Hydrogels For Peptide Hormones Delivery: Therapeutic And Tissue Engineering Applications

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Mohsen Doostmohammadi¹
Atefeh Ameri¹
Reza Mohammadinejad¹
Negar Dehghannoudeh²
Ibrahim M Banat³
Mandana Ohadi¹
Gholamreza Dehghannoudeh^{1,4}

¹Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; ²Faculty of Arts and Science, University of Toronto, Toronto M5S3G3, Ontario, Canada; ³School of Biomedical Sciences, Faculty of Life & Health Sciences, University of Ulster, Coleraine BT52 ISA, Northern Ireland, UK; ⁴Department of Pharmaceutics, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran

Abstract: Peptides are the most abundant biological compounds in the cells that act as enzymes, hormones, structural element, and antibodies. Mostly, peptides have problems to move across the cells because of their size and poor cellular penetration. Therefore, a carrier that could transfer peptides into cells is ideal and would be effective for disease treatment. Until now, plenty of polymers, e.g., polysaccharides, polypeptides, and lipids were used in drug delivery. Hydrogels made from polysaccharides showed significant development in targeted delivery of peptide hormones because of their natural characteristics such as networks, pore sizes, sustainability, and response to external stimuli. The main aim of the present review was therefore, to gather the important usages of the hydrogels as a carrier in peptide hormone delivery and their application in tissue engineering and regenerative medicine.

Keywords: hydrogels, peptides hormones, tissue engineering, drug delivery

Introduction

Peptides and proteins represent an opportunity for therapeutic intervention that closely mimics natural pathways for many physiological functions. Until now, over 60 drugs with peptide structure have been approved in the United States.¹ For example, insulin as a macromolecule has played a notable role in medical practice since 1920s.¹ Formulation, route of the administration, rate and pharmacokinetic profile of peptide drugs in the body showed the vital role in the success of protein drug delivery.^{2,3} The oral administration of drugs was the most preferred but unsuccessful. So developing an alternative delivery system for peptides are needed.⁴ Studies showed that peptide analogs that were loaded on the desirable systems had the better absorption profile.⁵ For example, in hormone therapy, continuous dosing of hormones induces down-regulation of hormone receptors on the target cells that escape the usefulness of the drugs. Thus, discontinuous delivery is rather than continuous.⁶ The smart delivery systems that release drugs by triggering through external stimuli are of great use in the case of peptide hormones (PHs) such as insulin, calcitonin, and human growth hormones (hGH).^{7,8} Hydrogels are cross-linkable water-swollen polymeric network, produced by the simple reaction of one or more monomers.⁹ Hydrogels protect and deliver peptides due to their unique physical properties include continuous release that resulted in the maintaining a high local concentration of peptides over a long period of time.^{10,11} Nowadays, natural hydrogels are gradually replaced by synthetic hydrogels, which have a long half-life, high capacity water absorption, and high gel strength.¹²

Correspondence: Mandana Ohadi;
Gholamreza Dehghannoudeh
Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
Tel +98 34 31325243; +98 34 31325014
Fax +98 34 31325003
Email mandanaohady@gmail.com;
ghr_dehghan@kmu.ac.ir

Gels are formed by chemical or physical process.¹³ Chemical gels are formed by cross-linked polymeric network while physical gels formed by non-covalent interactions between the chains.¹⁴ Physical gels known as stimuli-sensitive or smart gels are divided into thermo-sensitive, pH-sensitive or analytic-sensitive hydrogels.¹⁵ Thermo-sensitive hydrogels are very interested in biomedical applications, because of temperature can be controlled easily.^{16,17} So, the main goal of this review was to summarize some important hydrogels used for delivery of hGH, calcitonin, and insulin as some examples for sustain release of PHs.

Hormones

Hormones are produced by endocrine glands and secreted into the blood circulation and could act at some distance organs.¹⁸ According to the chemical structures, hormones are categorized into two main groups of steroids, e.g., cortisol, aldosterone, and progesterone; and peptides, e.g., insulin, growth hormone, and calcitonin.^{19,20}

Peptide Hormones (PHs)

The PHs are a group of small molecules with the polypeptide chain. The hypothalamus, besides its thermo-regulation activity, is responsible for the production of some PHs. PHs are transported and stored in the pituitary gland and have regulatory effects on the synthesis of other hormones produced by the anterior pituitary.²¹ This function was caused to calling them as releasing factors or inhibitory factors.^{22–24} Some PHs like insulin are stored within vesicles inside the cells and secreted into the blood after receiving specific simulations like high blood glucose.²⁵ Regarding PHs are hydrophilic, they cannot cross through phospholipid membrane, so the specific receptors on the target cells are needed.⁵ Nowadays, PHs with the special ligand are produced by applying the genetic engineering technologies. However, these targeted PHs showed significant healing effects on patients, but there are still some limitations such as degradation in the gastrointestinal (GI) tract, short half-life, and poor adsorption.^{19,22}

Stability Features Of PHs

The secondary, tertiary and quaternary structures of peptides are maintained by weak non-covalent interactions, so any changes in these interactions could lead to destabilization or denaturation of peptides.²⁶ For example, the changes of pH, temperature, ionic strength, and pressure

parameters could induce the aggregation, precipitation, and inactivation of the PHs.^{26–28} Therefore, to improve the chemical and physical stability of the PHs several strategies are developed. For instance, the site-specific mutagenesis to reduce peptides enzymatic degradation or glycosylation to enhance oral absorption, stability, and activity of the therapeutic peptides are being used (chemical strategy; PEGylation).^{28,29} Adding absorption enhancers such as surfactants, chelating agents, and fatty acids are commonly used (physical strategy; nanoparticles, liposomes, pH-responsive composites).^{26,30} Further strategies to improve the activity of the PHs are listed in Table 1.

New Drug Delivery Systems For Peptides

Polymer conjugation is used to reduce the proteins immunogenicity and their physiological environment stability through covalent interactions and retention in circulation, respectively.⁴⁵ For instance, pluronic hydrogels were used as carriers in several routes of administration.⁴⁶ Also, pluronic micelles could concentrate the proteins at the particle surface and the stability and bioactivity of the proteins are higher than the aqueous phase.⁴⁷ Polystyrene beads are also proposed to be safest particles for protein adsorption.⁴⁸ Liposomes are reported as good proteins carriers because of their size, aqueous core, biocompatibility and biologically inertness, weak immunogenic nature, and limited toxicity that protect peptides in the GI.^{49,50} For instance, Archaeosomes™ is reported as a new formulation of liposome that shows high stability.⁵¹ Combination of the silica and liposomes could protect proteins (insulin) from enzymatic degradation (lipolytic) and prolong protein release in simulated GI conditions.⁵² Respiratory tract is a non-invasive route to adsorb the macromolecular drugs due to reduce acidity and proteolytic activities and a thinner mucus layer. Inhalation of the peptides with relatively low molecular mass such as insulin is well, while systemic administration of these macromolecules through the respiratory tract is often a challenging problem due to biological barriers.⁵³ Nanoparticles show the potential to overcome the biological barriers because of their small size, avoiding clearance and phagocytosis.⁵⁴ For instance, insulin was encapsulated by using the layer-by-layer technique, administrated through the respiratory tract, resulted in a good-sustained level of drug in the blood.⁵⁵ Also, the inhaled insulin in the

Table I Using Strategies To Improve The Activity Of The PHs

Type of PHs	Therapeutic Class	Instability Agents	Solution	Delivery Route	Ref.
Growth hormone	Growth hormone	Physiological barriers: enzymes, mucus, and pH of the GI tract	Nanoparticles To improve the biologicals absorption by the permeation enhancers and defeating metabolism of peptides in the GI tract	Oral	26
			Liposomes Liposomes formulated with cetylpyridinium-chloride (CpCl) improved hGH oral bioavailability	Oral	31
			PEGylation Thiol modification by PEGylation improvement of their chemical stability and biological properties	Oral	26
		Metal-catalyzed-oxidation (MCO)	Minimizing exposure to oxygen, by reducing the headspace in the vial	Oral	23
		Deamidation-asparagine, glutamine	The pH value of the solution was reported to have an effect on the deamidation of Asn residues in hGH	Oral	28,32
	Growth hormone	Isoimerization-Asparagine-X, Aspartic Acid-X	-Changes in temperature and pH buffer species-Ionic strength	Oral	28,32
		Disulfide-scrambling/oligomerization cysteine	Changes in temperature and pH buffer species Ionic strength	Oral	28,32
Salmon calcitonin	Antiestoporotic	Physiological barriers: enzymes, mucus, and pH of the GI tract	Peptelligence technology Citric acid and lauroyl-L-acylcarnitine to improve the biologicals absorption by the permeation enhancers	Oral	29,33,34
			Eligen technology 8-(N-2-hydroxy-5-chloro-benzoyl)-amino-caprylic acid (5-CNAC) to improve the biologicals absorption by the permeation enhancers	Oral	34
			Axcess technology Improvement through the permeation enhancers such as Hydrophobic aromatic alcohols, butylated hydroxyalcohol (BHA), butylated hydroxytoluene (BHT) and propyl-gallate solubilized	Oral	34
	Antiestoporotic	Physiological barriers: enzymes, mucus and pH of the GI tract	Citric acid, phenylethyl alcohol, benzyl alcohol, PEG sorbitan monooleate, to improve the biologicals absorption by the permeation enhancers		34
			PEGylation The enhanced resistance against pancreatic peptidases and brush-border peptidases and improvement of their chemical stability and biological properties	Nasal	26
			Oracal™ have the permeation enhancer and the coated vesicle in its structure		26

(Continued)

Table 1 (Continued).

Type of PHs	Therapeutic Class	Instability Agents	Solution	Delivery Route	Ref.
			N-acetylation Improved oral bioavailability, marginally improved resistance to trypsin and leucine aminopeptidase, enhanced membrane permeability	Oral	30
	Regulating calcium ion concentration	β -Elimination	Changes in temperature and pH Buffer species oxidizing agent	Oral	32
		High elimination rates of the drug in contact with its absorption sites	Calcitonin-containing chitosan-aprotinin multilamellar vesicles (MVL) Chitosan-aprotinin coated liposomes	Oral	31
		Deamidation	The pH value of the solution was reported to have an effect on deamidation. pH 3–5, increased solvent viscosity	Oral	32
		Oxidation	Deamidation reaction is dependent on varying pH values. The pH value (pH < 7) was significant in the reaction medium. Other factors, including air exclusion, antioxidants, chelating agents, and polyols affected oxidation	Oral	32
		Aggregation/fibrillation	Lower concentration, minimal mechanical stress, organic solvents, alkyl saccharide, alkyl polyglycoside	Oral	32
Insulin	Antidiabetic, hypoglycemic hormone	Physiological barriers: enzymes, mucus, and pH of the GI tract	POD (patented protein oral) delivery technology system To improve the biologicals absorption by the permeation enhancers	Oral	34
			Axcess™ delivery technology system To improve the biologicals absorption by the permeation enhancers	Oral	34
			RapidMist technology Mixed micelles made up of a combination of enhancers. To improve the biologicals absorption by the permeation enhancers	Oral	34
			CPE-215 (Cyclopenta decalactone) technology To improve the biologicals absorption by the permeation enhancers	Oral	34
			pH-responsive nanoparticles Helping to release the protein in the small intestine and reduce the degradation by enzymes as well as enhance of absorption.	Buccal	26,30
	Antidiabetic, hypoglycemic hormone	Physiological barriers: enzymes, mucus and pH of the GI tract	pH dependent and mucoadhesive nanoparticles (Dextran sulfate/chitosan nanoparticles) To enhance intestinal residence time and release target protein in intestine	Nasal	26,35

(Continued)

Table 1 (Continued).

Type of PHs	Therapeutic Class	Instability Agents	Solution	Delivery Route	Ref.
			Micro particles Mucin and sodium alginate have used to prepare Insulin-loaded micro particles	Peroral/ Oral	31,36
			Liposome An alkyl/alkenyl sulfate comprised of protein/peptide	Peroral/ Oral	36,37
			Insulin-containing multivesicular liposomes (MVLs), adding of novel chitosan and carbopol to MVLs as sustained release protein delivery systems	Oral	31
	Antidiabetic, hypoglycemic hormone		Microsphere A protein microencapsulated with a water-based enteric coating	Oral	38
			Microparticles Enteric-coated capsules/tablets for oral delivery of protein, polypeptide or a peptide drug	Nasal; ocular	39
	Antidiabetic, hypoglycemic hormone	Physiological barriers: enzymes, mucus and pH of the GI tract	Microemulsion Fatty acid formulations for oral delivery of proteins and peptides	Oral	39
			Oral capsule using non-acylated amino acids as carriers (Eligen™)	Oral	29,39
			Macrosol™ W/O (Water/Oil) microemulsion technology (Macrulin™)	Oral	29
			Oral formulation of recombinant human insulin (AI-401)	Oral	29,39
			Mixed micellar solution (Oral-lyn™)		26,29
		Deamidation	Deamidation reaction in peptides or proteins is pH-dependent	Oral	32,40
		Isoelectric precipitation	Stresses such as temperature and moisture resulted in conformational changes, linear aggregation, and formation of a viscous gel	Oral	32
	Antidiabetic, hypoglycemic hormone	Physiological barriers: enzymes, mucus and pH of the GI tract	To reduce degradation by enzymes and enhance absorption in the small intestine	Buccal	39
			Nanoparticles Ligand-specific binding and uptake. The effectiveness of insulin conjugated vitamin B12-coated nanoparticles in diabetic rats. (Oradel™)	Oral	26,30
		Physiological barriers: enzymes, mucus and pH of the GI tract	PEGylation PEGylated products of insulin exhibited low degradation rate by elastase and pepsin in contrast to unmodified products of insulin	Oral	26,30

(Continued)

Table 1 (Continued).

Type of PHs	Therapeutic Class	Instability Agents	Solution	Delivery Route	Ref.
	Antidiabetic, hypoglycemic hormone	Physiological barriers: enzymes, mucus and pH of the GI tract	Insulin analog NNI954, GIPET (GI permeation enhancement technology): To improve the biologicals absorption by the permeation enhancers	Intestine	24
			Hexyl-insulin monoconjugate 2 (HIM2), hydrophobization, to increase lipophilicity of peptides Conjugation of the insulin molecule to the 1,3-dipalmitoylglycerol containing a free amino acid groups of glycine, phenylalanine and lysine molecule. Improvement of their stability against the enzymatic degradations.	Oral	41,42
			Oral HDV-insulin (HDV-I), Nanocarriers liposomal delivery system		26
Glucagon-like peptide-I (GLP-I)	Anti-diabetic medication (type 2 diabetes mellitus)	Physiological barriers: enzymes, mucus and pH of the GI tract	GIPET® technology (Gastrointestinal Permeation Enhancement Technology) To improve the biologicals absorption by the permeation enhancers.	Oral	37
			Emisphere™ (n-(8-[2-hydroxybenzoyl]amino)caprylic acid) (SNAC) carrier molecule improved membrane penetrability	Oral	30
			Glp-I/iDPP4 delivery multifunctional composite system At first, GLP-I loaded in PLGA nanoparticles and encapsulated within the HPMC-as pH-sensitive polymer, and followed by loaded with the dipeptidyl peptidase-4 inhibitor (iDPP4)		43
			Novel hybrid hyaluronan (HA) hydrogel encapsulating nanogels Protein nanocarrier used for sustained delivery of glucagon-like peptide-I	Oral	44
			Cell penetrating peptides L- and D-penetratin		43
	Anti-diabetic medication (type 2 diabetes mellitus)	Physiological barriers: enzymes, mucus and pH of the GI tract	PEGylation PEGylated products of Glp-I exhibited enhancing absorption and enzyme resistance	Oral	43
			Biotinylation To modify the peptide surface using biotin (vitamin H). To resist in intestinal enzymes To increase the intestinal absorption		43

form of lyophilized powders based on the amphiphilic polymers was developed.⁵⁶

Cell-penetrating peptides (CPPs) also known as short peptide sequences with positive charge that synthesized easily and have the potential for sequences modification. The high internalization and low cytotoxicity are the bold characteristics of cell-penetrating peptides.⁵⁷ To improve peptides and proteins delivery to the central nervous system, combination of the systemic administration of the drug and transient osmotic opening of the blood–brain barrier (BBB) was applied as a strategy.⁵⁸

Hydrogels For Peptide Hormone Delivery

Different types of materials have been developed for the hormone formulation industries.⁵⁹ PHs have great role in the development of the human brain by regulating specific genes expression. PHs have low stability during formulation and storage, which make their formulation challenging.⁶⁰ Controlled delivery of peptide drugs, especially agents with high molecular weight has great importance.¹¹ Recently concerted efforts have been made for the development of a stable formulation for non-invasive delivery of hormones through oral, skin or nasal methods.³⁴

Hydrogels are a network of cross-linked, water-soluble polymer chains that are insoluble in water and biological fluids but have water as their dispersion medium.⁶¹ The most advantages of hydrogels are biocompatibility potential, low toxicity, large-scale bioactivity and multi-functionality, controlled drug release, hydrophobicity, smart drug delivery, and biodegradability.⁶² Hydrogels structurally are too similar to natural tissue because of their significant water content. Drugs could be loaded on the hydrogels as well because of their porous nature. For instance, interpenetrating polymer networks (IPN) hydrogels were applied as drug delivery controller due to their mechanical strength and swelling/de-swelling response.²⁷ Also, hydrogels could be used as injectable due to their biocompatible and biodegradable features. Hydrogels are able to tolerate changes in the pH and temperature which could protect drugs against harsh environmental conditions. For example, Phan et al⁶³ used the biodegradable, temperature, and pH-sensitive injectable hydrogels that resulted in the sustained delivery of hGH and well-ordered degradation of the gel matrix without any swelling at the injection site and its surrounding tissue.⁶³ In another study, Schoener et al⁶⁴ applied a hydrophilic pH-responsive

hydrogel hybrid with hydrophobic nanoparticles due to cytocompatibility.

Disadvantages of the hydrogels include non-adherent to cells and tissues; they may need to be protected by a secondary covering. They are expensive for tissue engineering and regenerative medicine. Sterilization of hydrogels is difficult and time-consuming. Loading of drugs and cells into hydrogels are difficult.⁶⁵

Hydrogels As Carrier For Human Growth Hormone

Growth hormone is applied to treat children with growth hormone deficiency (GHD) that caused due to isolated hormonal deficiency, central nervous tumor, pituitary hormone deficiency, and cranial irradiation. Also, growth hormone is used for Prader–Willi and Turner syndromes, AIDS-associated weight loss, and renal insufficiency.^{66,67} The accepted administration way of hGH is injection.⁶⁸ Even hGH replacement therapy is accepted by scientists as a good treating protocol, but still, injection is the best way.⁶⁹ Till now, more studies were done to find the developed delivery systems that have a low initial burst and high bioavailability and therapeutic effects.^{70,71} Hydrogels seem to act as an excellent delivery systems for hGH because of their biodegradable, thermo-sensitive, and pH-dependent gelation.⁶³ PACU poly(amino carbonate urethane)-based pH-/temperature-sensitive injectable hydrogel was synthesized for sustain delivery of hGH.⁶³ The poly(methacrylic acid-g-ethylene glycol) [P(MAA-g-EG)] is one of the most extensively used hydrogels for oral peptide delivery.^{72–74} Mucoadhesive and retention were enhanced in the small intestine by PEGylation of the methacrylic acid (MMA) hydrogels. Copolymerization of MMA with hydrophilic monomers such as PEG and N-vinyl pyrrolidone could trigger the pH.⁷¹ Poly(methacrylic acid-co-N-vinyl pyrrolidone) hydrogel could act as an efficient delivery system for hGH orally, which showed the good release of the drug during the first hours of the administration in the upper small intestine.⁷⁵ In another study, poly(methacrylic acid-co-N-vinyl pyrrolidone) microparticles showed no release of hGH under gastric conditions, so it can conclude that the synthesized composite is suitable for high molecular weight drugs.⁷⁵

Hydrogels of poly(vinyl alcohol) (PVA) and poly(acrylic acid) (PAA) in combination with collagen (C) and hyaluronic acid were used in hGH delivery.⁷⁶ The release profile was in the linear phase during the first

3 days and then followed rapidly. A delivery system composed of negatively charged; poly-b-amino ester urethane (PAEU) copolymer hydrogel and positively charged; 2D-layered hydroxide nanoparticle (LDH), was developed to overcome the limitation of hGH such as premature degradation and low half-life. Releasing profile of hGH loaded on the mention hydrogel showed the sustained rate of drug release in in vitro and in vivo; 13 and 5 days, respectively.⁷⁷

Sucrose acetate iso-butyrate was combined with polylactic acid (SABER) as a new composite was investigated to deliver hGH that acted as weight-based dosing.⁷⁸ hGH burst release was significantly reduced by enhancing the content of polylactic acid that is because of the diffusional barriers around the proteins after floating in the aqueous environment.⁷⁸ Also, combination of the hydrogels such as poly(lactic acid-co-glycolic acid) (PLGA) reduced the immunogenicity response.⁶³ The surface erosion characteristics of the hydrogels could be controlled by incorporation of PEG into them.⁶³ For instance, PEGylation of fluorocarbon end groups attachment (R_f -PEG) hydrogel was done to deliver hGH.⁷⁹ In the presence of N-methyl pyrrolidone, R_f -PEG made gel quickly, because organic solvent that diffuses into the environment. Studies showed that hGH maintained in the active form inside the hydrogels and was released during 2 weeks without any aggregation. Furthermore, the ability of this hydrogel in the delivery of two other proteins including bovine serum albumin (BSA) and g-globulin was reported.⁸⁰

Hydrogels As Carrier For Calcitonin

Injection and oral use are the conventional administration of the calcitonin.^{81,82} Since the oral administration is too much favorable and improves the life quality, using hydrogels to formulate calcitonin hormone act as an interesting way to protect hormone from the harsh condition of the gut and intestine. Moreover, hydrogels could act as a control release composite.^{81,83} Since the upper small intestine and dominant stimulus are the most interesting parts to absorption of the oral administration peptide, because of the acidic environment. So, the attention to use of the pH-sensitive hydrogels is increasing. For example, salmon calcitonin was loaded on (P(MAA-g-EG)) hydrogel as a pH-sensitive compound that acted as constant release.⁸⁴ The polymerization in this hydrogel is performed by the interaction of oxygen from graft chain and acidic groups. This lead to hydrogel condensation and the collapse of the network in an acidic environment, while gradually with

enhancing the pH, the inter-polymeric bonds dissociate after ionization of groups and the water is allowed to enter to the network.⁸⁵ In another study, the high loading efficiency of complexation hydrogels (P (MAA-g-EG)) which was previously used for insulin oral delivery was demonstrated.⁸⁶ The polymer loaded with calcitonin showed pH-sensitive and complexation/decomplexation release behavior. The entrapped calcitonin retained in the polymeric matrix at pH 1.2, but it released immediately at high pH (pH 6.8). Furthermore, it was shown that P(MAA-g-EG) hydrogels showed the high affinity to peptide drugs with bulky structure.⁷⁸ P(MAA-g-EG) also presented some adhesive properties to the mucosal membrane with no adverse effect on this tissue.⁸⁶

Hydrogels As Carrier For Insulin

Insulin had been used in several ways such as pulmonary, nasal, buccal and oral, rectal, ocular and transdermal, vaginal, intrauterine, and injection.^{87,88} Oral administration of insulin has been estimated to be more convenient in enhancing patient adherence and its absorption imitates insulin secretion under physiological conditions and is effective on the hepatic glucose production and reduce the danger of hypoglycemia-related to the peripheral insulin injection.⁸⁹ But new oral insulin administration devices include liposomes, microcapsules, beads, hydrogels, and chemical modifications of the molecule showed the numerous challenges that have failed to improve their outcomes.^{90,91} Therefore, co-polymeric hydrogel microparticle of P (MAA-g-EG) and pH-sensitive nanoparticles have been demonstrated.^{33,92-95} For instance, the oral form of the pH-sensitive nanoparticles composed of poly(g-glutamic acid) and chitosan showed high bioavailability in diabetic animal models.⁹⁶

Also, super porous hydrogel (SPH) and SPH composite (SPHC) polymers enhanced the gut absorption of insulin in healthy pigs.⁹⁷ Further, a pH-temperature-sensitive hydrogel composed of poly(β -amino ester)-poly(ϵ -caprolactone)-poly(ethylene glycol)-poly(ϵ -caprolactone)-poly(β -amino ester) (PAE-PCL-PEG-PCL-PAE) pentablock copolymer, as a sustained injectable system was assessed.⁹⁸

The pH/thermosensitive polymeric beads based polymers of N-isopropylacrylamide (NIPAm), butyl methacrylate (BMA), and acrylic acid (AA) were applied to release insulin.⁹⁹ Results showed that the molecular weight of the polymers effects on the rate of release.¹⁰⁰ The insulin was entrapped in polymeric matrix by cross-linking through condensation of hydroxyl groups of Kappa carrageenan (KC) and vinyltriethoxysilane

(VTESI). Furthermore, studies on the structure of hydrogel revealed that by enhancing the crosslinker (KC) concentration, marked decrease in swelling ration of hydrogel was obtained which finally led to slow peptide release rate of hydrogel.⁹⁹ James and coworkers have prepared a smart polymeric “intelligent” delivery systems capable of sustained release of therapeutic macromolecules.¹⁰¹ The nanocarriers, polymeric nanoparticles, have shown benefits for peptide drug delivery following oral, nasal, pulmonary, parenteral, transdermal, and ocular doings.¹⁰² PCL-PEG-PCL, chitosan (CS), and poly(l-lactide) NPs achieved higher insulin loading and were employed to improve bioavailability and hypoglycemic activity of insulin *via* oral route.^{103,104} The chitosan-N-acetyl-L-cysteine (CS-NAC) NPs and hybrid poly-oligosaccharide NPs comprising CS and cyclodextrins were applied as nanocarriers for nasal insulin delivery.^{105,106} Nanocarriers such as CS NPs have been suggested as an excellent formulation for local and systemic delivery of insulin following pulmonary route.^{102,107} The polymeric nanocarriers have been used to enhance solubility, bioavailability, and prolonged circulation times of insulin.¹⁰⁸ The rectal form of insulin composed of acrylic hydrogels containing absorption enhancers was applied in *in vitro* and *in vivo* environment.¹⁰⁹

Hydrogels As Carrier For Glucagon-Like Peptide-I Receptor Agonist's

Application of the anti-diabetic drugs has been faced with several problems. For example, injection of insulin should be done before the meal, need repetitive every day and may lead to hypoglycemic symptoms. Therefore, to solve these problem GLP-1 analogs and their corresponding receptors (exendins) can be used.¹¹⁰ Exendin-4 is the safest exendin drug and belongs to incretin mimetics. It is demonstrated that this compound is able to enhance glucose-dependent insulin secretion and induce satiety.^{111,112} Byetta[®], a synthetic formulation of Exendin-4 (Exenatide (EXT)) has been approved for type II diabetes. Microsphere formulation (Bydureon[®]) has been developed with only one injection per week.¹¹³ Furthermore, less side effects were reported for Bydureon[®] than Byetta[®]. Until now several kinds of thermos-gels such as PEG and poly-phosphazene have been developed for protein delivery.^{114,115} The potency of poly(lactic acid-co-glycolic acid)-poly(ethylene

glycol)-poly(lactic acid-co-glycolic acid) triblock copolymers in delivery of EXT has been investigated by Li et al.¹¹⁶ In this formulation, zinc acetate was introduced to this formulation to improving drug release of EXT.¹¹⁶ A new hydrogel system of EX-4 using poly(organophosphazenes) was developed which is hydrophilic, easy and has high capacity of protein loading and easy administration.¹¹⁷ Poly(organophosphazenes) by conjugating protamine was used to enhance hydrogel interaction with EX-4.¹¹⁸ In another study, poly(ϵ -caprolactone-co-glycolic acid)-poly(ethylene glycol)-poly(ϵ -caprolactone-co-glycolic acid) (PCGA-PEG-PCGA) triblock thermos-sensitive co-polymers were used to sustain delivery of Liraglutide (Lira).¹¹⁹

Hydrogel-Based Peptide Hormone Delivery For Tissue Engineering And Regenerative Medicine

Due to their unique structural and physicochemical characteristics of hydrogels, they are considered as pioneer candidate in the tissue engineering.^{120,121} Gels are widely used in cell culture because of their 3D network structure and high permeability. For instance, hydrogel scaffolds are used for simultaneous seeding of cells because of their shape, porosity, and surface morphology.¹²² Since the hydrogel scaffolds are too similar to the extracellular matrix, an opportunity was created to overcome various challenges in tissue engineering.⁷

Calcitonin has diverse physiological functions such as regulation of calcium homeostasis and bone metabolism. The peptide hormone effectively prevents bone loss. Calcitonin upregulates collagen expression and inhibits metalloproteases.¹²³ Calcitonin affects extracellular matrix synthesis and has therefore been clinically used in the treatment of postmenopausal osteoporosis.¹²⁴ Recently, Liu et al¹²⁴ prepared the salmon calcitonin and oxidized calcium alginate (sCT-OCA)-loaded poly(d,l-lactic acid-co-glycolic acid)-b-poly(ethylene glycol)-b-poly(d,l-lactic acid-co-glycolic acid) (PLGA-PEG-PLGA) hydrogel. The thermo-sensitive triblock copolymer hydrogel exhibited sol-gel transition at body temperature and has therefore been used for long-term anti-osteopenia treatment in rats. sCT was released by degradation of the hydrogel. The system reduced serum calcium and bone trabecula reconstruction in the treatment of glucocorticoid-induced osteopenia in rats (Figure 1).

Figure 1 The PLGA-PEG-PLGA hydrogel for controlled sCT release.

Note: Data from Liu et al.¹²⁴

The FDA approved regulator of calcium homeostasis, parathyroid hormone (PTH) possesses anabolic effects on bone and therefore plays an important role in bone metabolism and regeneration.¹²⁵ Numerous studies have shown that once-daily injections of PTH enhance the bone healing in vivo.¹²⁶ In a study, Park et al¹⁰ presented a new strategy for improved clinical application of parathyroidectomized (PTX). They enhanced the sustained release of PTX using in situ-forming gelatin-hydroxyphenyl propionic acid hydrogels (GHH) to control mechanical stiffness. They reported the best-sustained release of PTH in GHH-embedded differentiated tonsil-derived mesenchyme stem cells (dTMSCs). The hydrogels improved blood calcium homeostasis and treated hypoparathyroidism effectively. Interestingly, undifferentiated TMSCs also incorporated into GHH have released PTH in a sustained manner. PTH was used to enhance osteoblasts proliferation.¹²⁷ They showed that stromal precursor antigen-1 (STRO-1) human periodontal ligament stem cells (hPDLSCs) expressed higher levels of the PTH-1 receptor (PTH1R) than STRO-1(–) hPDLSCs. In addition, intermittent PTH treatment enhanced the expression of PTH1R and osteogenesis-related genes in STRO-1(+) hPDLSCs. The results showed that the mineralization ability and alkaline phosphatase activity increased in PTH-treated cells. Intermittent PTH treatment improved the capacity for STRO-1(+) hPDLSCs to repair damaged tissue and ameliorate the symptoms of periodontitis. The effects of parathyroid hormone-related protein (PTHrP) (1–37) were investigated and the degradable implant was suggested as an attractive strategy for improved bone regeneration in aged and diabetic rats.¹²⁸

Conclusion

The usages of the pharmaceutical proteins (large molecule) as a therapeutic agent have been increased strangely because of their advantages. Because of the high proteolytic activity and low pH of the stomachs, proteins are destabilized and degraded oral, which resulted in the loss of biological activities. So, oral administration of proteins is a challenging route. Hydrogels seem to be suited to enhance efficacy, reduce dosing interval, and provide a more convenient dosage route for large and labile proteins. So, protein-loaded hydrogels are explored to increase the therapeutic outcome. Critically, biocompatibility depends on the interactions between tissue and material interface. Therefore, hydrogels have potential in overcoming the unique formulation challenges of biotherapeutics.

Acknowledgment

The authors would like to thank Kerman University of Medical Sciences (KMU) for facilitating relevant research into the conclusions related to this study.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Lau JL, Dunn MK. Therapeutic peptides: historical perspectives, current development trends, and future directions. *Bioorg Med Chem*. 2018;26(10):2700–2707. doi:10.1016/j.bmc.2017.06.052
2. Frokjaer S, Otzen DE. Protein drug stability: a formulation challenge. *Nat Rev Drug Discov*. 2005;4(4):298. doi:10.1038/nrd1876
3. Verma M, Furin J, Langer R, Traverso G. Making the case: developing innovative adherence solutions for the treatment of tuberculosis. *BMJ Global Health*. 2019;4(1):e001323. doi:10.1136/bmjgh-2018-001323
4. Park K. Controlled drug delivery systems: past forward and future back. *J Controlled Release*. 2014;190:3–8. doi:10.1016/j.jconrel.2014.03.054
5. Deb PK, Al-Attaqchi O, Chandrasekaran B, Paradkar A, Tekade RK. Protein/peptide drug delivery systems: practical considerations in pharmaceutical product development. In: *Basic Fundamentals of Drug Delivery*. Elsevier; 2019:651–684.
6. Shaji J, Patole V. Protein and peptide drug delivery: oral approaches. *Indian J Pharm Sci*. 2008;70(3):269. doi:10.4103/0250-474X.42967
7. El-Sherbiny I, Khalil I, Ali I, Yacoub M. Updates on smart polymeric carrier systems for protein delivery. *Drug Dev Ind Pharm*. 2017;43(10):1567–1583. doi:10.1080/03639045.2017.1338723
8. Ghasemi R, Abdollahi M, Zadeh EE, et al. mPEG-PLA and PLA-PEG-PLA nanoparticles as new carriers for delivery of recombinant human Growth Hormone (rhGH). *Sci Rep*. 2018;8(1):9854. doi:10.1038/s41598-018-28092-8
9. Nikolić LB, Zdravković AS, Nikolić VD, Ilić-Stojanović SS. Synthetic hydrogels and their impact on health and environment. In: *Cellulose-Based Superabsorbent Hydrogels*. Berlin: Springer; 2018:1–29.
10. Park YS, Lee Y, Jin YM, et al. Sustained release of parathyroid hormone via in situ cross-linking gelatin hydrogels improves the therapeutic potential of tonsil-derived mesenchymal stem cells for hypoparathyroidism. *J Tissue Eng Regen Med*. 2018;12(3):e1747–e1756. doi:10.1002/term.2430

11. Bandopadhyay S, Bandyopadhyay N, Deb PK, Singh C, Tekade RK. Preformulation studies of drug substances, protein, and peptides: role in drug discovery and pharmaceutical product development. In: *Dosage Form Design Considerations*. Elsevier; 2018:401–433.
12. Ahmed EM. Hydrogel: preparation, characterization, and applications: a review. *J Adv Res*. 2015;6(2):105–121. doi:10.1016/j.jare.2013.07.006
13. Mohammadinejad R, Maleki H, Larrañeta E, et al. Status and future scope of plant-based green hydrogels in biomedical engineering. *Appl Mater Today*. 2019;16:213–246. doi:10.1016/j.apmt.2019.04.010
14. Du G, Peng Y, Pei Y, Zhao L, Wen Z, Hu Z. Thermo-responsive temporary plugging agent based on multiple phase transition supramolecular gel. *Energy Fuels*. 2017;31(9):9283–9289. doi:10.1021/acs.energyfuels.7b01691
15. Ullah F, Othman MBH, Javed F, Ahmad Z, Akil HM. Classification, processing and application of hydrogels: a review. *Mater Sci Eng C*. 2015;57:414–433. doi:10.1016/j.msec.2015.07.053
16. Tomar N, Tomar M, Gulati N, Nagaich U. pHEMA hydrogels: devices for ocular drug delivery. *Int J Res Health Allied Sci*. 2012;1(4):224. doi:10.4103/2278-344X.107844
17. Peppas N, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulations. *Eur J Pharm Biopharm*. 2000;50(1):27–46.
18. Norman AW, Litwack G. *Hormones*. Academic Press; 1997.
19. Khpal M, Singer M. Inflammation, Hormones, and Metabolism. In: *Inflammation: From Molecular and Cellular Mechanisms to the Clinic*. New York: John Wiley & Sons, Inc; 2017:915–946.
20. Law S, Huang K, Chou V. Stability of desmopressin loaded in liposomes. *J Liposome Res*. 2003;13(3–4):269–277. doi:10.1081/LPR-120026392
21. Gavrilu A, Hollenberg AN. The hypothalamic-pituitary-thyroid axis: physiological regulation and clinical implications. In: *The Thyroid and Its Diseases*. Springer; 2019:13–23.
22. Parsons JA. *Peptide Hormones*. University Park Press; 1976.
23. Avanti C. *Innovative Strategies for Stabilization of Therapeutic Peptides in Aqueous Formulations*. 2012.
24. Varamini P, Toth I. Recent advances in oral delivery of peptide hormones. *Expert Opin Drug Deliv*. 2016;13(4):507–522. doi:10.1517/17425247.2016.1142526
25. Schulinkamp R, Pagano T, Hung D, Raffa R. Insulin receptors and insulin action in the brain: review and clinical implications. *Neurosci Biobehav Rev*. 2000;24(8):855–872.
26. Pawar VK, Meher JG, Singh Y, Chaurasia M, Reddy BS, Chourasia MK. Targeting of gastrointestinal tract for amended delivery of protein/peptide therapeutics: strategies and industrial perspectives. *J Controlled Release*. 2014;196:168–183. doi:10.1016/j.jconrel.2014.09.031
27. Dragan ES. Design and applications of interpenetrating polymer network hydrogels. A review. *Chem Eng J*. 2014;243:572–590. doi:10.1016/j.cej.2014.01.065
28. Manning MC, Chou DK, Murphy BM, Payne RW, Katayama DS. Stability of protein pharmaceuticals: an update. *Pharm Res*. 2010;27(4):544–575. doi:10.1007/s11095-009-0045-6
29. Muheem A, Shakeel F, Jahangir MA, et al. A review on the strategies for oral delivery of proteins and peptides and their clinical perspectives. *Saudi Pharm J*. 2016;24(4):413–428. doi:10.1016/j.jsps.2014.06.004
30. Bruno BJ, Miller GD, Lim CS. Basics and recent advances in peptide and protein drug delivery. *Ther Deliv*. 2013;4(11):1443–1467. doi:10.4155/tde.13.104
31. Tan ML, Choong PF, Dass CR. Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery. *Peptides*. 2010;31(1):184–193. doi:10.1016/j.peptides.2009.10.002
32. Cholewinski M, Lückel B, Horn H. Degradation pathways, analytical characterization and formulation strategies of a peptide and a protein calcitonine and human growth hormone in comparison. *Pharm Acta Helv*. 1996;71(6):405–419.
33. Chaturvedi K, Ganguly K, Nadagouda MN, Aminabhavi TM. Polymeric hydrogels for oral insulin delivery. *J Controlled Release*. 2013;165(2):129–138. doi:10.1016/j.jconrel.2012.11.005
34. Zelikin AN, Ehrhardt C, Healy AM. Materials and methods for delivery of biological drugs. *Nat Chem*. 2016;8(11):997. doi:10.1038/nchem.2629
35. Sarmiento B, Ribeiro A, Veiga F, Ferreira D, Neufeld R. Oral bioavailability of insulin contained in polysaccharide nanoparticles. *Biomacromolecules*. 2007;8(10):3054–3060. doi:10.1021/bm0703923
36. Martinho N, Damgé C, Reis CP. Recent advances in drug delivery systems. *J Biomater Nanobiotechnol*. 2011;2(05):510. doi:10.4236/jbnt.2011.225062
37. Niu Z, Conejos-Sanchez I, Griffin BT, O'Driscoll CM, Alonso MJ. Lipid-based nanocarriers for oral peptide delivery. *Adv Drug Del Rev*. 2016;106:337–354. doi:10.1016/j.addr.2016.04.001
38. Carino GP, Jacob JS, Mathiowitz E. Nanosphere based oral insulin delivery. *J Controlled Release*. 2000;65(1–2):261–269. doi:10.1016/S0168-3659(99)00247-3
39. Khafagy E-S, Morishita M, Onuki Y, Takayama K. Current challenges in non-invasive insulin delivery systems: a comparative review. *Adv Drug Del Rev*. 2007;59(15):1521–1546. doi:10.1016/j.addr.2007.08.019
40. Wang W. Instability, stabilization, and formulation of liquid protein pharmaceuticals. *Int J Pharm*. 1999;185(2):129–188.
41. Savale SK. Protein and peptide drug delivery system. *World J Pharm Pharm Sci*. 2016;5(4):1–19.
42. Clement S, Still JG, Kosutic G, McAllister R. Oral insulin product hexyl-insulin monoconjugate 2 (HIM2) in type 1 diabetes mellitus: the glucose stabilization effects of HIM2. *Diabetes Technol Ther*. 2002;4(4):459–466. doi:10.1089/152091502760306544
43. Ismail R, Csoka I. Novel strategies in the oral delivery of antidiabetic peptide drugs – insulin, GLP 1 and its analogs. *Eur J Pharm Biopharm*. 2017;115:257–267. doi:10.1016/j.ejpb.2017.03.015
44. Hirakura T, Yasugi K, Nemoto T, et al. Hybrid hyaluronan hydrogel encapsulating nanogel as a protein nanocarrier: new system for sustained delivery of protein with a chaperone-like function. *J Control Release*. 2010;142(3):483–489. doi:10.1016/j.jconrel.2009.11.023
45. Pasut G, Veronese FM. State of the art in PEGylation: the great versatility achieved after forty years of research. *J Controlled Release*. 2012;161(2):461–472. doi:10.1016/j.jconrel.2011.10.037
46. Batrakovaa EV, Kabanov AV. Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers. *J Controlled Release*. 2008;130(2):98–106. doi:10.1016/j.jconrel.2008.04.013
47. Suthiwangcharoen N, Nagarajan R. Nanoarmoring of proteins by conjugation to block copolymer micelles. *Methods Enzymol*. 2017;590(Elsevier):277–304. doi:10.1016/bs.mie.2017.01.013
48. Labonté V, Marion A, Virgilio N, Tavares JR. Gas-phase surface engineering of polystyrene beads used to challenge automated particle inspection systems. *Ind Eng Chem Res*. 2016;55(27):7362–7372. doi:10.1021/acs.iecr.6b01573
49. Swaminathan J. C. E. Liposomal delivery of proteins and peptides. *Expert Opin Drug Deliv*. 2012;9(12):1489–1503. doi:10.1517/17425247.2012.735658
50. Li X, Kuromi H, Briggs L, et al. Bicaudal-D binds clathrin heavy chain to promote its transport and augments synaptic vesicle recycling. *Embo J*. 2010;29(5):992–1006. doi:10.1038/emboj.2009.410
51. Kaur G, Garg T, Rath G, Goyal AK. Archaeosomes: an excellent carrier for drug and cell delivery. *Drug Deliv*. 2016;23(7):2497–2512. doi:10.3109/10717544.2015.1019653

52. Mohanraj VJ, Barnes TJ, Prestidge CA. Silica nanoparticle coated liposomes: a new type of hybrid nanocapsule for proteins. *Int J Pharm.* **2010**;392(1–2):285–293. doi:10.1016/j.ijpharm.2010.03.061
53. Dombu CY, Betbeder D. Airway delivery of peptides and proteins using nanoparticles. *Biomaterials.* **2013**;34(2):516–525. doi:10.1016/j.biomaterials.2012.08.070
54. Fröhlich E. Value of phagocyte function screening for immunotoxicity of nanoparticles in vivo. *Int J Nanomedicine.* **2015**;10:3761–3778. doi:10.2147/IJN.S83068
55. Amancha PK, Balkundi S, Lvov Y, et al. Pulmonary sustained release of insulin from microparticles composed of polyelectrolyte layer-by-layer assembly. *Int J Pharm.* **2014**;466(1–2):96–108. doi:10.1016/j.ijpharm.2014.02.006
56. Andrade A, Rossi RC, Stival VP, et al. Different supplements for finishing of Nellore cattle on deferred *Brachiarum decumbens* pasture during the dry season. *Bol Ind Anim.* **2015**;72(2):91–101. doi:10.17523/bia.v72n2p91
57. Jafari G, Wasko BM, Tonge A, et al. Tether mutations that restore function and suppress pleiotropic phenotypes of the *C. elegans* isp-1(qm150) Rieseke iron-sulfur protein. *Pnas.* **2015**;112(45):E6148–E6157. doi:10.1073/pnas.1509416112
58. Teleanu DM, Negut I, Grumezescu V, Grumezescu AM, Teleanu RI. Nanomaterials for drug delivery to the central nervous system. *Nanomaterials.* **2019**;9(3):371. doi:10.3390/nano9071000
59. Pawar R, Ben-Ari A, Domb AJ. Protein and peptide parenteral controlled delivery. *Expert Opin Biol Ther.* **2004**;4(8):1203–1212. doi:10.1517/14712598.4.8.1203
60. Hutchinson J, Burholt S, Hamley I. Peptide hormones and lipopeptides: from self-assembly to therapeutic applications. *J Pept Sci.* **2017**;23(2):82–94. doi:10.1002/psc.2954
61. Parhi R. Cross-linked hydrogel for pharmaceutical applications: a review. *Adv Pharm Bull.* **2017**;7(4):515–530. doi:10.1517/apb.2017.064
62. Hoffman AS. Hydrogels for biomedical applications. *Adv Drug Deliv Rev.* **2012**;64:18–23. doi:10.1016/j.addr.2012.09.010
63. Phan VG, Thambi T, Duong HT, Lee DS. Poly (amino carbonate urethane)-based biodegradable, temperature and pH-sensitive injectable hydrogels for sustained human growth hormone delivery. *Sci Rep.* **2016**;6:29978. doi:10.1038/srep29978
64. Schoener CA, Hutson HN, Peppas NA. pH-responsive hydrogels with dispersed hydrophobic nanoparticles for the delivery of hydrophobic therapeutic agents. *Polym Int.* **2012**;61(6):874–879. doi:10.1002/pi.4219
65. Batista RA, Otoni CG, Espitia PJ. Fundamentals of chitosan-based hydrogels: elaboration and characterization techniques. In: *Materials for Biomedical Engineering*. Elsevier; **2019**:61–81.
66. Los E, Rosenfeld RG. Growth and growth hormone in turner syndrome: looking back, looking ahead. Paper presented at: American Journal of Medical Genetics Part C: Seminars in Medical Genetics; **2019**.
67. Dao LN, Lippe B, Laird M, Beierle I. Human growth hormone. In: *Pharmaceutical Biotechnology*. Springer; **2019**:437–449.
68. Amato G, Mazziotti G, Di Somma C, et al. Recombinant growth hormone (GH) therapy in GH-deficient adults: a long-term controlled study on daily versus thrice weekly injections. *J Clin Endocrinol Metab.* **2000**;85(10):3720–3725. doi:10.1210/jcem.85.10.6881
69. Cai Y, Xu M, Yuan M, Liu Z, Yuan W. Developments in human growth hormone preparations: sustained-release, prolonged half-life, novel injection devices, and alternative delivery routes. *Int J Nanomedicine.* **2014**;9:3527.
70. Webster R, Xie R, Didier E, et al. PEGylation of somatropin (recombinant human growth hormone): impact on its clearance in humans. *Xenobiotica.* **2008**;38(10):1340–1351. doi:10.1080/0049250802413856
71. Goto T, Morishita M, Kavimandan NJ, Takayama K, Peppas NA. Gastrointestinal transit and mucoadhesive characteristics of complexation hydrogels in rats. *J Pharm Sci.* **2006**;95(2):462–469. doi:10.1002/jps.20566
72. López JE, Peppas NA. Effect of poly (ethylene glycol) molecular weight and microparticle size on oral insulin delivery from P (MAA-g-EG) Microparticles. *Drug Dev Ind Pharm.* **2004**;30(5):497–504. doi:10.1081/DDC-120037480
73. Atayde EC Jr, Montalbo RCK, Arco SD. Temperature- and pH-dependent drug release of block copolymers of methacrylic acid and poly (ethylene glycol) methyl ether methacrylates. *Philipp J Sci.* **2018**;147(3):363–372.
74. Atayde EC Jr, Montalbo RCK, Arco SD. Linear and hyperbranched copolymers of PEG-based acrylates and methacrylic acid as pH-responsive hydrophobic drug carriers. Paper presented at: Materials Science Forum; **2018**.
75. Carr DA, Peppas NA. Assessment of poly (methacrylic acid-co-N-vinyl pyrrolidone) as a carrier for the oral delivery of therapeutic proteins using Caco-2 and HT29-MTX cell lines. *J Biomed Mater Res Part A.* **2010**;92(2):504–512.
76. Cascone MG, Di Silvio L, Sim B, Downes S. Collagen and hyaluronic acid based polymeric blends as drug delivery systems for the release of physiological concentrations of growth hormone. *Jmsmm.* **1994**;5(9–10):770–774.
77. Veldhuis JD. A tripeptidyl ensemble perspective of interactive control of growth hormone secretion. *Horm Res Paediatr.* **2003**;60(Suppl. 1):86–101. doi:10.1159/000071232
78. Okumu FW, Dao LN, Fielder PJ, et al. Sustained delivery of human growth hormone from a novel gel system: SABERTM. *Biomaterials.* **2002**;23(22):4353–4358. doi:10.1016/s0142-9612(02)00174-6
79. Tae G, Lammertink RG, Kornfield JA, Hubbell JA. Facile hydrophilic surface modification of poly (tetrafluoroethylene) using fluoroalkyl-terminated poly (ethylene glycol)s. *Adv Mater.* **2003**;15(1):66–69. doi:10.1002/(ISSN)1521-4095
80. Burdick JA, Anseth KS. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. *Biomaterials.* **2002**;23(22):4315–4323. doi:10.1016/s0142-9612(02)00176-x
81. Nir Y, Paz A, Sabo E, Potasman I. Fear of injections in young adults: prevalence and associations. *Am J Trop Med Hyg.* **2003**;68(3):341–344.
82. Liu L, Yang H, Lou Y, et al. Enhancement of oral bioavailability of salmon calcitonin through chitosan-modified, dual drug-loaded nanoparticles. *Int J Pharm.* **2019**;557:170–177. doi:10.1016/j.ijpharm.2018.12.053
83. Li N, Li X-R, Zhou Y-X, et al. The use of polyion complex micelles to enhance the oral delivery of salmon calcitonin and transport mechanism across the intestinal epithelial barrier. *Biomaterials.* **2012**;33(34):8881–8892. doi:10.1016/j.biomaterials.2012.08.047
84. Torres-Lugo M, Peppas NA. Molecular design and in vitro studies of novel pH-sensitive hydrogels for the oral delivery of calcitonin. *Macromolecules.* **1999**;32(20):6646–6651. doi:10.1021/ma990541c
85. Donini C, Robinson D, Colombo P, Giordano F, Peppas N. Preparation of poly (methacrylic acid-g-poly (ethylene glycol)) nanospheres from methacrylic monomers for pharmaceutical applications. *Int J Pharm.* **2002**;245(1–2):83–91.
86. Kamei N, Morishita M, Chiba H, Kavimandan NJ, Peppas NA, Takayama K. Complexation hydrogels for intestinal delivery of interferon β and calcitonin. *J Controlled Release.* **2009**;134(2):98–102. doi:10.1016/j.jconrel.2008.11.014
87. Owens DR, Zinman B, Bolli G. Alternative routes of insulin delivery. *Diabet Med.* **2003**;20(11):886–898. doi:10.1046/j.1464-5491.2003.01076.x
88. Bahman F, Greish K, Taurin S. Nanotechnology in insulin delivery for management of diabetes. *Pharmaceutical Nanotechnology.* **2019**;7:113–128. doi:10.2174/2211738507666190321110721

89. Gordon Still J. Development of oral insulin: progress and current status. *Diabetes Metab Res Rev*. 2002;18:S1. doi:10.1002/dmrr.207
90. Li J, Wang Y, Han L, Sun X, Yu H, Yu Y. Time-action profile of an oral enteric insulin formulation in healthy Chinese volunteers. *Clin Ther*. 2012;34(12):2333–2338. doi:10.1016/j.clinthera.2012.11.004
91. Morales-Burgos AM, Carvajal-Millan E, Sotelo-Cruz N, et al. Polysaccharides in alternative methods for insulin delivery. In *Biopolymer Grafting*. Elsevier; 2018:175–197.
92. Morishita M, Lowman AM, Takayama K, Nagai T, Peppas NA. Elucidation of the mechanism of incorporation of insulin in controlled release systems based on complexation polymers. *J Controlled Release*. 2002;81(1):25–32. doi:10.1016/S0168-3659(02)00019-6
93. Morishita M, Goto T, Peppas NA, et al. Mucosal insulin delivery systems based on complexation polymer hydrogels: effect of particle size on insulin enteral absorption. *J Controlled Release*. 2004;97(1):115–124. doi:10.1016/j.jconrel.2004.03.008
94. Foss AC, Peppas NA. Investigation of the cytotoxicity and insulin transport of acrylic-based copolymer protein delivery systems in contact with Caco-2 cultures. *Eur J Pharm Biopharm*. 2004;57(3):447–455. doi:10.1016/j.ejpb.2004.02.008
95. Fukuoka Y, Khafagy E-S, Goto T, et al. Combination strategy with complexation hydrogels and cell-penetrating peptides for oral delivery of insulin. *Biol Pharm Bull*. 2018;41(5):811–814. doi:10.1248/bpb.b17-00951
96. Sonaje K, Chen Y-J, Chen H-L, et al. Enteric-coated capsules filled with freeze-dried chitosan/poly (γ -glutamic acid) nanoparticles for oral insulin delivery. *Biomaterials*. 2010;31(12):3384–3394. doi:10.1016/j.biomaterials.2010.01.042
97. Dorkoosh F, Verhoef JC, Borchard G, Rafiee-Tehrani M, Verheijden J, Junginger H. Intestinal absorption of human insulin in pigs using delivery systems based on superporous hydrogel polymers. *Int J Pharm*. 2002;247(1):47–55.
98. Nguyen MK, Lee DS. Controlling the degradation of pH/temperature-sensitive injectable hydrogels based on poly (β -amino ester). *Macromolecular Research*. 2010;18(2):192–199. doi:10.1007/s13233-009-0182-0
99. Rasool N, Yasin T, Heng JY, Akhter Z. Synthesis and characterization of novel pH-, ionic strength and temperature-sensitive hydrogel for insulin delivery. *Poly*. 2010;51(8):1687–1693. doi:10.1016/j.polymer.2010.02.013
100. Vermonden T, Censi R, Hennink WE. Hydrogels for protein delivery. *Chem Rev*. 2012;112(5):2853–2888. doi:10.1021/cr200157d
101. James HP, John R, Alex A, Anoop K. Smart polymers for the controlled delivery of drugs—a concise overview. *Acta Pharm Sin B*. 2014;4(2):120–127. doi:10.1016/j.apsb.2014.02.005
102. Patel A, Patel M, Yang X, K Mitra A. Recent advances in protein and peptide drug delivery: a special emphasis on polymeric nanoparticles. *Protein Pept Lett*. 2014;21(11):1102–1120.
103. Rastogi R, Anand S, Koul V. Evaluation of pharmacological efficacy of ‘insulin-surfoplex’ encapsulated polymer vesicles. *Int J Pharm*. 2009;373(1):107–115. doi:10.1016/j.ijpharm.2009.01.022
104. Elvassore N, Bertucco A, Caliceti P. Production of insulin-loaded poly (ethylene glycol)/poly (l-lactide)(PEG/PLA) nanoparticles by gas antisolvent techniques. *J Pharm Sci*. 2001;90(10):1628–1636. doi:10.1002/jps.1113
105. Reis CP, Veiga FJ, Ribeiro AJ, Neufeld RJ, Damgé C. Nanoparticulate biopolymers deliver insulin orally eliciting pharmacological response. *J Pharm Sci*. 2008;97(12):5290–5305. doi:10.1002/jps.21347
106. Teijeiro-Orsorio D, Remuñán-López C, Alonso MJ. New generation of hybrid poly/oligosaccharide nanoparticles as carriers for the nasal delivery of macromolecules. *Biomacromolecules*. 2008;10(2):243–249. doi:10.1021/bm800975j
107. Al-Qadi S, Grenha A, Carrión-Recio D, Seijo B, Remuñán-López C. Microencapsulated chitosan nanoparticles for pulmonary protein delivery: in vivo evaluation of insulin-loaded formulations. *J Controlled Release*. 2012;157(3):383–390. doi:10.1016/j.jconrel.2011.08.008
108. Fleige E, Quadir MA, Haag R. Stimuli-responsive polymeric nanocarriers for the controlled transport of active compounds: concepts and applications. *Adv Drug Del Rev*. 2012;64(9):866–884. doi:10.1016/j.addr.2012.01.020
109. Uchida T, Toida Y, Sakakibara S, et al. Preparation and characterization of insulin-loaded acrylic hydrogels containing absorption enhancers. *Chem Pharm Bull (Tokyo)*. 2001;49(10):1261–1266. doi:10.1248/cpb.49.1261
110. Eng J, Kleinman W, Singh L, Singh G, Raufman J-P. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J Biol Chem*. 1992;267(11):7402–7405.
111. Nielsen LL, Young AA, Parkes DG. Pharmacology of exenatide (synthetic exendin-4): a potential therapeutic for improved glycaemic control of type 2 diabetes. *Regul Pept*. 2004;117(2):77–88. doi:10.1016/j.regpep.2003.10.028
112. Ionut V, Zheng D, Stefanovski D, Bergman RN. Exenatide can reduce glucose independent of islet hormones or gastric emptying. *Am J Physiol Endocrinol Metab*. 2008;295(2):E269–E277. doi:10.1152/ajpendo.90222.2008
113. DeYoung MB, MacConell L, Sarin V, Trautmann M, Herbert P. Encapsulation of exenatide in poly-(D, L-lactide-co-glycolide) microspheres produced an investigational long-acting once-weekly formulation for type 2 diabetes. *Diabetes Technol Ther*. 2011;13(11):1145–1154. doi:10.1089/dia.2011.0050
114. Bae SJ, Suh JM, Sohn YS, Bae YH, Kim SW, Jeong B. Thermogelling poly (caprolactone-b-ethylene glycol-b-caprolactone) aqueous solutions. *Macromolecules*. 2005;38(12):5260–5265. doi:10.1021/ma050489m
115. Zhang Z, Ni J, Chen L, Yu L, Xu J, Ding J. Biodegradable and thermoreversible PCL-PEG-PCL hydrogel as a barrier for prevention of post-operative adhesion. *Biomaterials*. 2011;32(21):4725–4736. doi:10.1016/j.biomaterials.2011.03.046
116. Li K, Yu L, Liu X, Chen C, Chen Q, Ding J. A long-acting formulation of a polypeptide drug exenatide in treatment of diabetes using an injectable block copolymer hydrogel. *Biomaterials*. 2013;34(11):2834–2842. doi:10.1016/j.biomaterials.2013.01.013
117. Seo B-B, Park M-R, Chun C, Lee J-Y, Song S-C. The biological efficiency and bioavailability of human growth hormone delivered using injectable, ionic, thermosensitive poly (organophosphazene)-polyethylenimine conjugate hydrogels. *Biomaterials*. 2011;32(32):8271–8280. doi:10.1016/j.biomaterials.2011.07.033
118. Seo B-B, Park M-R, Song S-C. Sustained release of exendin 4 using injectable and ionic-nano-complex forming polymer hydrogel system for long-term treatment of type 2 diabetes mellitus. *ACS Appl Mater Interfaces*. 2019;11(17):15201–15211. doi:10.1021/acsami.8b19669
119. Chen Y, Luan J, Shen W, Lei K, Yu L, Ding J. Injectable and thermosensitive hydrogel containing liraglutide as a long-acting antidiabetic system. *ACS Appl Mater Interfaces*. 2016;8(45):30703–30713. doi:10.1021/acsami.6b09415
120. Naahidi S, Jafari M, Logan M, et al. Biocompatibility of hydrogel-based scaffolds for tissue engineering applications. *Biotechnol Adv*. 2017;35(5):530–544. doi:10.1016/j.biotechadv.2017.05.006
121. Eslahi N, Abdorahim M, Simchi A. Smart polymeric hydrogels for cartilage tissue engineering: a review on the chemistry and biological functions. *Biomacromolecules*. 2016;17(11):3441–3463. doi:10.1021/acs.biomac.6b01235

122. Sood N, Bhardwaj A, Mehta S, Mehta A. Stimuli-responsive hydrogels in drug delivery and tissue engineering. *Drug Deliv*. 2016;23(3):748–770. doi:10.3109/10717544.2014.940091
123. Naot D, Musson DS, Cornish J. The activity of peptides of the calcitonin family in bone. *Physiol Rev*. 2018;99(1):781–805. doi:10.1152/physrev.00066.2017
124. Liu Y, Chen X, Li S, et al. Calcitonin-loaded thermosensitive hydrogel for long-term antiosteopenia therapy. *ACS Appl Mater Interfaces*. 2017;9(28):23428–23440. doi:10.1021/acsami.7b05740
125. Pettway GJ, Meganck JA, Koh AJ, Keller ET, Goldstein SA, McCauley LK. Parathyroid hormone mediates bone growth through the regulation of osteoblast proliferation and differentiation. *Bone*. 2008;42(4):806–818. doi:10.1016/j.bone.2007.11.017
126. Rowshan HH, Parham MA, Baur DA, et al. Effect of intermittent systemic administration of recombinant parathyroid hormone (1-34) on mandibular fracture healing in rats. *JOMS*. 2010;68(2):260–267.
127. Wang X, Wang Y, Dai X, et al. Effects of intermittent administration of parathyroid hormone (1-34) on bone differentiation in stromal precursor antigen-1 positive human periodontal ligament stem cells. In: *Stem Cells International*. 2016:2016.
128. Ardura JA, Portal-Núñez S, Lozano D, et al. Local delivery of parathyroid hormone-related protein-derived peptides coated onto a hydroxyapatite-based implant enhances bone regeneration in old and diabetic rats. *J Biomed Mater Res Part A*. 2016;104(8):2060–2070. doi:10.1002/jbm.a.35742

Drug Design, Development and Therapy

Dovepress

Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also

been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/drug-design-development-and-therapy-journal>