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ARTICLE

A CMP-based Method for Tunable, Cell-mediated Gene Delivery from Collagen Scaffolds

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The use of collagen-based biomaterials in regenerative medicine has rapidly increased over the past decade. The unique structural and biochemical properties of collagen make it a particularly promising material for delivering both protein and DNA-based therapeutics. Although many collagen modification techniques have been developed, the majority of them require multi-step chemical treatments that can modify the natural favourable properties of collagen. We have developed a promising biomimetic modification technique employing collagen-mimetic peptides (CMP)s to control the retention and delivery of DNA polyplexes from collagen structures, including both monomeric 2-D collagen films and fibrous, 3-D gels. Variations in the concentration of CMPs displayed on polyplexes enabled tuning of polyplex retention *vs.* release over periods of at least 2 weeks on films and a month on gels. Retention of CMP-modified polyplexes (20 days) was substantially improved compared to non-modified polyplexes, which were retained for only 2 days. The activity of bound polyplex in collagen gels was shown, through a series of transfection studies, to be maintained in the presence of serum for a minimum of 2 weeks. Only matrix metalloproteinase (MMP)-stimulated cells exhibited significant levels of transfection suggesting that cell mobility within the gel was vital and that collagen remodelling played a role in stimulating gene release and expression. To our knowledge, this study is the first to deliver genes with CMP-modified polyplexes and to examine the effects of CMP display on DNA release. The results suggest that this technique may be used more broadly to create tuneable, collagen-based delivery systems.

Introduction

Collagen is the most abundant mammalian protein, and is the principal component of all connective tissues. It provides both mechanical strength and structural integrity, while also playing an essential role in the regulation of key processes underlying tissue development and regeneration.[1,2] In addition, collagen serves as a cell-responsive reservoir for a wide array of essential, highly potent growth factors (GF)s and other signalling molecules which allows for rapid, on-demand, and highly localized cellular responses without *de novo* synthesis.[3,4]

Collagen's natural potential in regenerative medicine applications has long been recognized.[1-7] Its earliest uses as an absorbable suture date back as far as the early second century A.D.[8] Over the past two decades, collagen-based biomaterials have become widely used in both research and medicine due to the structural tuneability and functional versatility of collagen.[1-2] Collagen-based devices have been tailored to present chemotactic and/or structural gradients among other features.[1-2,9-10] However, while collagens and other biomaterial scaffolds generally improve the regeneration of many tissues such as skin and bone, the incidence of complete healing after treatment remains low.[9,11-13] For example, only ~50% of diabetic foot ulcers were found to fully close during a therapeutic trial employing artificial skin.[14]

Incomplete healing and a failure to meet clinical standards have led to an increased interest in combining biomaterial scaffolds with GFs and other key signalling molecules. The topical and sustained application of various GFs has been examined for many applications,[14-15] and GF therapies such as FDA-approved REGRANEX® Gel (containing PDGF) have been shown to enhance repair in tissues such as chronic wounds.[14] However, REGRANEX® and other GF therapies exhibit modest clinical utility overall.[15-20] The high GF doses necessary for improved repair increase the risk of cancer,[21] and inefficient GF delivery, GF instability, and off-target effects are key issues hindering development.[22-24] Gene-based GF approaches have enormous potential to address these issues. Gene delivery can promote the sustained, localized production of fresh, bioactive GF, and spatial control may be attained through the use of tissue-specific promoters.[25] Gene-based approaches to deliver PDGF, VEGF, and other factors can promote enhanced healing at greatly reduced doses as compared to topical GF, [26-28] yet gene-based strategies often fail to reach the clinic due to inefficient gene transfer and concerns about off-target expression.[26-35]

Biomaterial-mediated delivery of therapeutic proteins and genes provides an opportunity to combine the benefits of the structural/bioactive properties of the biomaterial with improved delivery of GFs and other factors. Delivery from a substrate mimics natural mechanisms of GF and viral delivery, increases spatial and temporal control over release, and often improves stability and prevents immune recognition.[22,36-42] Indeed, collagen-based systems have previously been used to mediate gene delivery in many past studies based on electrostatic or physical immobilization.[40,43-48] Despite promising results in terms of improving therapeutic efficacy when compared to bolus delivery,[43-48] key issues with electrostatic and physical methods, including insufficiencies in promoting strictly local delivery and tuneable release, have decreased the efficacy of these approaches and led to safety concerns.[35] Chemical modification, such as the chemical cross-linking of collagen containing therapeutic DNA,[49,50] or the covalent modification of collagen to increase affinity for a therapeutic molecule (i.e. biotinylation),[37] has allowed for greater control over release, allowing the local delivery of DNA encoding factors such as bFGF and PDGF.[51] Despite these encouraging results, many chemical modifications techniques damage the bioactive compounds,[37,52] and the complexity of collagen often makes covalent modification difficult.

A recently developed collagen modification technique, based on the affinity of collagen-mimetic peptides (CMP)s for native collagen, offers many appealing advantages. Specifically, short peptides comprised of collagen-like $(GXY)_n$ motifs can be thermally annealed with collagens, and *via* strand exchange, these peptides can be incorporated into the native collagen triple helix. The melting behaviour of CMP-collagen assemblies is highly tuneable through manipulation of CMP amino acid composition and molecular weight.[5,53-57] Both *in vitro* and *in vivo* studies have demonstrated that CMPs can selectively detect tiny quantities of collagen (e.g. 5 ng), target regions with increased collagen remodelling, such as tumours and joints, and significantly improve retention of a myriad of cargoes.[54, 56-57] CMPs containing bioactive sequences, such as the integrin-binding sites GEKGER, can be used to modulate cell behaviour, such as the adhesive/migratory responses in human MSCs.[56-57] However, while proline-rich CMPs were shown to aid in bolus transport of plasmid DNA into cells,[55] no study has examined the benefits of using CMPs to increase gene retention in collagen.

In this paper, we introduce a novel, CMP-based approach for creating DNA polyplex-modified collagens with tailorable release profiles and improved gene transfer, as shown in Figure 1. Controlled release was achieved by varying the display of CMPs on the polyplexes. The CMPs used in this study were designed to act as both adjustable tethers to control the affinity between the collagen and the polyplexes, as well as adhesive/endocytic ligands, as these proline-rich CMPs contained the amino acid sequence GEKGER that was previously shown to engage $\alpha_2\beta_1$ integrin.[56,57] We anticipated that the use of CMPs would allow highly stable, tuneable

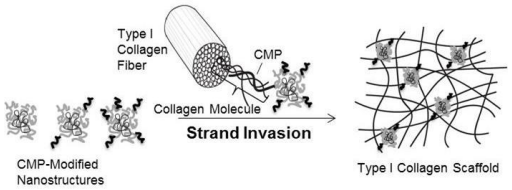


Figure 1. Tailorable CMP-based approach for producing DNA polyplex – modified collagen scaffolds. CMP-modified polyplexes are bound to collagen via thermally induced annealing that induces CMP strand invasion and CMP-collagen triple helical hybridization. The versatility of the CMP allows it to perform as both a reversible tether and an integrin-binding ligand. Controlled retention and release may be achieved through variation of CMP display on the nanostructures.

attachment of DNA polyplexes to various collagen-based substrates, as well as CMP-linked polyplex release during MMP-mediated collagen remodelling. Accordingly, herein we demonstrate the versatile modification of different collagen structures with CMP-linked polyplexes. CMPs were synthesized by solid-phase peptide synthesis methods, and these CMPs were used to modify DNA polyplexes *via* Michael-type addition chemistry.[57] Retention of fluor-modified CMPs on 2-D and in 3-D collagen scaffolds illustrated a direct dependence between polyplex retention and CMP incorporation, where polyplexes remained attached to collagen scaffolds for days to over a month depending on the level of CMP incorporation and the CMP sequence. Additionally, the CMP-linked polyplexes exhibited significantly higher stability and activity than electrostatically sequestered polyplexes or free polyplexes, indicating that the CMP/collagen system had significant benefits as compared to existing collagen-based gene delivery systems by acting as a gene-stabilizing depot to promote transfection with reduced dosing and a lower probability of off-target effects. The ability to tailor nanostructure retention in collagen over prolonged time periods *via* physical modification, combined with the capacity to provide "on-demand" release and collagen-mediated uptake, has broad implications for enhanced healing in a variety of applications in regenerative medicine.

Experimental

Materials

Fmoc-protected amino acids were purchased from Anaspec (Fremont, CA) and H-Rink amide ChemMatrix® resin was purchased from PCAS Biomatrix (Quebec, Canada). *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and the rink amide 4-methylbenzhydrylamine (MBHA) resin were purchased from Novabiochem (San Diego, CA). High-performance liquid chromatography (HPLC)-grade *N*,*N*-dimethyl formamide (DMF), acetonitrile, trifluoroacetic acid (TFA), and cell culture reagents, including Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (PBS), and

tryspin were purchased from Fisher Scientific (Fairlawn, NJ). Piperidine, 4-methylmorpholine, all cleavage cocktail components, and branched polyethylenimine (PEI, 25 kDa) were purchased from Sigma-Aldrich (St. Louis, MO). Type I bovine collagen was purchased from Advanced BioMatrix (San Diego, CA) and AlexaFluor555 was purchased from Life Technologies (Carlsbad, CA). pCMV-GLuc plasmid was purchased from New England Biolabs (Ipswich, MA). The plasmid was amplified in NEB 5-α electrocompetent *E. coli* purchased from New England Biolabs and purified from bacterial culture using a Qiagen Megaprep Kit (Valencia, CA), according to the manufacturer's protocols.

Collagen-mimetic Peptide (CMP) Synthesis

Two CMP sequences (GPP and GPO) and a scrambled CMP (GPO^S) were synthesized using automated Fmoc solid-phase peptide synthesis (SPPS) using a PS3 or Tribute™ peptide synthesizer (Protein Technologies Inc., Tuscon, AZ). The sequences are summarized in Table 1.

The GPP sequence was synthesized as previously described[56-57] on a Rink amide MBHA resin, and the GPO and GPO^S sequences were synthesized in a similar manner on a Rink amide ChemMatrix® resin. Specifically, for all three sequences, amino acid residues were activated for coupling with HBTU in 0.4 M methylmorpholine in DMF and de-protected using 20% piperidine in DMF for 10 min. For the GPP sequence, 1 h coupling times were used and all residues after the 15^{th} residue were double coupled. For the GPO and GPO^S sequences, 2 h coupling times were used and all amino acids after the 10^{th} residue were double coupled. The GPP sequence was cleaved from the resin using a cocktail of 94:1:2.5:2.5 TFA/triisopropylsilane (TIS)/water/1,2-ethanedithiol (EDT) for 6 h, whereas the GPO and GPO^S sequences were cleaved from the resin in the same cocktail for 3 h. After cleavage, the cocktail was evaporated and the cleaved peptides were precipitated in ethyl ether, dissolved in water, and lyophilized. Fluorescently labeled CMPs were produced by using carboxyl-amine chemistry to label the N-terminus of the CMPs with Alexa Fluor 555 on resin.

Crude peptides were purified using reverse phase-high performance liquid chromatography (HPLC) on a Prominence chromatography instrument (Shimadzu, Inc., Columbia, MD) equipped with a Viva C18 (4.2 mm × 50 mm, 5 μm particle diameter) column from Restek (Lancaster, PA). Water with 0.1% TFA (Solvent A) and acetonitrile with 0.1% TFA (Solvent B) were employed as HPLC solvents with a gradient of solvent B from 25-35% over 30 minutes. The eluent absorbance was monitored at λ=210 nm. Electrospray ionization mass spectrometry (ESI-MS) was used to confirm the product, using a Thermo Fisher Scientific LCQ Mass Spectrometer. For GPP: m/z =1610.6 [$(M + 2H)^{2+}$= 1610.85]; for GPO and GPO^S: m/z =1543.6 and 1543.4, respectively [$(M + 2H)^{2+}$= 1543.6] (**Figure S1**). Circular dichroism studies were used to confirm the triple helical structure and melting temperatures (T_m)s of the CMPs (Table 1), as previously described.[56,57]

CMP Retention on Collagen Films

To generate stable collagen films, 100 μL of 5 mg/mL bovine collagen type I solution was added to each well of an 8-well cell culture plate and allowed to air-dry. This process was repeated a total of four times. Subsequently, the resulting films were rinsed with distilled water, neutralized with cell-culture grade Dulbecco's phosphate buffered saline (PBS) solution (pH=7.4) and allowed to air-dry after additional distilled water washes to remove salts. To monitor CMP hybridization to the collagen films, preheated solutions of Alexa Fluor 555-labeled CMPs (2 mM in PBS) were added to the dry collagen films. The films were incubated with the CMP solutions for 3 h at room temperature, allowed to air-dry, and then thoroughly washed with PBS solution and distilled water until free peptide was no longer detected in washes as measured using a GloMax®-Multi Detection System (Promega). A standard curve was generated using CMP solutions of known concentration to enable calculation of the amount of CMP that was initially bound to the films *vs.* the unbound peptide that was detected in the washes. Subsequently, to monitor release *vs.* retention over time, films were incubated in PBS at a range of temperatures and CMP release was monitored through the detection of Alexa Fluor 555 or Alexa Fluor 350 in the washes.

CMP-Polyplex Formation

GPP-PEI conjugate was synthesized using a sulfo-succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) crosslinker. A solution of SMCC in dimethyl sulfoxide (DMSO) was added to a solution of PEI dissolved in PBS such that the SMCC:PEI molar ratio was 1:1 and the final concentrations of SMCC and PEI were 1 mM. These conditions were designed to allow the crosslinker NHS esters to react with PEI primary amines and form stable amide bonds as described by the manufacturer. After an hour, GPP was added to the solution at a molar ratio (PEI:GPP) of 1:3 and the mixture was incubated at room temperature for 24 h to facilitate Michael-type addition of the thiol-containing peptide to the maleimide-functionalized PEI. The product was purified with an Amicon Ultra-0.5

Sequence Name	Peptide Sequence	Melting Temperature (T_M)
GPP	$(GPP)_3$GPRGEKGERGPR$(GPP)_3$GPCCG	43°C
GPO	*$(GPO)_4$GEKGER$(GPO)_4$GGCG*	45 °C
GPO^S	*GGGPCPEGGOPOPPGPEPOGKGOOPOGGROGGOG*	N/A

Table 1. CMP Sequences and Melting Temperatures (T_m)

centrifugal filter device and formation of the product was confirmed with gel permeation chromatography (GPC) as shown in **Figure S2**.

CMP-modified polyplexes were formed by self-assembling GLuc plasmid with mixtures of non-modified PEI and the GPP-PEI conjugate, according to established, although slightly modified, protocols.[58] Briefly, equal volume solutions of plasmid and PEI were prepared in 20 mM HEPES, pH 6.0, and the PEI solutions were added drop-wise to the DNA solutions so that the final DNA concentration was 20 μg/mL. The PEI concentrations in the mixture were varied such that the N:P ratio, defined as the ratio of the number of amines (N) in the polymer to the number of phosphates (P) in the plasmid, would be as specified. CMP incorporation was varied by varying the ratio of GPP-PEI to total PEI in the PEI solution. The polyplex solutions were incubated for 10 minutes at room temperature to allow polyplex formation to occur. To confirm CMP incorporation into the polyplexes, polyplexes were formed with AF555-labeled GPP-PEI. The relative amount of fluorescence in the polyplexes was determined using a GloMax®-Multi Detection System (Promega; Madison, WI).

Polyplex Characterization

To confirm that GPP-PEI was incorporated into the polyplexes, polyplexes were created using different amounts of AF555-labeled GPP-PEI. Subsequently, the polyplex solutions (N:P=10; 20 μg/mL DNA in 10 mM HEPES buffer, pH 6.0) were purified by centrifuging the solutions through a Centricon-100 membrane (Millipore, Eschborn, Germany) for 10 minutes at 500 g and diluting the retentate in HEPES buffer. This process was repeated five times. Following purification, the fluorescence of the DNA and GPP-PEI in the retentate solution containing the polyplexes was quantified using a standard curve with a Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen; Carlsbad, CA) and detection of the AF555 label, respectively.

DNA condensation efficiency and polyplex size were analyzed using agarose gel electrophoresis and dynamic light scattering (DLS), respectively. For electrophoresis assays, polyplexes were made with 0.2 μg of DNA, and either the N:P ratio or the fraction of GPP-PEI:total PEI was varied. The polyplexes were analyzed in 1% agarose gels containing 0.5 μg ethidium bromide per mL of tris/borate/ethylenediaminetetraacetic acid (EDTA) (TBE) buffer. Twenty microliters of each polyplex solution was added to 4 μL of gel loading buffer, and each well of the gel was loaded with 20 μL of the polyplex mixture. The gels were run at 100 V for 1 h and imaged with a BioRad Gel Doc XR (Hercules, CA). To determine the hydrodynamic radii of the polyplexes, a Brookhaven Instruments (Brookhaven, CT) ZETAPals with the 90Plus addition was used. Experiments were conducted using a 658 nm wavelength solid-state laser at an angle of 90° and a temperature of 25 °C. A second order cumulant fit was used to obtain the average hydrodynamic diameters of the polyplexes.

Polyplex Retention on Collagen Films

In order to quantify the amount of polyplex that initially bound to the collagen films, and to determine the amount of polyplex that was retained on the films over time, polyplex retention studies were conducted after addition of different amounts of GPP-PEI polyplex or PEI polyplex to pre-made collagen films. To melt the CMPs and thereby enable efficient hybridization of the GPP-modified polyplexes to collagen, the GPP-PEI polyplexes were pre-incubated at 55 °C for 30 min. The control – produced with GPP-free PEI polyplexes – was treated similarly to ensure that any differences in collagen binding were caused by interactions of the GPP with collagen. Electrophoresis assays and DLS were used to confirm polyplex integrity after incubation. Subsequently, the films were incubated with the polyplexes for 3 h at room temperature, allowed to air dry, and thoroughly washed with a total of 5 mL of distilled water and PBS until free polyplex was no longer detected in the washes. To calculate the amount of polyplex that was initially bound to the films, the amount of polyplex in these initial washes was quantified by fluorescence-based analysis of DNA recovered in the washes. Polyplexes in the washes were disassembled through incubation with 20 mM heparin for 30 minutes. The amount of recovered DNA was quantified using a Quant-iT™ PicoGreen® dsDNA Assay Kit and the result was used to back-calculate the amount of DNA/polyplex retained on the film. Subsequently, to monitor release *vs.* retention over time, the polyplex-modified films were incubated in PBS at 37 °C. Polyplexes released into the washes were quantified as a function of time using the same fluorescence-based analyses to detect and quantify DNA.

Polyplex Retention in Collagen Gels

Collagen gels with GPP-immobilized or GPP-free (physically encapsulated) polyplexes were constructed using acid-soluble, type I bovine collagen according to a modification of the manufacturer's procedures. GPP-PEI polyplex solutions or PEI polyplex solutions (20 μg/mL DNA) containing 2 mM of sucrose were preincubated at 50 °C for 30 minutes to melt the GPP. The polyplexes were then flash frozen using liquid nitrogen and lyophilized, as these procedures were previously shown to allow for dehydration while preventing aggregation and thereby preserving polyplex activity.[66] To formulate polyplex-containing collagen gels, the lyophilized polyplex was re-suspended in chilled DMEM at a concentration of 0.2 μg/μL of DNA in DMEM. Slowly, 100 μL of this chilled polyplex solution was added to 800 μL of chilled collagen solution while gently vortexing. The pH was then adjusted to 7.4 using sterile 0.1 M NaOH and the final volume was adjusted to 1.0 mL with sterile water, resulting in a solution containing collagen at a concentration of 4.0 mg/mL and DNA at a concentration of 20 μg/mL. The polyplex-collagen solutions were subsequently allowed to settle on ice for 3 h to allow any bubbles to rise and to enable CMP-collagen hybridization. To create each gel, 500 μL of neutralized polyplex-collagen solution was added to each well of an 8-well cell culture plate and the plates were incubated overnight at 37 °C. Gelation was confirmed visually, and subsequently, the gels were thoroughly washed with 7 mL of

PBS and water, after which polyplex was no longer detectable in the washes. Initial retention of polyplex as well as polyplex retention over time were quantified using fluorescence-based DNA quantification assays, as described for the polyplex/collagen film retention studies.

Cell Culture and Collagen Film Transfection Studies

NIH/3T3 cells (ATCC, Manassas, VA) were cultured at 37 °C and 5% CO_2 in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin−streptomycin (P/S), according to ATCC protocols. For collagen film transfection studies, cells were plated at a density of 8,000 cells/cm^2 on films modified with polyplexes containing the pCMV-GLuc plasmid. For comparison, cells were also plated at the same density on non-modified collagen films and transfected by the addition of non-CMP-linked polyplexes to the media, using either standard bolus polyplex dosing conditions (2.0 μg DNA/cm^2)[58] or dosing conditions that matched the maximum amount of DNA retained on any of the films (~0.2 μg DNA/cm^2). Transfection was monitored daily by quantifying luminescence in the media and cells were cultured under the same conditions. For these analyses, 10 μL of the media was analysed with a BioLux® Gaussia Luciferase Assay (NE Biolab®; Ipswich, MA), according to the manufacturer's protocol. MTT assays (Millipore; Billerica, MA) were used to assess cell viability and a Pierce BCA assay kit was used to determine total cell protein concentration at 1 and 4 days post-plating according to standard manufacturer-recommended protocols.

Collagen Gel Stability/Transfection Studies

To determine if cells could be transfected with polyplexes immobilized or encapsulated within collagen gels, GPP-PEI polyplex-modified collagen gels or PEI polyplex-encapsulating collagen gels were prepared with pCMV-GLuc plasmid. These gels were incubated in complete media maintained at physiological temperature and pH (DMEM with 10% FBS and 1% P/S at 37 °C and 5% CO_2) for a specified period of time ranging from 0 to 7 days. Subsequent to the incubation, NIH/3T3 cells were plated on the gels at a density of 15,000 cells/cm^2. Cells were incubated either in the presence or absence of 10 ng/mL tumor necrosis factor-alpha (TNF-α), a well-known stimulator of MMP production,[59] and gene expression was monitored over several days. MMP activity was confirmed *via* a SensoLyte® 520 Generic MMP Assay Kit *Fluorimetric*, using the manufacturer's protocol, and gene expression was detected by analysing the luminescence of GLuc secreted into the media, using the BioLux® Gaussia Luciferase Assay.

Results and discussion

CMP Film Retention Studies

Two different triple helix-forming CMPs, GPP and GPO (Table 1), were designed to enable modification of collagen *via* strand invasion. The GPP sequence was designed based on the principles outlined in work by Krishna *et al*.[56,57] $(GPP)_3$ triplets were incorporated for their propensity to promote triple helix formation and ability to provide an efficient folding nucleus.[56] GPR triplets, previously shown to impart similar stability to triple helices as GPO triplets, were incorporated to flank an $\alpha_2\beta_1$ integrin-binding sequence, GEKGER,[56] as sequence motifs such as GXKGEX have also been shown to electrostatically stabilize triple helices. Furthermore, the sequence was designed to form a type III collagen-mimetic cysteine knot at its C terminus. Cysteine knots have been shown to function as triple helix nucleation sites and to confer stability *via* covalently cross-linking the peptide strands of the triple helix.[56-57,60-61] The addition of reactive cysteine residues also provided a site for chemical conjugation in subsequent studies. Hydroxyproline was omitted so the sequence could potentially be mass produced *via E. coli* expression. The traditional GPO sequence was designed to contain standard GPO triplets known to initiate and form stable triple helices. This sequence also incorporated the same $\alpha_2\beta_1$ integrin-binding sequence, GEKGER.[56,57]

Peptides were synthesized using automated SPPS and standard Fmoc chemistry. The crude product was purified *via* RP-HPLC and its purity and identity confirmed using RP-HPLC and ESI-MS, respectively. In order to identify optimal hybridization conditions to maximize GPP and GPO retention on collagen, solutions containing the AF555-labeled GPP, GPO, or GPO^S, were pre-incubated at various temperatures and then allowed to hybridize to collagen films. As shown in **Figure 2a**, the quantity of GPP and GPO that was initially retained on the films increased as a function of increasing pre-incubation temperature up to a temperature of 65 °C, at which point GPP and GPO retention decreased with additional increases in temperature. The finding that CMP retention continued to increase with temperature at pre-incubation temperatures that were above the T_M values for the two CMPs (Table 1) suggested that pre-heating the CMP solutions did more than ensure that the CMPs were single stranded and available for strand invasion. As suggested in earlier studies, it is likely the solutions at higher temperatures destabilized the natural triple helical structure of collagen making it more accessible to strand invasion; meanwhile, at overly high temperatures, film denaturation may have disrupted or destroyed natural collagen triple helical structure reducing the possibility of strand invasion.[53,54] In contrast, a scrambled GPO sequence that did not form a triple helix was not retained, further suggesting that retention was due to strand invasion as opposed to non-specific interactions.

For applications in regenerative medicine, modifications to collagen must be stable at physiological temperatures over extended time periods. Hence, we next examined the fractional retention of the CMPs on collagen films after prolonged incubation at 25 °C or 37 °C. As shown in **Figure 2b**, GPP and GPO sequences achieved sustained release for approximately 12 and 14 days at 25 °C, and for 8 and 10 days at 37 °C, respectively. The sustained release suggests the collagen-CMP interaction is dynamic, likely due to incomplete triple-helical structure formation. While the release rate was essentially constant for both CMPs at 25°C, two release profiles appeared to exist at an

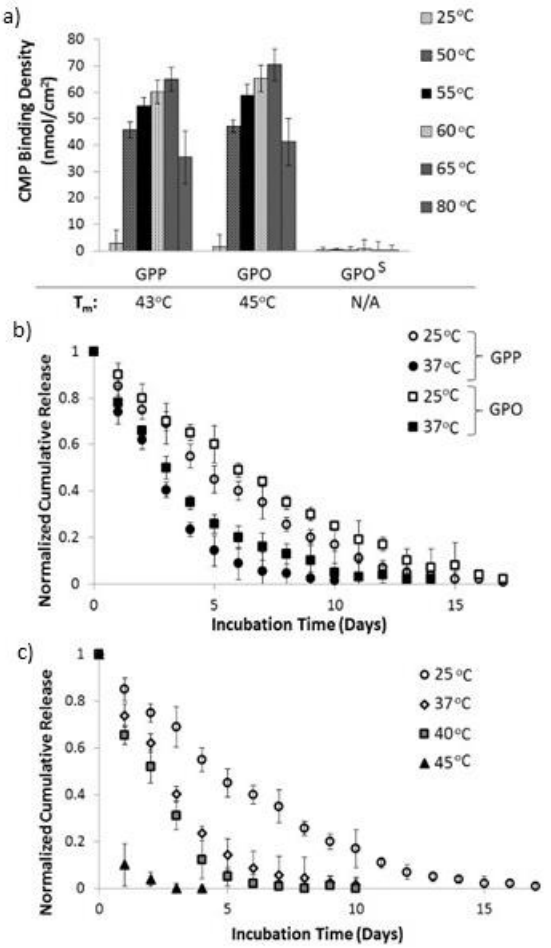


Figure 2. GPP/GPO Collagen Film Binding Studies. a) The optimal preheating temperature for maximizing CMP binding was determined through initial retention studies. b) The retention *vs.* release kinetics of GPP (circles) and GPO (squares) were compared at 25 °C (white) and 37 °C (black). (c) Retention *vs.* release of GPP as a function of temperature (25 °C (white diamonds); 37 °C (grey diamonds); 40 °C (grey squares); 45 °C (black triangles)). Each data point represents the mean ± standard deviation for a total of three separately prepared and analysed samples.

incubation temperature of 37 ºC. Both GPO and GPP were initially released at a faster rate for the first five days (~75% of GPO and 85% of GPP were released), at which point the rate of release slowed by over a factor of 4. The existence of two profiles suggested that the more slowly released population might represent a fraction of the GPP-collagen complexes that was hybridized to a collagen domain with high thermal stability.[16,17] Furthermore, it was found that GPO peptides were consistently retained for longer periods than GPP peptides at both of the different incubation temperatures. This finding was not unexpected based on the slightly higher triple helical stability of GPO (T_M=45ºC) as compared with GPP (T_M=43ºC), and because hydroxyproline residues impart increased stability by enabling the formation of additional stabilizing hydrogen bonds through their γ-hydroxyl group.[60-62] Because GPP-based sequences are less expensive and can be expressed in *E. coli*, the remainder of this work focused on the GPP sequence that has been extensively studied in our lab.[56,57]

GPP peptide retention was also examined at elevated temperatures (**Figure 2c**). As shown in the figure, GPP was retained for approximately 2 weeks when incubated at room temperature but only 3 days at 45 ºC. At an incubation temperature of 45 ºC, approximately 87% of the initially retained GPP was released after one day of incubation, which is similar to the amount released from the films incubated at 37 ºC over the initial 5 days. Sustained released was then maintained for the remainder of the incubation. These results suggested that at the higher incubation temperature, only the most stable fraction of GPP-collagen complexes remained after incubation, and that this highly stable fraction comprised ~15% of the GPP-collagen complexes. These findings also confirmed that the GPP-collagen interaction was thermally sensitive and likely triple helical in nature.

Characterization of GPP-PEI Polyplex

We next sought to establish a versatile method to incorporate CMPs in DNA polyplexes. A GPP-PEI conjugate was synthesized using an SMCC crosslinker and purified *via* treatment with an Amicon Ultra-0.5 centrifugal filter device. The successful production of the GPP-modified product was confirmed by GPC as shown in **Figure S2** (SI). To functionalize polyplexes with GPP, a specified percentage of the PEI typically used to condense DNA was replaced with the GPP-PEI conjugate and polyplex formation was conducted by standard self-assembly procedures.[58] To quantify how much GPP was incorporated into the polyplex, AF555-tagged GPP-PEI conjugates were synthesized and used to make polyplexes. Following removal of free GPP-PEI from the polyplex solutions *via* ultrafiltration, the fluoresence intensity in solutions containing polyplexes was quantified. As shown in **Figure 3a,** the 10% GPP-PEI sample showed fluorescence that was approximately half that of the 20% GPP-PEI sample and approximately one fifth that of the 50% GPP-PEI sample. These data indicated that varying the percent of GPP-PEI/PEI used during polyplex formation could be used to directly manipulate the amount of CMP incorporated into the polyplex.

Because linkage of polymers or peptides to PEI lowers the charge density and can affect complexation,[58, 63-66] the binding stability and structure of the GPP-modified polyplexes were further characterized. To assess the maximal effects on stability/structure imparted by large amounts of GPP, polyplexes containing 50% GPP-PEI were analysed. Polyplexes were prepared at a range of N:P ratios and the resulting structures were assessed by agarose gel electrophoresis and ethidium bromide staining. In these studies, polyplex formation is detected as a reduction in DNA mobility, and when DNA is sufficiently condensed, binding by the intercalating dye ethidium bromide is reduced. The electrophoretic assay showed that fluorescence in the well noticeably faded as the N:P ratio was increased, and fluorescence was effectively absent at N:P=10 (**Figure 3b**), an

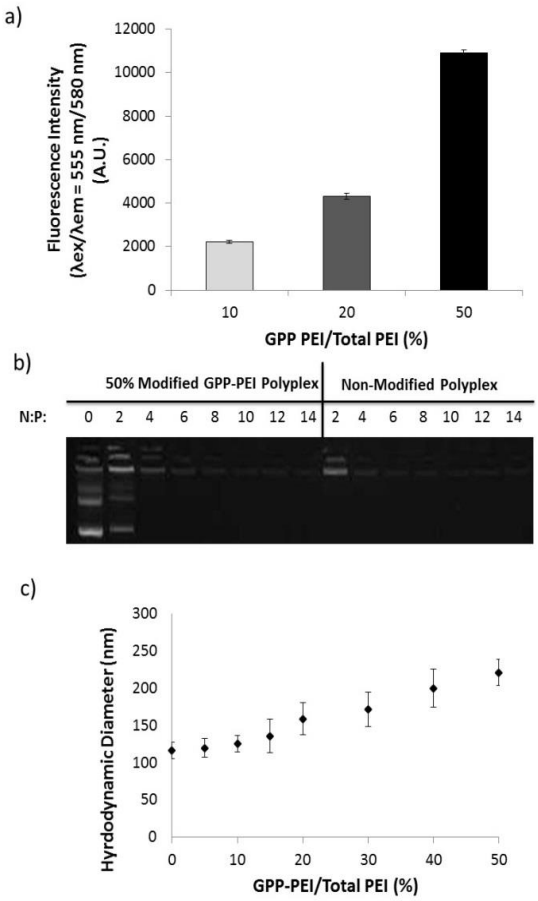


Figure 3. Polyplex Characterization. a) The relative fluorescence of AF555-labeled CMP in purified polyplexes as a function of the percent GPP included in the polyplex formulation. Each data point represents the mean ± standard deviation for a total of four separately prepared and analysed samples. b) Agarose gel electrophoresis and ethidium bromide staining analysis of DNA binding efficiency by 50% GPP-PEI polyplexes and non-modified polyplexes. c) DLS analysis of the hydrodynamic diameters of the polyplexes as a function of the percent of GPP-PEI. The data represent the mean ± standard deviation for nine separately prepared and analysed samples.

N:P ratio commonly employed for PEI-DNA polyplexes.[58] This behaviour was consistent with the electrophoretic behaviour in polyplexes formed with non-modified PEI, and indicated that GPP-PEI incorporation had minimal effects on DNA binding capacity.

To confirm that the polyplex size was within a reasonable range for efficient endocytosis, DLS was performed to determine the hydrodynamic radii of the polyplexes. As shown in **Figure 3c**, the hydrodynamic radius increased as the GPP-PEI/PEI ratio increased, indicating that incorporating the conjugate had some effect on complexation. The polyplexes are likely less compact due to the added molecular weight of the CMP and the slight reduction in charge density of the PEI, or alternatively, the presence of the peptide may have caused a modest amount of polyplex aggregation.[38,58, 64-66] However, the size increases were relatively minimal and the polyplex diameters remained within a size range that permits endocytic internalization.[58, 64]

CMP-Polyplex Collagen Film Retention Studies

To determine whether the amount of immobilized polyplex could be tuned through variations in the fraction of GPP-PEI in the polyplex, GPP-polyplex binding and retention studies were conducted on collagen films using polyplexes that contained various amounts of GPP. The polyplexes were preheated and incubated with pre-made collagen films at room temperature, and initial polyplex binding was quantified by using fluorescence measurements. The retention of GPP-PEI polyplex *vs.* PEI polyplex is shown in **Figure 4a**. Consistent with previously reported studies,[38, 67-68] a small amount of PEI polyplexes were retained on the collagen films, even in the absence of the GPP modification. This non-specific retention is likely due to electrostatic interactions between the positively charged polyplexes and collagen (pI < 8).[49, 67-68] By comparison, when 10% GPP-PEI was used, the amount of DNA initially retained increased by almost an order of magnitude (9-fold greater than the retention of non-modified PEI polyplex). When larger amounts of GPP were incorporated (e.g. 20% or 50% GPP-PEI), the amount of initial retention also increased, by 11- and 15-fold, respectively. We suspect that this increased retention occurred because the larger amounts of GPP displayed on the polyplex increased the likelihood of the GPP-collagen interaction, or because the incorporation of GPP increased the avidity of the polyplex for collagen. In fact, the total amount of polyplex retained on the modified films was very similar to that retained on covalently-modified substrates that were used successfully to achieve high levels of transfection in past studies.[41,42] The lack of perfect linearity in the retention may result from the heterogeneities in the distribution of GPPs on the polyplex, such that 2-D surfaces like collagen films may not be able access all of the GPPs displayed. Studies examining the retention of biotinylated polyplexes on a streptavidin-modified surface noted a similar dependence of initial retention on biotin display.[41,42]

Polyplex retention at 37 °C was monitored over a two-week period *via* similar fluorescence-based approaches to detect DNA released into the supernatant as a function of time. As shown in **Figure 4b**, polyplexes modified with 10%, 20%, or 50% GPP-PEI were retained on collagen films for 8, 9, and 12 days, respectively, whereas non-modified polyplexes were only retained for 2 days. The GPP-polyplexes were released at a significantly slower, sustained rate as compared with the non-modified polyplexes, as evidenced by the slopes of the retention curves. The release rates of the GPP-polyplexes were relatively constant after an initial large release during the first day of incubation. Eventually, the rate of release became similar for each GPP-polyplex as shown by the nearly identical slopes of the retention curves after 4 days. The initial burst release after 1 day of incubation likely consisted primarily of polyplexes that were interacting non- specifically with the collagen. Unlike when the

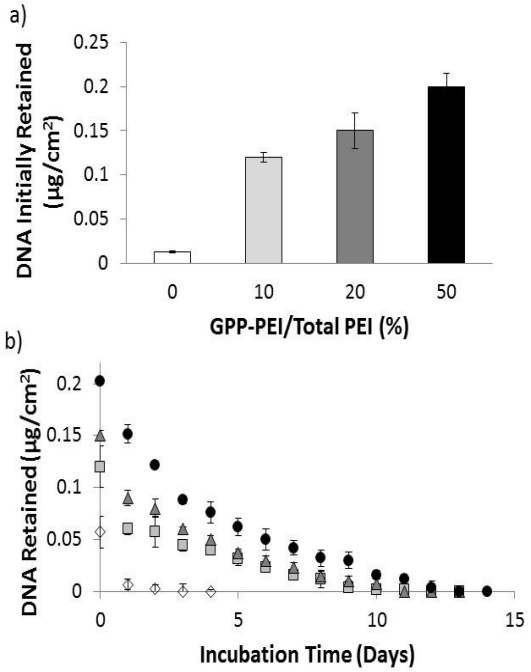


Figure 4. Polyplex Film Retention Studies. a) Initial retention studies to quantify the amount of DNA retained on collagen films as a function of the percent GPP-PEI in the polyplex. b) Retention of DNA on collagen films with time, as a function of the percent GPP-PEI within the polyplex including 0% (white diamond), 10% (grey square), 20% (dark grey triangle), and 50% (black circle). Each data point represents the mean ± standard deviation for a total of four separately prepared and analysed samples.

collagen films were modified with GPP alone, the retention curves for the GPP-polyplexes on collagen films did not have two distinct slopes.

In this case, more factors contributed to sustained release including GPP-collagen interactions, different degrees of GPP availability on each polyplex surface, and variability in polyplex size and charge. The direct dependence between the amount of GPP on the polyplex and retention was consistent with expectations, given that, as in the peptide studies above, the GPP-modified polyplexes should integrate into the natural collagen structure through specific, physical interactions previously shown to be stable at 37ºC. Thus the GPP-modified polyplexes were expected to be retained longer as compared with the non-modified polyplexes. Meanwhile, the increased retention as a function of increased GPP functionalization level was presumed, as before, to be caused either by the increased probability of binding of a single GPP chain in the more highly functionalized polyplexes, or by the increased avidity imparted by multiple GPP chains interacting simultaneously with collagen.

To further examine the release kinetics and distinguish these two possibilities, the polyplex retention data were normalized by the initial amount of DNA that was retained and a normalized retention curve was created (**Figure S3**). No significant difference was noted in the normalized slopes of polyplex retention *vs.* time after 4 days, suggesting that on a per polyplex basis, the release rates were similar, regardless of the amount of GPP incorporation. This result indicated that the primary effect of incorporating multiple GPP chains per polyplex was to increase the likelihood of GPP-collagen binding but not the number of GPP chains bound within a given polyplex. The fact that the normalized 50% GPP-PEI modified film release rate was faster for 4 days before slowing and becoming identical to the 10% and 20% GPP-PEI modified release rates suggested that incorporating large amounts of GPP increased the amount of unstable, partial GPP-collagen binding. Such an occurrence is likely the result of the 2-D architecture, as films in this geometry may not have access to all of the GPPs displayed on the spherical polyplex. This finding is contrary to what was found for the release of biotin-modified polyplexes from streptavidin-coated surfaces as a function of biotinylation level.[41,42] The difference is likely because the GPP-collagen interaction is more reversible and the fact that biotin can be displayed more densely on the surface of a polyplex due to its smaller size.

CMP-Polyplex Collagen Gel Retention Studies

Given that many applications in regenerative medicine require 3-D scaffolds, retention studies were conducted using collagen gels in addition to collagen films. With these studies, we also sought to determine whether increased levels of GPP-polyplex functionalization would translate into increased probability of binding and/or avidity to collagen in the 3-D format. Polyplexes were mixed into neutralized collagen solutions, and after gelation, the initial polyplex retention was quantified after heparin was used to disassociate the non-retained polyplex in solution and a Quant-it™ PicoGreen® Kit was employed to detect the liberated DNA. The gels were then incubated at 37ºC in PBS and retention over time was monitored in a similar fashion.

As shown in **Figure 5a**, at least 7 μg/cm^2 of DNA was initially retained when GPP modification was employed, corresponding to 3.5 μg DNA per mg of collagen. The levels of initial retention on all GPP-PEI-modified polyplex gels were higher than the level of initial retention on gels with non-modified PEI polyplex, with 16-28% more retention depending on the amount of GPP modification. As was observed on the films, increasing the number of the GPPs in the polyplexes enhanced the probability of the GPP-collagen interaction, and thereby increased the initial polyplex retention. However, while the 3-D findings were similar to the trends observed on films, notably, much larger amounts of non-modified PEI polyplex were retained in the gels as compared with the films. This is consistent with the entrapment of non-modified polyplexes in the collagen gels, as has been demonstrated in past studies.[41-43]

Polyplex retention over time was also monitored by using fluorescence approaches to quantify DNA in the supernatant. As shown in **Figure 6b**, DNA was detected in the supernatants of

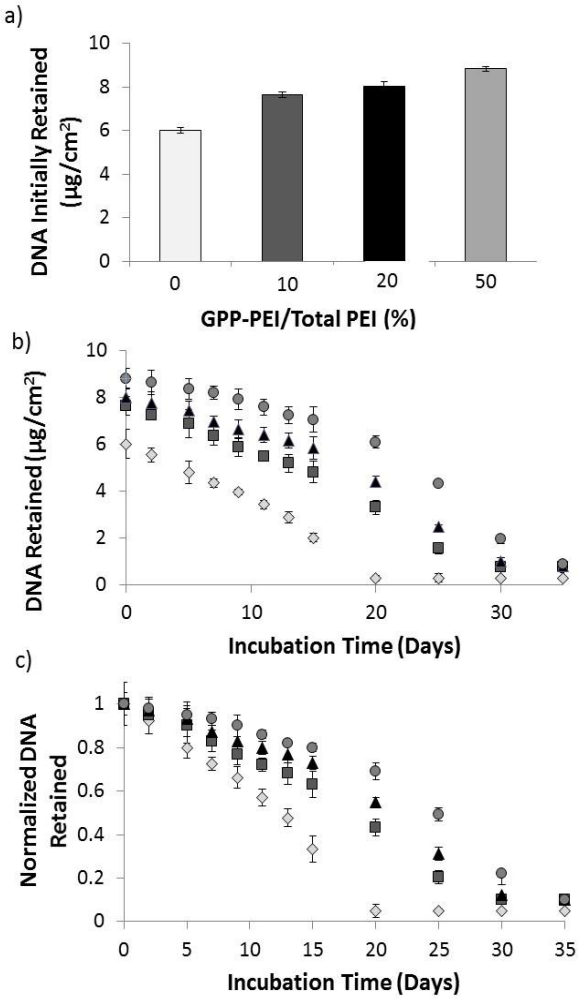


Figure 5. Polyplex Gel Retention Studies. a) Initial retention studies to quantify the amount of DNA retained in collagen gels as a function of the percent GPP-PEI in the polyplex. b) Retention over time of DNA in collagen gels as a function of the percent GPP-PEI in the polyplex including 0% (white diamond), 10% (grey square), 20% (dark grey triangle), and 50% (black circle). c) Normalized retention data showing release kinetics on a per polyplex basis. Each data point represents the mean ± standard deviation for a total of four separately prepared and analysed samples.

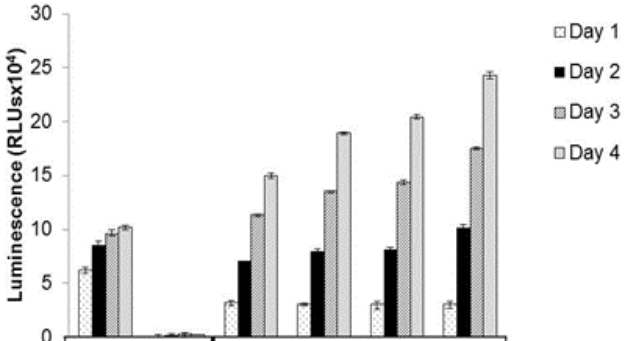
both the 10% and 20% GPP-PEI polyplex-modified gels for approximately 10 days longer than it was detected in the supernatant of the non-modified polyplex gels. The 50% GPP-PEI polyplex was retained for 15 days longer than the non-modified polyplex. The same data were used to generate normalized retention curves, as presented in **Figure 5c**. The rate of release, as represented by the slopes of the curves, was significantly affected by GPP modification. Comparisons of the slopes suggest that the release of the 10%, 20%, and 50% GPP-polyplexes was reduced ~1.7, 2.3, and 3.1-fold, respectively. As was previously observed in the 2-D film system, PEI polyplexes appeared to non-specifically interact with collagen, yet modification with GPP still enabled the more stable modification of collagen.

CMP-Polyplex Collagen Transfection Studies on Films

A series of transfection studies were conducted to determine whether the polyplexes retained their activity after immobilization, and whether the inclusion of the integrin-binding GPP linkage altered gene expression efficiency and/or kinetics. NIH/3T3 cells were plated on films that were freshly modified with polyplexes containing a Gluc-encoding plasmid. Luciferase expression was monitored daily, and expression in cells on the modified films was compared to expression in cells transfected by bolus delivery of polyplex. BCA protein assays were used to ensure that similar numbers of cells were present in each sample for accurate comparison of gene transfer efficiency.

As shown in **Figure 6**, the largest amount of luminescence at 1-2 days post-transfection was detected in the sample transfected using typical conditions and dosing for bolus delivery (2 μg/cm^2).[58] In contrast, by 3 days post-transfection, the luminescence in the samples transfected by the polyplex-modified collagens (0%, 10%, 20% or 50% GPP-PEI modified) was 18%, 41%, 49%, and 83% greater, respectively, than the luminescence detected in the 2 μg/cm^2 bolus delivery sample. Luminescence increased significantly, on a daily basis, in samples transfected with the bound polyplex, whereas luminescence in the bolus delivered polyplex samples was essentially constant after day 2; transfections were halted after 4 days due to high cell confluence, and accordingly reduced cell viability in all samples. Negligible luminescence was detected in the media extracted from the sample that received a bolus delivery of 0.2 μg/cm^2, a dose corresponding to the largest quantity of DNA delivered from the modified films. These results indicated that bound polyplex remained viable on the collagen films, and that active polyplexes were released even after multiple days of exposure to the culture conditions, in contrast to the bolus transfection induced only in the initial days. This is consistent with the literature showing that polyplexes are not stable, even for short time periods (<1 h), in media containing serum.[69] Hence, retention on collagen appeared to stabilize the polyplexes. The connections to collagen may prevent the polyplexes from aggregating with one another, in addition to slowing their release from the films.

Delivering the polyplex *via* collagen films as opposed to bolus delivery also induced higher transfection levels, despite the use of an order of magnitude less DNA. The magnitude of the improvements in transfection efficiency *via* substrate-mediated delivery were higher in the studies here than in those observed for other substrate-mediated delivery systems in the literature, particularly in the presence of serum.[70,71] For instance, in a citric acid-based polyester elastomer system that achieved sustained transgene expression through controlling biomaterial degradation, bound polyplexes achieved higher levels of transfection than samples receiving bolus delivery at 3 days post-transfection. High levels of transgene expression were also noted at 12 days post-transfection, while samples that received a bolus delivery of polyplex achieved high levels for only 7 days. While



	Bolus		Bound			
DNA (μg/cm²)	2	0.2	0.05	0.11	0.15	0.20
CMP-PEI/Total PEI (%)	N/A	N/A	0	10	20	50

Figure 6. Film Transfection Experiments. Cells were transfected with either bolus or bound polyplexes and the levels of luciferase expression were monitored by luminometry analyses. Each bar represents the mean ± standard deviation for a total of six separately prepared and analyzed samples.

similar to our results, the study used reduced serum media making the environment more stable for the polyplex.[71]

The effect of the level of GPP-PEI modification was also examined. At 1 day post-transfection, no significant difference was detected in the luminescence readings from the films containing different types of immobilized polyplex. However, after day 1, luminescence readings for samples drawn from the GPP-PEI modified films increased more rapidly when the polyplexes contained greater quantities of GPP-PEI; for example, between days 1 and 2, the luminescence readings increased by 2.2, 2.5, 2.7, and 3.4-fold in the 0%, 10%, 20%, and 50%-modified samples, respectively. The readings continued to increase in a similar manner for the entire monitoring period, with greater transgene expression at each day on films modified with polyplexes containing higher percentages of GPP-PEI. Notably, while the amount of DNA initially retained on the films increased as a function of the amount of GPP-PEI, transfection was initially similar on all the films, likely because GPP-modified polyplexes were released from collagen at a slower rate when more GPPs were present; therefore, less DNA would have been available at the earlier time points. Additionally, because the films that were modified with GPP-PEI polyplexes exhibited slower release rates, larger amounts of polyplex were available at later times; hence, by 3 days post-transfection, larger increases in luminescence were observed. These results indicated that transgene expression kinetics could be controlled through altering GPP display on polyplexes.

CMP-Polyplex Collagen Transfection Studies on Gels

In many tissue repair environments, the ability to maintain stable and localized gene constructs over long periods is essential, yet standard methods for forming gene-activated matrices can cause losses in activity and/or escape of polyplexes.[70-74] Ideally, gene-containing depots can maintain gene functionality and localization until cellular invasion activates the depots. Hence, to test transfection under conditions that mimic *in vivo* cell invasion, cells were plated on CMP-polyplex-modified collagen gels that had been pre-incubated in serum-containing media. Cells were cultured on the gels in the presence or absence of TNF-α, a well-known stimulator of MMP production.[59] The production of active MMP was confirmed *via* a SensoLyte® 520 Generic MMP Assay Kit *Fluorimetric*.

GPP-polyplex-modified collagen gels induced significantly reduced transgene expression levels in the absence of active MMP, whereas expression was robust in TNF-α stimulated cells (**Figure 7**). Specifically, while the trends in transgene expression were similar with and without MMP induction, the levels of transgene expression in all TNF-α stimulated cell samples were approximately 3-fold larger than the levels in the corresponding samples in the absence of TNF-α stimulation. When TNF-α stimulated cells were plated on gels after short pre-incubation periods (0-3 days), the cells exhibited similar luminescence regardless of the percentage GPP-PEI. In contrast, luminescence in cells plated on gels pre-incubated for longer periods exhibited large differences as a function of the percentage of GPP-PEI. After 5 days pre-incubation, the luminescence induced by the non-modified polyplex gels was 43% lower than the luminescence on this gel after 3 days pre-incubation, whereas the luminescence induced by the 10%, 20%, and 50% GPP-PEI polyplex gels remained constant or increased between 3 and 5 days pre-incubation. Even when the GPP-PEI polyplex gels were pre-incubated for 7 days, transfection remained constant (10% gel) or increased (20% gel and 50% gel). In contrast, luciferase induction activity decreased to less than half of the original (0 day) level after a 7 day incubation of the non-modified polyplex gels. Hence, incorporating GPP-PEI into the polyplexes increased the amount of active polyplex accessible to cells for periods of over a week.

Given that earlier works show that coating polyplexes with gelatin (denatured collagen) can be used to improve transfection

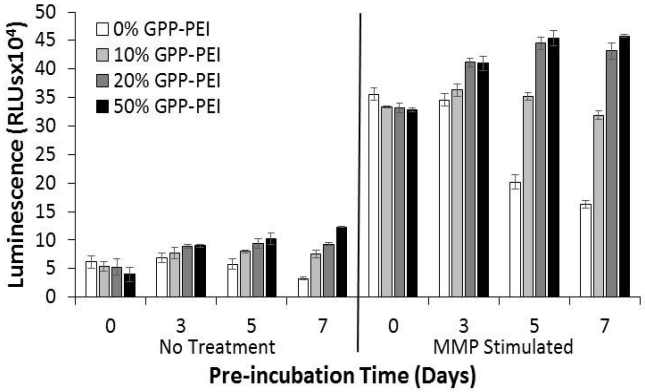


Figure 7. Gel Transfection Experiments. NIH/3T3 cells were plated on fresh polyplex-modified gels or polyplex-modified gels that were pre-incubated under physiological conditions for up to a week. Cells were treated with TNF-α to stimulate MMP expression as specified. The data represent the luminescence in the media due to luciferase expression by the cells after 4 days on the gels. Each data point represents the mean ± standard deviation for a total of eight separately prepared and analyzed samples.

efficiency through enhancing stability[75] and improving cellular uptake,[76] it is possible that the GPP modification of polyplexes improved stability/activity by mediating encapsulation of the polyplex within a stable layer of collagen. Previous studies have shown that polyplexes can be stabilized for similar periods *via* absorption onto polymer scaffolds [69,71] and chemical-modification of collagen,[37,50] but using GPPs, we obtained prolonged association with collagen using a physical modification technique and a natural substrate. Additionally, as similar experiments performed in the absence of TNF-α stimulation induced negligible gene expression, it was clear that polyplex release was MMP-mediated and that cell mobility within the gel played a significant role in controlling polyplex availability.

Other studies have also successfully demonstrated that substrate-modification can be used to obtain sustained gene delivery, but in previous studies, DNA retention and release were generally coupled, as both were typically controlled by DNA-substrate affinity. Hence, in most examples, increasing the quantity of DNA retained decreased the release rate and availability of the DNA to cells.[36-40] In contrast, the GPP-collagen interaction allowed stable polyplex retention for over a month under physiological conditions, and increased retention (mediated by increased GPP functionalization levels) did not negatively affect the rate of MMP-mediated release. A related approach to employ MMP activity to stimulate gene release from polyplexes encapsulated in an enzymatically degradable hydrogel similarly found proteolytic degradation allows cellular infiltration, promotes cell growth and allows for long term delivery of DNA, which is essential for the regeneration of functional tissues.[77] The use of CMPs such as GPP instead of encapsulation alone should allow for increased control over retention *vs.* release, and should impart a higher degree of tailorability as compared with physical modification techniques that rely on electrostatics and nonspecific interactions. CMPs can also provide additional functionality by acting as both adjustable tethers to control the affinity between the collagen and the polyplexes, as well as adhesive/endocytic ligands or cell penetrating peptides.

Conclusions

Collagen is an abundant, versatile material with intrinsic properties that make it a promising biomaterial for use in regenerative medicine. While a greater understanding of both its physical and biochemical properties has aided in creating a myriad of medical technologies already in use, most modification techniques require multi-step chemical treatments which prevent the full capitalization of collagen's regenerative potential. In this work, we have developed a promising new biomimetic, collagen modification technique and are the first to our knowledge to demonstrate its efficient application in gene delivery. By mimicking collagen's natural structure, collagen can be modified with DNA through physical modification to allow delivery of genes *via* collagen.

The CMP-based delivery method was shown to be highly tuneable and versatile, as changes in the number of CMPs in the polyplex readily altered collagen binding *vs.* release, and alterations in the CMP sequence offer a readily accessible platform to change CMP-collagen affinity or incorporate integrin-binding functionality. As a prototypical CMP, GPP could be used to incorporate DNA polyplexes into collagen structures including both 2-D collagen films and fibrous 3-D gels for approximately 2 weeks and a month respectively. The incorporation of GPPs increased the efficiency of initial polyplex retention by an order magnitude on films and by over 25% in gels, with increases in retention of at least 50%. CMP linkage retained polyplexes on collagen films for a 5-fold longer period than polyplexes lacking CMPs, and CMP linkage retained polyplexes within collagen matrices for over a month. This is a markedly longer period than the vast majority of simple collagen-based systems that rely solely on physical modifications, in which retention is typically on the order of a few days.[79] Polyplex activity was also consistently and fully maintained in the presence of serum for at least a week, whereas

most bolus and substrate-mediated gene delivery approaches report rapid and significant serum-induced reductions in activity within hours to a few days. Additionally, transfection in the gels was found to be cell-mediated and to require the presence of MMPs, suggesting that the system will be ideal for coordinating cellular invasion processes with localized, on-demand gene delivery and expression of healing factors during tissue regeneration. The robustness and stability of the modification as well as its versatility suggests that this technique may be used more broadly to create tuneable, collagen-based delivery systems for regenerative medicine applications requiring highly localized gene retention, infiltration/adhesion of a diversity of cells, and on-demand gene expression over different periods of time.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Electro Spray Ionization Mass Spectrometry, Circular dichroism studies, and MMP expression studies are included.]. See DOI: 10.1039/b000000x/

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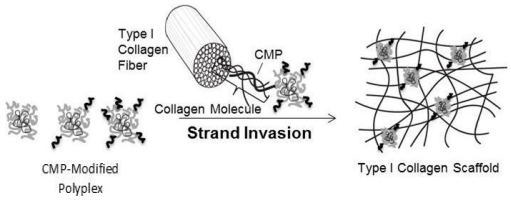

Collagen mimetic peptides (CMP)s were used to tailor release *vs.* retention of DNA polyplexes from collagen while preserving polyplex activity.