrogen bonding-driven nanoparticle assembly. Free from electrostatic interactions, our fluid nanoparticle-assembled coacervate demonstrates significant pH- and salt-independent structural stability and forms a physically adhesive coating on a large surface area of intestinal tract with an extended residence time of more than 2 days to mediate the sustained release of preloaded water-soluble small molecule drugs in vivo. The orally administered drug-laden nanoparticle-assembled coacervate significantly mitigates the symptoms of inflammatory bowel disease, restores the diversity of gut microbiota, reduces systemic drug exposure, and improves the therapeutic efficacy in a rat acute colitis model compared with the oral administration of the same amount of drug in solution form. We suggest that the nanoparticle-assembled coacervate provides a promising drug delivery platform for management and treatment of numerous gastrointestinal diseases where controlled drug release with extended residence time is desired.
Inflammatory bowel disease (IBD) including Crohn’s disease (CD) and ulcerative colitis (UC) causes chronic relapsing inflammation of the gastrointestinal (GI) tract. IBD significantly increases the risk of developing colorectal cancer and imposes a lifelong healthcare burden on millions of patients worldwide. Oral dosage formulations of drugs such as corticosteroids are the most desirable delivery systems available to treat IBD and are vastly superior to enema and subcutaneous and intravenous injection in terms of patient compliance. However, many patients require lifelong administration of drugs to control IBD symptoms; therefore, reducing the severity of side effects attributable to systemic drug exposure resulting from frequent oral dosing via conventional delivery systems, such as enteric-coated tablets and capsules, is essential.

Although many forms of oral drug delivery vehicles have been developed, the insufficient residence time within the intestinal tract and the complex design of delivery vehicles limit their widespread clinical applications. Endowing oral drug delivery vehicles with wet bioadhesion may prolong intestinal residence time. However, solid adhesives with high adhesion energy on wet tissues may not be suitable as orally administered intestine-coating drug delivery platforms. For example, we recently reported a bioadhesive hydrogel, which can adhere to gastric ulcer sites upon in situ gelation. However, such adhesive hydrogel cannot form the extensive and uniform adhesive coating layer on the convoluted intestinal tract surfaces due to its solid nature after rapid gelation.

Distinct from the solid bioadhesives, conventional complex coacervates are polymer-concentrated and water-immiscible liquids formed by fluid–fluid phase separation of complexed polyanions and polycations and represent an exciting class of drug delivery vehicles. However, the complex coacervation process is driven by electrostatic attraction between polyanions and polycations within narrow pH/salt ranges. Considering that the GI tract routinely undergoes substantial changes in motility, fluid content, and acidity (from pH 1.5 in the stomach to pH 6.15–7.88 in intestines), conventional complex coacervates can be easily disrupted by pH/salt variations. One recent groundbreaking work reported the precise engineering of low molecular weight sucralose into complex coacervate to increase the residence time in intestines to several hours. Despite these advances, the limited choices of polyelectrolytes species for preparing complex coacervates that can adapt to the harsh in vivo environment, together with the insufficient intestinal residence time, greatly hamper the development of fluid coacervates as the orally administered drug delivery systems.

Here we report a universal strategy to prepare water-immiscible, bioadhesive, and noncomplex liquid coacervates derived from bidentate hydrogen bonding–driven self-assembly of nanoparticles as an orally administered intestinal-coating formulation. Driven by the gastrointestinal peristalsis, the nanoparticle-assembled fluid coacervate (named NPA coacervate hereafter) can effectively spread to coat and adhere on the large intestinal surface area with a prolonged residence time of more than 2 days to mediate the sustained release of loaded drugs. Using the water-soluble dexamethasone sodium phosphate (Dex-P) as a model small molecule drug, we demonstrated that the oral administration of drug-laden NPA coacervate significantly enhanced the IBD therapeutic outcomes, improved the richness and diversity of gut microbiota, and reduced the systemic drug exposure compared with the treatment with Dex-P aqueous solution in a rat model of dextran sulfate sodium (DSS)-induced acute colitis—one of the most widely used models due to its shared clinical and histopathological characteristics with human IBD and its validation as a relevant model to treat human diseases.

Results and discussion

The design of noncomplex NPA coacervates via nanoparticle assembly. Nanoparticles required for fabricating NPA coacervates contain a hydrophobic core and end-functionalized (e.g., catechol) hydrophilic polyethylene glycol (PEG) chains as the shell. In this study, MALDI-TOF mass spectrometry was used to confirm the successful synthesis of the hydrophobic core (Supplementary Fig. 2). The molecular weight distribution of the hydrophilic PEG chains was further determined by gel permeation chromatography (GPC, Supplementary Fig. 2). Dynamic light scattering (DLS) analysis confirmed the formation of the as-prepared NP1, NP2, and NP3 nanoparticles with hydrodynamic radii of ~50–150 nm (Supplementary Fig. 3). The bidentate hydrogen–bonding and π–π interactions derived from the catechol and phenyl end groups of the hydrophilic PEG chains provided the driving force to induce the assembly of NP2 and NP3 nanoparticles and formation of the liquid NPA2 and NPA3 coacervates (G′ < G″), respectively (Fig. 1d and Supplementary Fig. 4). In contrast, the control NP1 nanoparticles with no end groups failed to form coacervates via fluid–fluid phase separation, thereby confirming the importance of the physical interactions of end groups to the formation of NPA coacervates (Supplementary Fig. 5).

The NPA2 coacervate was chosen as the model for further investigations because hydrogen–bonding interactions provided by the functionalized catechol groups that drive the coacervation of NP2 nanoparticles can mediate adhesion of the NPA2 coacervate to wet tissue surfaces. The coacervation behaviors of NPA2 can be visualized by fluid–fluid phase separation and further examined by transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR), and DLS (Fig. 1e). The dark spots marked by red circles in the TEM images are supposed to be the hydrophobic cores of the assembled NP2 nanoparticles, while the catechol-functionalized hydrophilic PEG shell acts as a flexible polymer matrix that connects the hydrophobic cores to form the stable NPA2 coacervate dense phase. It is noted that the free hydroxyl (–OH) groups generally exhibit narrow and intense absorption bands, while –OH groups involved in hydrogen bonding show a broader absorption band in FTIR and DLS (Fig. 1e). The FTIR result of freeze-dried NPA2 coacervate showed an obvious broad –OH band, indicating the existence of hydrogen bonding interactions derived from catechol groups (Supplementary Fig. 6). DLS analysis further showed hydrogen bonding-mediated assembly of NP2 nanoparticles to form coacervate micro-droplets as confirmed by the increasing hydrodynamic radius of NP2 nanoparticles after 3.5 h of dialysis (Supplementary Fig. 7).

Free from electrostatic interactions, our noncomplex NPA2 coacervate is believed to be pH- and salt-independent. Compared with conventional complex coacervates that depend on pH values, our NPA2 coacervate remained stable after 2 days and did not become a single-phase solution under a wide range of pH conditions (Fig. 1f). The frequency-dependent storage (G′) and loss (G″) moduli of the liquid NPA2 coacervate as revealed by rheological analysis demonstrates its highly dynamic polymeric structure due to the presence of supramolecular interactions, such as hydrogen bonding derived from the catechol groups (Supplementary Fig. 8). Furthermore, our noncomplex NPA2 coacervate exhibited a salting-out effect and the shear moduli (G′ and G″) and viscosity increased with increasing salt concentrations (Fig. 1g and Supplementary Fig. 9). It should be noted that electrostatic interactions between polyanions and polycations weaken with increasing salt concentrations; therefore, a critical salt concentration of 0.8–2.0 M NaCl generally can lead to the rapid dissociation of complex coacervates. However, our NPA2 coacervate remained as a viscous liquid (G′ < G″) in saline.
5.0 M NaCl, further confirming that the noncomplex NPA coacervation should be attributed to hydrogen bonding-induced nanoparticle assembly rather than electrostatic interactions. The rheological analysis confirmed the shear-thinning behavior of the NPA coacervates (Supplementary Figs. 8, 9). The viscosity of the NPA2 coacervate decreased quickly with an increasing shear rate from 0.1 to 20.0 s\(^{-1}\), thus insuring the easy injection of the NPA2 coacervate through a 21 G needle. In contrast, the NPA3 coacervate demonstrated a much lower viscosity than that of the NPA2 coacervate, and this can be attributed to the weak interaction energy of π–π interactions (<10 kJ mol\(^{-1}\)) (Fig. 1d)\(^{30}\).

**Biocompatible and digestive enzyme-resistant NPA2 coacervate possesses reversible wet bioadhesion.** Fluid NPA2 coacervate with a solid content of about 15 wt% flowed out from a tilted glass bottle and formed a durable adhesive coating on the medical nitrile glove (Fig. 2a and Supplementary Fig. 10). Furthermore,
Fig. 2 Biocompatible and fluid NPA2 coacervate is wet bioadhesive and resistant to digestive enzymes. a Fluid NPA2 coacervate formed a durable adhesive coating on the medical nitrile glove. b NPA2 coacervate can glue two ribbons of porcine skin tissue together and hold the weight of the tissues. c Schematic of the lap-shear test to measure the adhesive energy (\(G_{ad}\)). Scale bar: 10 mm. d The adhesion performance of NPA2 coacervate was much better than that of the controls. NPA2 coacervate showed adhesive energy (\(G_{ad}\)) comparable with the previously reported values (around 2–10 J m\(^{-2}\)) for nanoparticle-based adhesives and polymeric adhesives. \(n = 5\) independent lap-shear tests per group. f The hydrogen-bonding interactions derived from catechol groups ensure the stable and reversible adhesion energy level of NPA2 coacervate during the successive bonding/debonding cycles. g The viability staining of human mesenchymal stem cells (hMSCs) also suggested excellent cytocompatibility of the NPA2 coacervate. Scale bar: 200 \(\mu \text{m}\). h No significant differences in body weights were observed between the rats receiving oral administration of NPA2 coacervate on days 1, 3, 5, 7, and 9, and untreated healthy rats during the 10-day toxicity evaluation. \(n = 3\) biologically independent rats in control group, \(n = 4\) biologically independent rats in NPA2 group. i, j The fluid NPA2 coacervate coating (stained by Fast Green FCF) can adhere to the fresh and wet mucosa, flow down slowly, and remain stable after soaking in simulated gastric fluid (Ga) and simulated intestinal fluid (In) at 37 °C for 2 h, respectively. Scale bar: 15 mm. k, l GPC data of the fresh (Ga, In) and used simulated gastric or intestinal fluid (Ga’/In’) incubated with NPA2 coacervate. No degraded or dissolved NPA2 coacervate components were found in the simulated gastric or intestinal fluid, respectively. Data were presented as mean ± SD. ***p < 0.001 (Ordinary one-way ANOVA). Source data are provided as a Source Data file for Fig. 2d–f, h.
the catechol-mediated wet bioadhesion of NPA2 coacervate was sufficiently strong to glue two ribbons of the porcine skin tissue together and hold the tissue weight (Fig. 2b)\textsuperscript{15}. The adhesive energy ($G_{ad}$) of NPA2 coacervate was estimated to be ~7.07 J m$^{-2}$, similar to the previously reported values (around 2–10 J m$^{-2}$) for nanoparticle-based\textsuperscript{14} and polymeric adhesives (Fig. 2c, d, e and Supplementary Fig. 11)\textsuperscript{15,16}. The NPA2 coacervate-bonded porcine skin joints can withstand an applied force five times greater than that of the homolog control groups (NPA1 nanoparticle solution and NPA3 coacervate). Furthermore, the peak adhesion force of the NPA3 coacervate was achieved at a much larger deformation, indicating the capability to effectively dissipate a large amount of energy under a mechanical challenge (Fig. 2d). The low adhesion energy of the control homologs can be attributed to the weak bulk cohesion (NPA1 solution) or interfacial adhesion (NPA3 coacervate), respectively. Therefore, although the π–π interaction can facilitate coacervation, the negligible bioadhesion of the NPA3 coacervate makes it less suitable for drug delivery in the intestinal tract. Furthermore, reversible hydrogen bonding interactions derived from the catechol groups ensure the stable adhesion energy level of the NPA2 coacervate during the bonding/debonding cycles (Fig. 2f). Consistent with the observed bioadhesion on porcine skins, the NPA2 coacervate showed decent mucoadhesion (~2.74 J m$^{-2}$) on the luminal surface of porcine intestines (Supplementary Fig. 12), which can be attributed to the physical interactions between the catechol/PEG structure of coacervate and glycosylated mucins\textsuperscript{47,48}.

To evaluate the safety of oral administration, we first tested the cytotoxicity of our NPA2 coacervate by incubating it with human mesenchymal stem cells (hMSCs). Viability staining and cell metabolic assays (MTT) of hMSCs incubated with NPA2 coacervate for 7 days suggested excellent cytocompatibility of the NPA2 coacervates (Fig. 2g and Supplementary Fig. 13). Furthermore, Sprague Dawley (SD) rats were gavaged with NPA2 coacervate (4 g per kg rat) every other day for 10 days. During the 10-day exposure period, the rats receiving oral administration of coacervate did not develop any abnormal symptoms such as diarrhea and showed no significant weight change compared with the untreated healthy rats, further confirming the minimal toxicity of NPA coacervates (Fig. 2h).

We next investigated the influence of the physical peristalsis and chemical environment (gastric acid and intestinal fluid) of the GI tract on NPA2 coacervate coating by using simulated ex vivo experiments. When deposited on the upright intestinal mucosa surface, the fluid NPA2 coacervate can adhere to the fresh and wet mucosa and steadily flow down driven by gravity, leaving a trailing adhesive coating layer (Fig. 2i). After soaking the NPA2 coacervate-coated intestinal mucosa tissues in simulated gastric fluid (Ga) or simulated intestinal fluid (In) at 37 °C for 2 h, respectively, the adherent coacervate coatings remained undiluted and maintained adhesion on the mucosa surface (Fig. 2) and Supplementary Fig. 14). The NPA2 coacervate also remained largely stable in the 25 mg/ml pig bile salt or under the high shear strain (up to 1000%) despite a slight dip in shear moduli toward the high shear range (Supplementary Figs. 15, 16). In addition, the digestive enzyme-resistant polymeric structure of the NPA2 coacervate was further confirmed by incubating NPA2 coacervate in simulated gastric fluid (Ga) and simulated intestinal fluid (In) at 37 °C for 3 days, respectively (Fig. 2k). GPC analysis showed that no degraded or dissolved NPA2 coacervate components were found in the simulated body fluids (Ga’, In’) after 3 days of incubation (Fig. 2i). It is noted that the nontoxic, biologically, and chemically inert polyurethanes are widely used in clinical practices\textsuperscript{49,50} and a polyurethane-based surgical adhesive (TissuGlu\®) has been approved by United States Food and Drug Administration (FDA) for various clinical applications.

Therefore, we believe that the stable polyurethane network of NPA2 coacervate helps establish the feasibility for the following in vivo evaluations. Besides, our laboratory-scale synthesis yielded ~300 ml of NPA2 coacervates per batch and showed stable batch-to-batch reproducibility, indicating the promising potential for large-scale production of NPA2 coacervates.

**NPA2 coacervate optimizes drug release with extended GI residence time in vivo.** We believe that the water-immiscible, bioadhesive, and liquid nature of our noncomplex NPA2 coacervate can adapt to the complex motility, fluid content, and sharp pH variation within the GI tract, and establish a large-area durable coating on the intestinal mucosa to ensure sufficient intestinal retention for sustained drug release. To further test this hypothesis, the capability of NPA coacervates as bioadhesive coatings on the gastrointestinal mucosa was further evaluated in vivo (Fig. 3a). Sprague Dawley (SD) rats with unrestricted access to water and standard laboratory diet received a single oral gavage of 1.0 ml NPA2 or NPA2 coacervate (modified with a Cy7 tag, a near-infrared fluorescent dye) and were sacrificed after 0, 6, 24, or 48 h to evaluate the fluorescence retention in the harvested GI tract (Fig. 3b). In agreement with the in vitro bioadhesive property, the NPA2 coacervate adhered to the rat GI tract for at least 48 h as evidenced by the intense fluorescence signal, whereas the NPA1 coacervate had limited retention in the GI tract after 1 day (Fig. 3b). Normal gastric emptying takes place within 2 h, and colonic arrival occurs after 5 h\textsuperscript{51}. Therefore, the in vivo intestinal retention experiments demonstrated the prolonged retention of our NPA2 coacervate in the GI tract.

We further evaluated the efficacy of the NPA2 coacervate to mediate sustained drug release both in vivo and in vitro. We utilized Dex-P, a water-soluble sodium phosphate salt of dexamethasone (Dex), as a model anti-inflammatory drug\textsuperscript{52}. The NPA2 coacervate showed a high Dex-P encapsulation efficiency (the amount of Dex-P loaded in the NPA2 coacervate divided by the total Dex-P input) of ~42% (Fig. 3c and Supplementary Fig. 17). The partition coefficient (K), which was defined as the ratio between the concentration of Dex-P in the NPA2 coacervate and the supernatant Dex-P concentration, was around 3.62 (Fig. 3d). In addition to the excellent drug loading capacity, our NPA2 coacervate showed prolonged release of preloaded Dex-P under both in vitro (Fig. 3e) and in vivo conditions (Fig. 3f). We next examined the drug release kinetics in vivo by monitoring the serum levels of Dex-P at selected time points after oral gavage of Dex-P-laden NPA2 coacervate or Dex-P aqueous solution (Fig. 3f). The serum concentration of Dex in SD rats spiked at 1 h after oral gavage of the Dex-P solution and then decreased rapidly (Dex-P/PBS). In contrast, the serum Dex concentration in the SD rats receiving oral gavage of Dex-P-laden NPA2 coacervate (Dex-P/NPA2) remained consistently at the lower therapeutic level for over 40 h (Fig. 3f). The total dosage of Dex-P administered in the two groups was the same. Therefore, our NPA2 coacervate can mediate sustained release and improve the pharmacokinetics of the preloaded drug in the SD rat model, thereby reducing dosing frequency and systemic drug exposure.

It is noted that ulcerative colitis is a chronic inflammatory disorder limited to the colonic and rectal mucosa\textsuperscript{53} and shows similar gastric and small intestinal transit time as that of healthy controls\textsuperscript{34,35}. Because absorption of the Dex-P, a water-soluble small molecule drug, mainly occurs in the small intestine in the absence of a drug carrier\textsuperscript{56}, the serum Dex concentrations of DSS-induced colitic rats and healthy rats receiving the equivalent amount of Dex-P in PBS (Dex-P/PBS) were similar (Supplementary Fig. 18). We further tested the capability of NPA2 coacervates...
as bioadhesive coatings to mediate the sustained release of drugs in a DSS-induced colitic rat model. The observed retention of fluorescently-label coacervate in the harvested GI tract revealed that the NPA2 coacervate can adhere to the GI tract of colitic rats for at least 48 h, thus confirming the similar retention time of our NPA2 coacervate in the GI tract of colitic rats compared with that of the healthy rats (Supplementary Fig. 19), and this explained the observed similar release and uptake kinetics of coacervate-delivered Dex-P (as indicated by serum Dex concentration) between the colitic and healthy rats (Supplementary Figs. 19, 20).

To demonstrate the versatility of NPA2 coacervate to mediate sustained drug release, other first-line small molecule drugs used to treat IBD including antibiotic metronidazole, anti-inflammatory 5-aminosalicylic acid (5-ASA), and immunoregulatory methotrexate disodium salt (MTX) were also encapsulated into the NPA2 coacervate to investigate the release kinetics.
Dex-P/NPA2 treatment against DSS-induced acute colitis. Dex-P/distal colon. such as interleukin proteins (ZO-1 and occludin-1) and pro-inflammatory cytokines, such as interleukin-10, IL-6, and TNF-α, were observed in untreated colitic SD rats (Control) or rats treated with the equivalent amount of Dex-P solution in PBS (Dex-P/PBS, Fig. 4e). Furthermore, histopathology scoring of H&E-stained tissue sections was used to evaluate the severity of colonic histological damage in a blinded fashion by a trained pathologist. Disease severity in colitic SD rats receiving Dex-P/PNA2 decreased significantly (mean histopathology score, 0.500) compared with colitic SD rats in the Dex-P/PBS group (mean histopathology score, 1.917) and the untreated control group (mean histopathology score, 3.000). Histopathology scores of colitic SD rats in the Dex-P/PBS group were not significantly different from the untreated control group (P = 0.056, Fig. 4f).

Colonic MPO activity in colitic SD rats receiving Dex-P/NPA2 was also significantly reduced compared with the untreated control group (Fig. 4g). Although we also observed a reduction of MPO activity in the Dex-P/PBS group due to the therapeutic activity of Dex-P against IBD, the high serum Dex level associated with such administration of Dex-P aqueous solution indicates an increased risk of complications related to severe systemic drug exposure (Fig. 3e). We have also tested the therapeutic efficacy of NPA2 alone to treat colitic rats via oral gavage on days 1, 3, and 5 and then analyzed the MPO activity and histopathology score of colon tissues on day 7 (Supplementary Fig. 23). The therapeutic efficacy of treatment with NPA2 alone did not show a significant difference compared with that of the non-treated colitic rats (Control) as evidenced by the similar MPO activity, histopathology score, and H&E staining, thus confirming that the NPA2 coacervate only served as a drug delivery vehicle and did not mitigate the colitic symptoms. In addition, colitic SD rats receiving Dex-P/NPA2 recovered the expression of tight junction-associated proteins including ZO-1 and occludin-1 and showed significantly reduced local levels of IL-1β, IL-6, and TNF compared with the untreated group and Dex-P/PBS group (Fig. 4h, i and Supplementary Figs. 24, 25). Taken together, oral delivery of Dex-P encapsulated in NPA2 coacervate to colitic SD rats showed significantly enhanced therapeutic outcomes and reduced systemic exposure than administering the equivalent amount of Dex-P in an aqueous solution (Supplementary Fig. 26).

Dex-P/NPA2 regulates innate immune responses and restores the gut microbiota. Macrophage activation can be broadly classified into the pro-inflammatory M1 polarization or anti-inflammatory M2 polarization, and macrophages play a key role in the maintenance of mucosal homeostasis by secreting many cytokines. Therefore, we next investigated the intestinal immune responses of colitic SD rats by analyzing macrophage polarizations in the harvested colon tissues from colitic rats receiving Dex-P/NPA2 or Dex-P/PBS and untreated colitic SD rats (Control) on day 5 (Fig. 5a). Many studies have shown that dexamethasone (Dex), a corticosteroid, can promote anti-inflammatory M2 macrophage polarization and suppress pro-inflammatory M1 polarization, thus enhancing the secretion of anti-inflammatory cytokines, such as IL-10, IL-6, and TNF-α. Furthermore, Dex-P/NPA2 treatment significantly reduced the local levels of pro-inflammatory IL-1β and IL-6 released by M1 macrophages (Fig. 5e) and increased the level of anti-inflammatory IL-10 (Fig. 5f). Taken together, oral administration of Dex-P/NPA2 to colitic SD rats promoted the M2 polarization of intestinal macrophages and mediated strong anti-inflammatory effects against acute colitis, thereby creating a favorable immune microenvironment to promote colon repair and regeneration (Fig. 5c, d).

Dysbiosis of gut microbiota, which is implicated in various inflammatory and immune diseases, plays a key role in the pathogenesis of IBD, while the chronic inflammation caused by IBD, in turn, promotes dysbiosis of gut microbiota. Therefore, restoring gut microbiota and recovery of gut immune homeostasis can complement each other in the successful treatment of acute colitis. Although orally administered Dex-P may not
**Fig. 4** Dex-P-laden NPA2 coacervate demonstrates enhanced therapeutic efficacy in a rat model of DSS-induced colitis. 

**a, b** SD rats were given 4.5% DSS in drinking water to induce acute colitis. The colitic rats received oral gavages of Dex-P-laden NPA2 coacervates (Dex-P/NPA2) or the equivalent amount of Dex-P in PBS (Dex-P/PBS) on days 1, 3, and 5. Untreated colitic SD rats were used as the negative control (Control). All SD rats were sacrificed on day 7. 

**c, d** Colonic edema and diarrhea caused by DSS-induced colitis in SD rats receiving Dex-P/NPA2 were significantly relieved compared with that of the untreated colitic SD rats (Control) and colitic SD rats receiving Dex-P in PBS (Dex-P/PBS). Scale bar: 10 mm. 

**e** Representative images of H&E staining demonstrated that histological inflammation was diminished in the colitic SD rats receiving Dex-P/NPA2, while histological damages were observed in untreated colitic SD rats (Control) or colitic SD rats receiving Dex-P/PBS. Scale bar: 150 μm. 

**f-i** On day 7, colon tissues were analyzed for histopathology score **f**, MPO activity **g**, mRNA levels of tight junction-associated proteins including ZO-1 and occludin-1 **h**, and pro-inflammatory cytokines including interleukin IL-1β and TNF **i**. Data were presented as mean ± SD. 

*n = 6* biologically independent rats per group. *p < 0.05, **p < 0.01, ***p < 0.001 (Ordinary one-way ANOVA). Source data are provided as a Source Data file for Fig. 4d, f-i.
directly modulate the gut microbiota\(^6^9\), we expected the enhanced gut immune microenvironment by Dex-P/NPA\(_2\) treatment can positively regulate the gut microbiota. Analyses of fecal samples collected from colitic SD rats on day 5 by sequencing the V4 region of the 16S ribosomal ribonucleic acid (rRNA) gene showed that Dex-P/NPA\(_2\) treatment indeed increased the bacterial richness (observed operational taxonomic units, OTUs) and diversity (Chao and Shannon indices) in colitic SD rats (Fig. 5g, h). In addition, a heatmap of β-diversity distance distribution was generated, and samples with similar β-diversity were clustered to reflect the similar compositions of gut microbiota (Fig. 5i). The β-diversities between colitic SD rats receiving Dex-P/NPA\(_2\) and healthy SD rats were clustered more closely compared with colitic SD rats in the Dex-P/PBS group and...
the untreated control group (Control), thus suggesting the enhanced recovery of gut microbiota in colitic SD rats by Dex-P/NPA2 treatment. This was further confirmed by the taxonomic bacterial distribution histogram and clustered heatmap based on the relative abundance of gut microbiota at the family level (Fig. 5j, k).

In conclusion, we have developed a simple yet highly efficient strategy for oral drug delivery by using a nanoparticle-assembled (NPA) biodhesive noncomplex coacervate. The NPA coacervate exhibited: (1) pH and salt-independent stability under the complex environment within the GI tract; (2) effective coating of large intestinal surface and prolonged intestinal retention for several days; and (3) enhanced therapeutic efficacy for IBD and gut microbiota restoration with reduced systemic drug exposure via the controlled release of a loaded drug. When used for clinical treatments, these highly desired attributes of NPA coacervate-based oral drug delivery will potentially enhance patient compliance and acceptance and therefore the eventual therapeutic outcomes, especially compared with conventional therapies such as enema, subcutaneous and intravenous injection. Although we used Dex-P as a model drug to treat IBD in this study, our biodhesive NPA2 coacervate can effectively deliver many other drugs, especially the water-soluble small molecule drugs, which are usually difficult to achieve sustained-release kinetics by using the existing oral drug delivery platforms. Therefore, we believe that the NPA coacervate-based drug delivery represents a promising approach to the treatment of numerous gastrointestinal diseases, including mucosal wound healing, gastrointestinal cancers, peptic ulcers, and viral infections.

Methods

In situ assembly of as-prepared core-shell nanoparticles into NPA coacervate.

The concentrated amphiphilic polymers composed of hydrophilic poly(ethylene glycol) (PEG) and hydrophobic segments in 20 mL DMF (27 wt%) were dropped into cold deionized water (60 mL) with 600 rpm magnetic stirring to form the core-shell nanoparticles (NP1, NP2, and NP3), respectively. The nanoparticle solutions then were dialyzed against cold deionized water (standard regenerated cellulose (RC) dialysis membrane with 3.5 kDa cutoff, Spectrum Chemical) at room temperature for 24 h to form NPA coacervates via nanoparticle assembly. To determine the successful synthesis and the molecular weight of the hydrophobic segment of the amphiphilic polymers, 1.5 μL of the hydrophobic segment in methanol (1 mg/mL) was premixed with 1.5 μL of matrix solution (saturated α-cyan-4-hydroxy cinnamic acid, Sigma). Then this mixture was applied to a ground steel target that was ready for mass spectrometry. Finally, the analyses of air-dried samples were performed in an AutoFlex Speed LRF MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) under reflector mode.

In vitro biocompatibility test.

Passage 4 hMSCs (8 × 10^4 cells/well, Lonza, Catalog #: PT-2501) were incubated in six-well plates. Each well was added 2 mL growth medium consisting of α-minimum essential medium (Gibco) supplemented with 16.7% fetal bovine serum (FBS, Gibco), 1% glutamine (Sigma), and 1% penicillin/streptomycin (Sigma). NPA2 coacervates with a final concentration of 5 mg/mL were added. The control groups were not added NPA2 coacervates. The medium was changed every 2 days. Live/dead staining (Thermo Fisher) was performed on day 7. hMSCs were imaged using a confocal laser scanning microscope (Nikon C2). For the MTT assay, hMSCs (0.6 × 10^4 cells/well) were incubated in a 96-well plate in the growth medium. About 5 mg/mL NPA2 coacervates were used to culture the cells for 7 days. After that, 10 μL MTT (0.3 mg/mL) was added to each well to produce formazan crystals according to the manufacturer’s instructions (Thermo Fisher), which were further dissolved by 200 μL of DMSO (Sigma). Finally, a Multiscan microplate reader (Thermo Fisher) was used to collect the absorbance value of each well at 540 nm for cell metabolic calculation.

Animals.

Sprague Dawley (SD) rats (200–300 g, female, 8–12 weeks) were obtained from the Laboratory Animal Services Centre (Chinese University of Hong Kong) and cohoused for a week before being randomly assigned to different groups. Animal experiments were approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong (19-152-MIS) and were carried out in accordance with the guidelines of the Animals Ordinance (Chapter 340), Department of Health, Hong Kong.

Oral toxicity test.

SD rats (200–300 g, female, 8–12 weeks) receiving oral administration of NPA2 coacervate (4 mg per kg rat) on days 1, 3, 5, 7, and 9 during the 10-day toxicity evaluation and untreated healthy rats were used as the control group. Body weights were measured. Any abnormal symptoms such as diarrhea were recorded.

Ex vivo adhesion experiments.

Fresh intestinal tissues were bought from Sha Tin Market (Hong Kong), stored on ice, and washed with PBS buffer (1×) three times before use. For ex vivo adhesion testing, NPA2 coacervate stained by Fast Green FCF (Sigma) was deposited on the upright wet intestinal mucosa surface to monitor gravity-induced fluid adhesion behaviors of NPA2 coacervate over time. Then the above NPA2 coacervate-coated intestinal tissues were soaked in simulated gastric fluid (Ga) or simulated intestinal fluid (In) at 37 °C for 2 h respectively. To monitor changes of the adherent coacervate coatings, photos were taken before and after the soaking and analyzed by Imagej 1.80_.172. Simulated gastric fluid (Ga) and simulated intestinal fluid (In) with enzymes were prepared following United States Pharmacopeias (USP).

Digestive enzyme-resistant test.

NPA2 coacervates were incubated in simulated gastric fluid (Ga) and simulated intestinal fluid (In) at 37 °C for 3 days, respectively. GPC (Aglent system, 1260 Infinity II) with a refractive index detector was used to detect whether the degraded or dissolved NPA2 coacervate components existed in the simulated body fluids (Ga’, In’) after 3 days of incubation. PBS (1×, Gibco) was used as the elution phase. NPA2 coacervate was also placed in 1× PBS buffer with pig bile salt (25 mg/mL, Solarbio) for 24 h at 37 °C to test its stability.

In vivo NPA coacervates adhesion experiments.

Sprague Dawley (SD) rats (female, 8–12 weeks) weighing around 250 g were housed in groups of three rats per cage. SD rats were allowed unrestricted access to water and a standard laboratory diet before and after oral gavage. SD rats weighing around 250 g received 4.5% Dextran sodium sulfate (DSS, 40 kDa, Alfa Aesar) supplemented in the drinking water for 7 days to induce colitis, followed by normal water during the treatment. Healthy SD rats or DSS-induced colitic rats received a single oral gavage of 1.0 mL NPA2 or NPA3 coacervate (modified with a Cy7 tag, a near-infrared MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) under reflector mode.

The color Chao diversity, and phylogenetic β diversity illustrated that colitic SD rats receiving Dex-P/NPA2 and healthy SD rats were clustered more closely, suggesting more similar bacterial compositions. The α diversity index means the greater differences between samples. A clustered heatmap of UniFrac values for measuring gut microbiota β diversity indicates that colitic SD rats receiving Dex-P/NPA2 and healthy SD rats were clustered more closely, suggesting more similar bacterial compositions. The color of the square shows the distance of evolution between each two samples. The range of blue to red corresponds to close to far distance, and the bigger index means the greater differences between samples.
fluorescent dye). Briefly, an amine-NCO reaction between Cyanine 7 amine (0.2 mg, Lumiprobe) and polymerized PEG with terminated NCO groups (1 at 50% w/w) was used to modify the hydrophilic shell of NP nanoparticles with a Cy7 tag. Then SD rats were sacrificed after 0, 6, 24, 48 h, and GI tracts were harvested to evaluate the fluorescence retention by an in vivo live imaging system IVIS200 (Xenogen) with an ICG filter channel to measure the fluorescent signal intensity.

**In vitro and in vivo drug release.** For in vitro Dex-P release, 2 ml of Dex-P in PBS (1.5 mg/ml, TCI) was added to 1 ml NPA2 coacervate followed by vortex for 15 s for Dex-P concentration. The changes of supernatant Dex-P concentrations were measured by a Shimadzu UV-3600 UV–vis-NIR spectrophotometer at 242 nm. Then 2 ml fresh PBS buffer (1×, Gibco) was added to the as-prepared Dex-P-laden NPA2 coacervate (1 ml). Dex-P concentrations in the above PBS were measured at 242 nm. The protocols for in vitro release of other first-line small molecule drugs used are similar. For in vivo release of antibacterial metronidazole (Mtro, J&K), anti-inflammatory 5-aminosalicylic acid (5-ASA, J&K), and immunoregulatory methotrexate disodium salt (MTX, Alfa Aesar) were the same as in vitro Dex-P release, but the release of Metrotro, 5-ASA, and MTX was measured at 320, 331, and 371 nm, respectively.

For in vivo Dex-P release, healthy SD rats or DSS-induced colitic rats (200–300 g, female, 8–12 week) received a single oral gavage of 1.0 ml as-prepared Dex-P-laden NPA2 coacervate (Dex-P/NPA2) or Dex-P aqueous solution (Dex-P/PBS). The total dosage of Dex-P administered in the two groups was the same (~1.15 mg). SD rats were allowed unrestricted access to water and a standard laboratory diet before and after oral gavage. Blood was collected from the tail vein at selected time points after oral gavage of Dex-P-laden NPA2 coacervate or Dex-P aqueous solution to measure the serum Dex concentrations by a commercial Dexamethasone ELISA assay (Neogen Corporation) following the manufacturer’s instructions.

**DSS-induced rat models of colitis.** SD rats (female, 8–12 weeks) weighing around 250 g received 4.5% Dextran sodium sulfate (DSS, 40kDa, Alfa Aesar) suspended in the drinking water for 7 days to induce colitis, foliated by normal water during the treatment. Healthy SD rats were provided with normal water only. Then NPA2 coacervate only, Dex-P-laden NPA2 coacervates (Dex-P/NPA2) or the equivalent amount of Dex-P (1.15 mg, MCE, USA) in PBS (Dex-P/PBS) on days 1, 3, and 5 was administered via an oral route into colitic SD rats. BI-DPO also tested to treat DSS-induced colitic rats with the equivalent amount of Dex-P (1.15 mg, MCE, USA) in PBS (Dex-P/PBS) via enema on days 1, 3, and 5. Dex-P was used at 1.15 mg per dose based on the published reports related to treatments in rodent colitis models. Untreated colitic SD rats were used as a negative control. All SD rats were allowed unrestricted access to water and a standard laboratory diet before and after oral gavage. All SD rats were sacrificed on day 7, and the entire colon was excised and gently washed with physiological saline for further evaluation of colon weight and length, histological severity, IBD-associated colonic myeloperoxidase (MPO)-activity, expression of related mRNAs, immunofluorescence staining against tight junction-associated proteins including ZO-1 and occludin-1 (Santa Cruz), and ELISA tests for IL-6 and TNF (MultiSciences).

On day 5 of the treatment, colon tissues from colitic rats receiving Dex-P/NPA2 or Dex-P/PBS on days 1, 3, and 5 and untreated colitic SD rats (Control) were harvested to study the intestinal immune responses of colitic SD rats by analyzing macrophage polarization. And fresh fecal samples were collected from the above colitic SD rats in each group on day 5 and stored at −80 °C in autoclaved sterile vials for further analysis of gut microbiota.

**Immunol staining.** Immunohistochemical staining for macrophage polarization was performed using an antibody against CD206 (M2 marker, Abcam, ab64693, dilution of 1:200) or iNOS (M1 marker, Abcam, ab283655, dilution of 1:200), followed by Vectastain ABC Kit (Vector Laboratories) and the DAB (3, 3-diaminobenzidine) Substrate Kit (Vector Laboratories). Briefly, paraffin sections (7 μm thick) of distal colon tissues were deparaffinized and rehydrated. Then antigen retrieval of sections was performed in microwave-assistant hot antigen retrieval buffer (1×, citrate buffer pH 6.0, Abcam). Sections were further incubated with 10% horse serum (Thermo Fisher) for 30 min to block nonspecific binding sites. Next sections were incubated at 4 °C overnight with primary antibodies directed against ZO-1 conjugated with Alexa Fluor® 594 (dilution of 1:200) and occludin-1 conjugated with Alexa Fluor® 488 (dilution of 1:200) and nuclei were stained with 4′,6-diamidino-2-phenylindole (Thermo Fisher, dilution of 1:1000).

**Statistical analysis.** All data were presented as mean ± standard deviation. Statistical analyses were performed by using ordinary one-way ANOVA with Tukey’s post hoc testing or two-tailed Student’s t-test to compare multiple groups or two groups (Graphpad Prism 7.0). In all cases, p values less than 0.05 were considered statistically significant differences among the compared groups, to which different asterisks were assigned (*p < 0.05; **p < 0.01; ***p < 0.001). For all multiple comparisons, the mean of each column with the mean of every other column was compared.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The datasets for gut microbiota generated in this study have been deposited in the GEO repository under accession code GSE167138, Ribosomal Database Project (RDP) Classifier is "publicly available [https://sourceforge.net/projects/rdp-classifier/files/rdp-classifier]/". All other relevant data supporting the key findings of this study are available within the article and its Supplementary Information files or from the corresponding author upon reasonable request. Source data are provided with this paper.

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