Review

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Drug Carriers for the Delivery of Therapeutic Peptides

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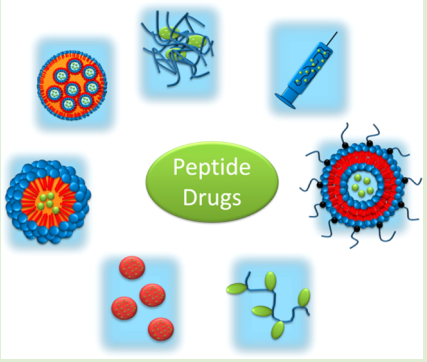
ABSTRACT: Peptides take on an increasingly important role as therapeutics in areas including diabetes, oncology, and metabolic, cardiovascular, and infectious diseases. In addition, many peptides such as insulin have been employed for many years. A challenge in the administration of peptide drugs is their often low hydrolytic stability, as well as other problems that are common to any drug treatment such as systemic distribution. There is a significant attention in the literature of protein drugs and their delivery strategies, but not many overviews are specifically dedicated to peptides. In this review, the different approaches to deliver peptides have been summarized where the focus is only on drug carriers based on organic materials. Initial discussion is on different methods of polymer−peptide conjugation before being followed by physical encapsulation techniques, which is divided into surfactant-based techniques and polymer carriers. Surfactant-based techniques are dominated by liposome, microemulsions and solid-lipid nanoparticles. The field widens further in the polymer field. The delivery of peptides has been enhanced using polymer-decorated liposomes, solid microspheres, polyelectrolyte complex, emulsions, hydrogels, and injectable polymers. The aim of this article is to give the reader an overview over the different types of carriers.



1. THE IMPORTANCE OF PEPTIDES IN NANOMEDICINE

Peptides are short chains built up from amino acids. The only difference between peptides and proteins is the size, and it is commonly accepted that polypeptides with approximately 50 amino acids are regarded as proteins. Peptides embody important functional molecules in nature, but they have been discovered in recent years as building blocks in medicinal chemistry and nanomedicine. The applications of polypeptides range from scaffolds for material design to molecules that can convey important biological information, to simply degradable structures. Ring-opening polymerization of amino acid *N*-carboxyanhydrides led to degradable polymers that also have helical structures, as found in proteins.[1−5] Peptides are popular in material design to introduce linkers that can degrade in the presence of proteases. Very often specific peptide linkers are employed that can be cleaved rapidly by proteases that are up regulated in certain diseases. This can be used to cleave a drug specifically at high protease concentrations.[6] Frequently applied is the use of cell-penetrating peptides that can enhance the uptake of drugs or drug carriers.[7−10] Certain peptides called homing peptides, also have the ability to selectively target cells.[11] A large impact in terms of market value has been made by peptides as drugs. Peptide drugs were initially isolated from natural sources, such as animals, but this area of research is now matured and several synthetic peptide drugs are on the market. The use of peptide drugs encompasses all diseases ranging from cancer to metabolic and cardiovascular diseases.

2. GENERAL STRATEGIES AND CONSIDERATIONS FOR THE DELIVERY OF PEPTIDE

The rise of peptide drugs is accompanied by challenges in their delivery.[12,13] One of the issues is that the drug does not reach its target. Similar to other drugs, elimination from the system through renal filtration, uptake by the reticuloendothelial system, and accumulation in nontargeted cells can result in a low therapeutic effect. Even more pronounced in peptide drugs, compared to many traditional drugs, is their low hydrolytic stability in the presence of enzymes. Moreover, their high molecular weight and chemical composition, which is often a cocktail of charges and hydrophobic groups, hamper cellular uptake.

To combat these limitations in the peptide administration, various systems have been developed. These range from direct injection of the peptide drug to the malignant site, to slow administration over an extended period of time using a pump.[12] However, these techniques are not feasible for everyday living since they required skilled medical personal to carry out the administration. Delivery by implants hold potential for further development and include the implantation of polymer disks loaded with peptides. Although these systems are commercially available, they require hospitalization and they are not suitable, in particular, for drugs that need to be administered on a daily basis.[12]

The search for suitable drug delivery carriers for peptide drugs led to the development of an array of drug carriers.

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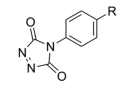
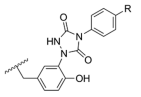
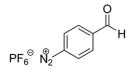
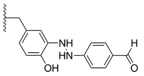
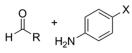
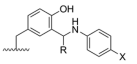
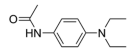
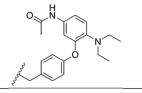
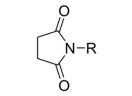
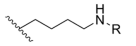
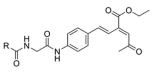
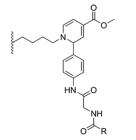
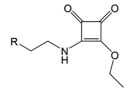
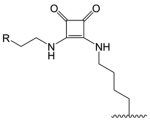
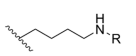
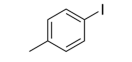
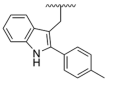
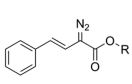
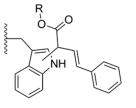
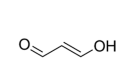
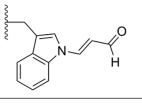
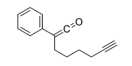
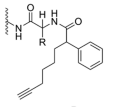
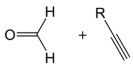
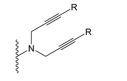
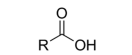
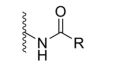
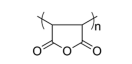
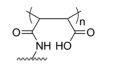
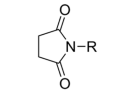
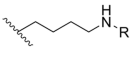
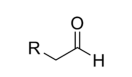
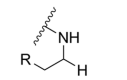
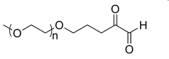
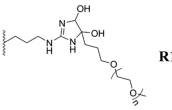
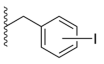
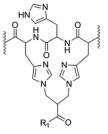
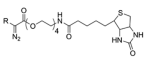
Table 1. Chemical Modification Methods, Categorized by Specific Amino Acid Residues Undergoing the Reaction

Amino Acid	Reactant	Example Conditions	Product	Ref
Tyrosine		PBS/CH_3CN (1:1) pH 7-7.4 RT		R1[16,17]
		0.1M PBS pH 8.0 30 min RT		R2[18]
		pH 6.5 18 hr RT – 37°C		R3[19]
		$(NH_4)_2Ce(NO_3)_6$ (cat.) 50mM bis(TRIS) buffer pH 6.0 1hr RT		R4[20]
Lysine		PBS with 0.3M NaCl and 5mM EDTA pH 7.1 1 hr		R5[21,22]
		0.1M PBS pH 7.4 10-30 min 40°C		R6[24]
		Borate buffer/DMSO (3:1) pH 9.1 20h RT		R7[26]
		TEA (cat.) DMF 60 min RT		R8[52]
Tryptophan		$AgBF_4$ (1 equiv.) 2-nitrobenzoic acid (1.5 equiv.) $Pd(OAc)_2$ (5mol%) PBS pH 6.0 Microwave irradiation (80W) 10min 80°C		R9[29]
		$Rh_2(OAc)_4$ tBuOH Buffer solution pH 6.0 24hr RT		R10[30,31]
		80% TFA 1,1,3,3-tetra-methoxypropane (10 equiv.) 1 hr RT		R11[32]
Terminal amine		H_2O/PBS (1:8) 15min RT		R12[33]
		CuCl (cat.) Aqueous solvent 18hr 35°C		R13[34]
		EDC DMAP (cat.) DMSO (anhyd.)/DCM(anhyd.) 1:2 30min RT		R14[45,46,49]
		0.5M borate buffer pH 8.5 3 hr RT		R15[48]
		DMSO (anhy.)/TEA (10:1) N_2 atmosphere 4 hr RT		R16[50]
		$NaCNBH_3$ (cat.) 100mM acetate buffer pH 5.0 4-24hr RT		R17[51]

Amino Acid	Reactant	Example Conditions	Product	Ref
Arginine		100mM PBS OR borate buffer pH 7.4 OR 9.0 (resp.) 1-7 days RT		R18[35,36]
Phenylalanine	IPy_2BF_4	HBF_4 (2 equiv. of IPy_2BF_4) CH_2Cl_2/TFA (100:10) 30min RT		R19[37]
Histidine		50mM sodium phosphate 150mM NaCl 10mM EDTA pH 6.7 3 h RT		R20[38]
Cysteine	O-mesitylenesulfonyl-hydroxylamine (MSH, 100 equiv.)	50mM PBS pH 8.0 20 min 4°C		R21[39]
		$Rh_2(OAc)_4$ $tBuOH_{(aq)}$ pH 6.2 5 hr 4°C		R22[40]
		AuCl/AgOTf (1:1) CH_3CN/H_2O (2:1) 1 hr RT		R23[41]
		H_2O_2 100mM PBS pH 3.5 2 hr RT		R24[55,56]
		Tris(2-carboxyethyl)-phosphine DMF/H2O (2:1) Overnight RT Under Ar atmosphere		R25[57]
		DMF Stirred together – no further reaction conditions given		R26[58]
Serine		H_2O/tBuOH (1:1) 1.5 hr RT or 37°C		R27[42]
Glycine		Tributylamine (cat.) Isobutylchloroformate (cat.) DMSO (anhyd.) 24 hr RT N_2 atmosphere		R28[53]
		EDC Borate buffer pH 7.0 24 hr 4°C		R29[54]
Threonine		$NaIO_4$ 25mM HEPES buffer/acetate buffer pH 4.8-7.2 overnight 6°C		R30[59]

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Although many of these drug carriers are used for both proteins and peptide drugs, the differences in size can mean that certain types of carriers are more successful for smaller peptide drugs than large proteins. The following article seeks to give an overview over different types or organic carriers, but it cannot act as a comprehensive review on this topic. For approaches to inorganic particles for the delivery of peptides the reader is referred to other review articles.[14]

When designing a drug delivery carrier for a certain peptide, the researcher is initially faced with the question of whether the peptide can be physically encapsulated or if chemical binding is necessary. This decision is often determined by the type of peptide drugs. Chemical conjugation requires the presence of a functional group that allows for efficient reaction. Often this group needs to be cleavable so as the macromolecular drug might be inactive. In contrast, physical encapsulation can be affected by the solubility of the drug. The solubility of peptides can range from water-soluble to hydrophobic depending on the amino acid composition, which may then in turn influence decisions about the carrier. Other considerations include the size of the carrier, whether low molecular weight ingredients, such as oils and surfactants, are sufficient or if more stable polymers are required. However, it is not only the physical parameter of the drug that plays a role, but also the administration route.

3. POLYMER–PEPTIDE CONJUGATES

While many peptides display promising results in in vitro studies, they often suffer from rapid clearance or enzymatic breakdown in in vivo studies. As a consequence, much attention has been devoted to synthesizing delivery systems to maintain, or increase, the bioactivity of peptide-based drugs once it has been introduced into the system. Chemical conjugation is one such technique and involves attachment of the peptide to its delivery vehicle via a chemical bond. It is hoped that this section of the review will showcase examples of recent techniques used in general peptide conjugation and the potential extension of these techniques for peptide–polymer conjugation for drug delivery.

3.1. Methods of Conjugation. Chemical of conjugation of peptides to the delivery carrier must be carefully planned. Just as peptides can become denatured upon exposure to environments encountered in physical encapsulation methods, destruction of the peptide activity may also occur if the conjugation site on the peptide is the same as its activation site. To this end, many conjugation methods focus on the reaction between a specific amino acid reside and its substrate, where modification of the chosen amino acid does not affect the activity of the peptide.

The conjugation technique utilized is then dependent upon the nature of the amino acid. Cysteine, for example, contains a thiol side group which provides excellent reactivity toward traditional thiol-based reactions. Thus, the chemical bond which can be established between the peptide and the substrate is highly dependent on the nature of the side group. The following section of the review aims to highlight some specific conjugation chemistries, categorized by the amino acid being targeted, with a focus on methodologies which have been reported within the past 5 years. The majority of the studies make use of a "linker" molecule which is capable of reacting with the amino acid side group, but is also functionalized with the desired substrate, thereby allowing the indirect conjugation of the peptide with the substrate. For a comprehensive look into peptide/protein-conjugate strategies, the reader is directed toward the excellent review written by Gauthier et al.[15] A table summarizing the majority of chemical reactions highlighted in the following section of this paper is given in Table 1.

3.1.1. Tyrosine. Desired groups can be introduced to the ortho position on the phenol group on tyrosine through the use of a novel linking molecule 4-phenyl-3*H*-1,2,4-triazole-3,5(4*H*)-dione or PTAD. PTAD is a diazondicarboxamide, where one nitrogen in the diazo bond adds to the ortho position on the aromatic ring. PTAD acts as a linker molecule between a peptide and a substrate as desired groups can be introduced on to the para position on the PTAD aromatic ring. Ban et al. successfully introduced a RGD-analogue to PTAD-PEG and linked the final PTAD-PEG-RGD moiety to a tyrosine residue on trastuzumab (Herceptin)[16] (Table 1, **R1**). In addition to azide functionality, groups attached to the aromatic ring on PTAD can contain alkyne or ketone functionality without impacting PTAD effectiveness and gave approximately 60% conjugation to tyrosine, regardless of the functional group attached on PTAD.[17]

Another linker molecule similar to PTAD is 4-formylbenzene diazonium hexafluorophosphate (FBDP). Although FBDP cannot carry other compounds at the para position, it does contain an aldehyde, allowing for subsequent reactions at that site once FBDP has reacted with a tyrosine residue. The main advantage of FBDP is the stability of the compound as it is solid at room temperature and stable for up to 3 months when stored at 4 °C. Under slightly basic conditions, it conjugates to N-acyl tyrosine methylamide with 99% percent efficiency (Table 1, **R2**) and is capable of introducing one FBPD linker per chymotrypsinogen A protein after 30 min.[18] A three-component, Mannich-like reaction has also shown selectivity toward tyrosine residues. The combination of tyrosine, substituted aldehyde and substituted aniline at low temperatures (25–37 °C) and pH 6.5 conjugates the aldehyde species to the ortho position on the tyrosine residue. The aniline component adds to the aldehyde in a condensation reaction (Table 1, **R3**), removing water and attaching both substituents on the aldehyde and aniline to the tyrosine residue. Joshi et al. performed the conjugation of aniline, substituted with rhodamine dye, and formaldehyde to chymotrypsinogen A under previously state conditions and achieved 66% conjugation[19]

Although most tyrosine conjugation methods facilitate attachment to the aromatic ring, direct conjugation to the hydroxyl group is also possible. The use of a cerium catalyst is required and the method is only applicable to phenylene diamine and anisidine derivatives. Seim et al. found that N-acyl phenylene diamine with structure shown in Table 1, **R4** was very promising and conjugated at 50% to melittin peptide.[20] However, drawbacks to this technique include its reactivity toward both tyrosine and tryptophan, as well as the formation of an ortho-substituted tyrosine residue as a minor product. The ability of N-acyl phenylene diamine to act as a linker is yet to be determined and, hence, the potential of the reaction to conjugate a desired substrate on to tyrosine is currently unknown.

3.1.2. Lysine. Lysine is an often-targeted amino acid side group due to the reactivity of the amine end-group and ease in which it can be modified. *N*-Hydroxylsuccinimide (NHS) is a reagent that is often used to conjugate a desired substrate to a lysine amine as it only requires the substrate to contain a NHS group at the desired conjugation site. One recent example incorporating NHS-functionalized PEG was seen where a PEG

chain was capped with NHS at one end and maleimide at the other to introduce bifunctionality. The polymer was initially reacted with a surface-exposed lysine on a HIV glycoprotein called gp120 to give a gp120-PEG-maleimide compound (Table 1, **R5**) before the maleimide was reacted with a surface-exposed thiol group on toll-like receptors 7/8 (TLR7/8, possibly from a cysteine residue) to give the final product shown in Figure 1.[21] This example shows the flexibility offered by NHS-facilitated amine reactions and the tolerance it has for other functional groups, including azides.[22]

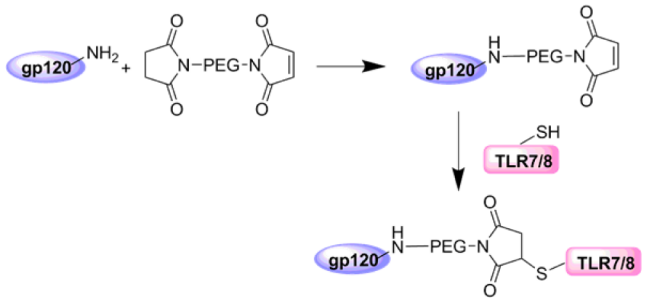

Figure 1. NHS-capped PEG and subsequent reaction with surface-exposed lysine on gp120 and thiol on TLR7/8.

Selectivity of a NHS-mediated reaction at a particular lysine site can potentially be controlled via kinetic means. This control is particularly important where more than one lysine residue exists, but conjugation is desired only at a specific location and highlights one of the major disadvantages of using NHS for conjugation. By controlling the ratio of biotin-amido-caproate-N-hydroxysuccinimidyl ester (biotin-LC-NHS) to lysozyme C to 0.5:1, Chen et al. managed to add only one biotin-LC molecule to a lysine residue with 94% yield.[23] Although the actual lysine site cannot be chosen, they have demonstrated that control over conjugation efficiency at the most reactive lysine is possible. Thus, control over conjugation to only one lysine site is possible.

Moving away from NHS-mediated lysine modification involves the 6π-azaelectrocyclisation as a reactant compound, as shown in Table 1, **R6**. Although Tanaka et al. have only used this method for lysine labeling on human serum albumin,[24] it is conceivable that other compounds of interest may be introduced on to a peptide via substitution of the R-group in the reactant.

Modern amine conjugation techniques have focused on the use of reagents which selectively target amine groups, even when in the presence of other nucleophiles, such as hydroxyl groups. Squaric acid, and its ester derivatives, have been shown to selectively react with amino functional groups to produce a vinyl amine connection. Dingels et al. modified one end of a PEG chain with diethyl squarate and then successfully PEGylated bovine serum albumin (BSA) with the activated PEG in mild aqueous conditions.[25] Conjugation of hyper-branched PEG chains was also shown to be successful when mediated with squaric acid functionality and the conditions can be found Table 1, **R7**.[26] Wurm et al. expanded on the previous work by showing that squaric acid, disubstituted with short PEG chains (for increased solubility characteristics), could be used to glycosylate BSA at multiple sites and that the glycosylation could be controlled via the ratio between the squaric acid linker and BSA.[27] The use of squaric acid for conjugation targeting amino sites has recently been reviewed by Wurm et al.[28]

3.1.3. Tryptophan. Like tyrosine, site-selective conjugation at tryptophan residues has been of particular interest due to its naturally low abundance in proteins[15] which enables the conjugation site to be more strictly controlled. Metal catalysts have been of significant importance and offer several different methods of tryptophan conjugation.

Palladium has allowed for the direct C-functionalization of the tryptophan indole through substitution with an aryl-iodide. Arylation of short peptides (up to 4 amino acid residues) with 4-iodotoluene at the somewhat harsh temperatures required (80 °C) for the reaction have given yields greater than 57%. For example, 4-iodotoluene was successfully conjugated to the peptide defined by RGWA at 94% (Table 1, **R9**).[29] Although only the attachment of toluene has been investigated, it may be that groups other than methyl groups can be attached at the para-position on the toluene aromatic ring and then introduced directly on to indole. The higher temperatures required, however, may mean that this technique is only applicable to short peptides as polypeptides and proteins may become denatured, regardless of the short reaction time (10 min).

Rhodium is another metal which has found success in catalyzing tryptophan conjugation involving vinyl metal-locarbenoids. Although the reaction was designed for the labeling of proteins, the scheme indicates that conjugation of a desired compound may be possible by taking advantage of reactant structure in Table 1, **R10** and replacing the R-group with the desired substrate.[30] Antos et al. generated 40% singly modified, and trace amounts of doubly modified, melittin using this method after reacting for 24 h at room temperature.[31]

Increased functionality can also be introduced on to tryptophan residues by adding an α,β-unsaturated aldehyde to the indole nitrogen. Under acidic conditions, reactions of tryptophan with malondialdehyde givean unsaturated aldehyde, allowing for further reaction of the tryptophan residue into species such as hydrazines or carbazides. The major advantage to this method is the reversible nature of the hydrazone group, which allows for the regeneration of the parent tryptophan residue and could act as a cleavable linker. Reaction of malondialdehyde with *N*-acetyltryptophan in 80% aqueous TFA gave >98% conversion after 1 h at room temperature, while reactions with model peptide PTHIKAWGD also appeared to be successful (yield not given), indicating that scaling up of this process to a more complicated peptide holds great potential (Table 1, **R11**).[32]

3.1.4. N-Terminus. The N-terminus of a peptide chain is often targeted due to the inherent functionality offered by the amine group. This is especially important in peptides where there are no other functional residues present, or where any present lysine groups is vital to the activity of the peptide (as is the case of Chen et al.[22]). Ketene-based reagents have shown excellent selectivity toward N-terminal amines, even in the presence amino acids which contain amino side groups. Chan et al. demonstrated that reaction of an alkyne-functionalized ketene not only retained the alkyne functionality of the reagent, but also reacted almost exclusively with the terminal amine in the majority of pentapeptides with structure XSKFR, where X was varied over the 20 natural amino acids (Table 1, **R12**).[33] Upon scaling up the reaction to peptide with structure YTSSSKNVVR, the yield was 40%.

An aldehyde-alkyne-amine (A3) system has also been developed to convert a terminal amine, in the presence of a

copper catalyst, into a tertiary amine that has been disubstituted with the alkyne species in the system. Although the alkyne species has only been tested with aryl, alkyl, and tetramethylsilane groups (Table 1, **R13**),[34] it stands to reason that unreactive "side groups", such as polymer chains, may be conjugated to the peptide via a substituted alkyne. In addition, the system is compatible with guanidine, disulfide, thioether, phenol, and hydroxyl side groups on the terminal amino acid residue.

3.1.5. Arginine. The guanidine group on arginine makes it an interesting, but often difficult, amino acid to process or modify. Reagents which are targeted for arginine reaction must also withstand possible reactions to other highly reaction amino acid residues such as lysine and cysteine. Gauthier et al. developed a system whereby they used an α-oxo-aldehyde to selectively modify arginine residues, even when in the presence of lysine or cysteine.[35] They take advantage of the irreversible nature of the reaction between methylgloxal and arginine to conjugate mPEG chain to the egg white lysosome (Table 1, **R18**). As the same reaction between methylgloxal and lysine or cysteine is reversible, they cleaved unwanted mPEG to these residues during processing and purification. Poly(methacrylates) manufactured using ATRP technique were shown to allow the incorporation of an α-oxo-aldehyde end group, thereby allowing conjugation of polymers such as PEGMA, DMAEMA, tBuMA, and MMA to arginine residues on egg white lysosome.[36]

3.1.6. Phenylalanine. Selective reactions on the phenylalanine aromatic ring are often difficult when tyrosine residues are present due to the higher reactivity of the phenol side group. However, when tyrosine is not present, iodonium can be employed to monoiodinate the phenyl group (Table 1, **R19**), leading to the possibility of further reactions using the halogen functionality. A total of 90% iodination of a peptide, with structure ADATF, was achieved when HBF_4 was used as an additive in conjunction with IPy_2BF_4.[37]

3.1.7. Histidine. It is possible to attach sulfone-based reagents to a histidine tag, where the final product is cross-conjugated to several histidine residues. A modified PEG chain (Table 1, **R20**, reactant) was attached to the 6-hisitidine tag on the C-terminus of the domain antibody (dAb) protein where 40−50% of the initial protein was monopegylated, 10−15% was dipegylated, and trace amounts were tripegylated.[38] Although the method of addition and structure of the final product is unclear, it is thought to be that of the product shown in Table 1, **R19**.

3.1.8. Cysteine. Cysteine is an amino acid that is often targeted for chemical conjugation due to the reactivity of its thiol side group and ability to undergo typical thiol-based reactions. As explored previously, maleimide readily undergo reactions with cysteine and is a method which is often employed in peptide conjugation where the substrate to be conjugated is functionalized with a maleimide group.[21]

Novel methods of cysteine modification include the use of O-mesitylenesulfonylhydroxylamine (MSH) to eliminate the thiol group and convert it to a dehydroalanine (Dha). Dehydroalanine is then capable of reacting with other thiolated species, with almost complete conversion. The initial oxidative elimination of cysteine thiol is very fast and occurs readily at low temperatures. When Bernardes et al. oxidized subtilisin *Bacillus lentus* (SBL), they achieved full conversion of the single surface-exposed cysteine after 20 min at 4 °C.[39] Further reaction of the dehydroalanine-functionalized SBL with small thiolated products such as sugars, short peptides, and alkene-functionalized alkyl compounds (**1**−**3**, Figure 2) achieved greater than 95% conversion (Table 1, **R21**).

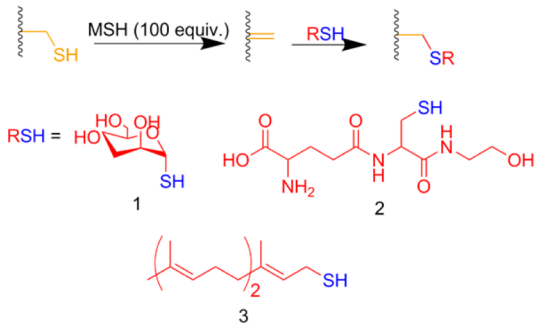

Figure 2. Oxidative elimination of cysteine thiol group into dehydroalanine (orange) using MSH and subsequent attachment of a thiolated species (7−9, blue/red) via a thioether.

Rhodium catalysts have also found use in cysteine conjugations where it allows the addition of diazo compounds. It has been successfully used to attach relatively large compounds to proteins, such as the attachment of the polymeric reactant in Table 1, **R22** to cystic fibrosis transmembrane conductance regulator-associated ligand (CAL) protein. As with tryptophan modification, a hydroxylamine additive was required and 98% of initial CAL protein was converted after 5 h at 4 °C.[40] Although conjugation at tryptophan residues is theoretically possible, Kundu et al. stated that due to the rarity of tryptophan, the side reaction has not been an issue in practice.

Cysteine modification using allene compounds have also been accomplished in the presence of gold-based catalysts. The reaction of a peptide with structure STSSSCNLSK with different allenes was catalyzed by gold chloride/silver trifluoromethanesulfonate (AuCl-AgOTf) and gave yields of at least 60%, depending on the allene species.[41] This includes a conjugation rate of when the peptide was reacted with the bulky compound shown in Table 1, **R23** and indicates that the system could potentially introduce other macromolecular compounds of interest to the allene structure.

3.1.9. Serine. Despite the relative lack of reactivity of serine resides, at least when compared to amino acids such as cysteine or lysine, there exists a unique opportunity for serine modification when it is located at the N-terminus. By using sodium periodate, the serine hydroxyl group can be oxidized to an aldehyde and then reacted with ylides to introduce a desired group via an alkene bond (Table 1, **R27**). Using this method, Han et al. introduced functionalities such as alkenes, alkynes, and trifluorocarbon to dipeptide Ser-Leu at yields of 84, 85, and 92%, respectively.[42]

3.2. Examples of Therapeutic Polymer−Peptide Conjugates. It can be seen from the previous section that many peptide conjugation methods are available and are capable of attaching relatively large substrate compounds. Some of the examples even focused on the attachment of polymers such as PEG[21,35,38] and various polymethacylates,[36] which is of particular interest for this review. This section of the review then focuses specifically on the attachment of therapeutic peptides to polymers, usually in an effort to enhance the bioavailability of the peptides. Again, this section will be organized by amino acid residue, which is being targeted for

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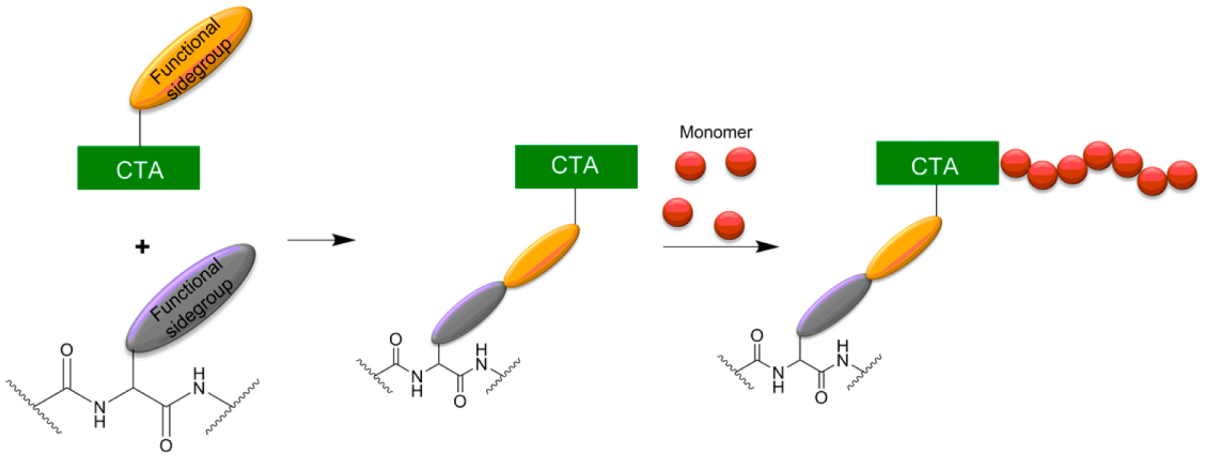

Figure 3. General scheme for the divergent approach.

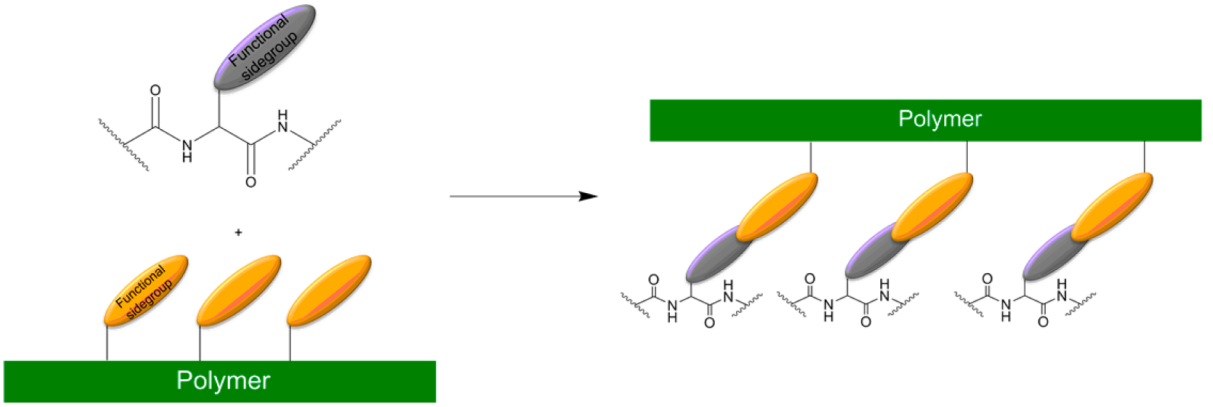

Figure 4. General approach for the convergent approach.

polymeric conjugation to hopefully provide an overview on different approaches possible when faced with only a set number of residues (hence, functionality) to choose from.

In general, there are two broad categories under which chemical conjugation falls. There is the divergent approach (Figure 3), where peptide conjugation occurs before polymerization and in general, only one peptide is introduced per polymer chain. This often involves the incorporation of the peptide into the chain-transfer agent, especially in the case of RAFT polymerization where RAFT-agents can be modified with relative ease without affecting RAFT functionality. For example, glutathione has been incorporated into the Z-group of a trithiol RAFT agent without affecting its ability to polymerize styrene and methaylacrylates as well as a range of acrylates and amides.[43]

The other approach is a convergent approach where the polymer substrate is already synthesized before attempts are made to conjugate the peptide moiety to the chain (Figure 4). The major advantage of this technique is the ability to attach more than one copy of the peptide to each polymer chain, thereby increasing the loading efficiency of the system. The polymeric system is then expected to contain necessary functional groups required for the conjugation reaction. The majority of peptide-polymer examples in literature deal with the convergent approach.

3.2.1. N-Terminus. A pentapeptide, defined by structure YIGSR, has been of interest in the treatment of B16-BL6 melanoma cells. Due to its short half-life, attempts have been made to increase its stability via attachment to a polymeric carrier. Yamamoto et al. conjugated YISGR to both PEG and PVP. Although it was not stated how conjugation to PEG was achieved, conjugation to PVP was through an amide bond between a carboxyl group on the polymer and the terminal amine on the peptide.[44] PVP-YIGSR demonstrated increased plasma half-life compared to unmodified YIGSR (15-fold increase) and PEG-YIGSR (7-fold) as well equal inhibitory effects on B16-BL6 cells at only 10% of the concentration required from PEG-YIGSR.

A similar reaction was used to conjugate the terminal amine on BH3 (a peptide derived from BCL2 Homology 3 domain, an apoptotic regulator in the cell) to a carboxylic acid group on to bis(2-carboxyethyl)-PEG, which had been end-functionalized with citric acid.[45] The introduction of citric acid was to introduce several copies of carboxyl functionality so that more than one reaction per polymer chain was possible. Carboxylic acid reactions were all mediated via a 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/4-dimethylaminopyridine (DMAP) system (Table 1, **R14**). This enabled the group to simultaneously attach an LHRH targeting peptide (through amide formation with a lysine residue) as well as the drug camptothecin (through ester formation with a hydroxyl group) to the other carboxyl groups. The conjugated moiety demonstrated significantly improved inhibition of ovarian carcinoma tumor size, especially when compared to the free camptothecin and camptothecin−PEG conjugate.[46]

Amide bond formation between an amine group and a polymer substrate can also be formed using anhydrides. The copolymer system composed of poly(styrene-*co*-maleic anhydride) (SMA) is very useful for reactions with amines and has been evaluated as a delivery system for anticancer proteins such as neocarzinostatin, better known as SMANCS.[47] The spontaneous reaction between the anhydride group and a free amine makes SMA a convenient system for drug conjugation. Due to the ability of the peptide YIGSR to inhibit lung metastatsis, Mu et al. attempted to decrease its degradation and clearance from the body by conjugating it to SMA. They found that simply combining the two reactants for 3 h at room temperature was sufficient for conjugation to occur (yield not given; Table 1, **R15**).[48] Ukawala et al., however, took a similar approach to Chandna et al. and conjugated YIGSR to carboxyl-functionalized PEG (catalyzed by an EDC/NHS system),[49] demonstrating different strategies which are possible when attempting to conjugate peptides to polymers at the terminal amine.

Carboxyl-functionalized PEG is often used for reactions with amine groups, but the requirement for an activating agent such as EDC has meant that "pre-activated" polymers have been designed to eliminate the extra step. Methoxypoly(ethylene glycol)-succinimidyl propionate (PEG-NHS) is one such example and is commercially available, demonstrating the usefulness of preparing intermediates with a succinimide end-cap. Such compounds are usually solid and as such, can be prepared, isolated and stored for later use. When insulin was coupled to PEG-NHS, amide formation at both the N-terminus and lysine residue occurred (Table 1, **R16**). However, it was observed that the half-life of insulin conjugated at the N-terminus (20.7 days) was significantly longer when compared to conjugation at the lysine residue (8.6 days) or for free insulin (0.4−0.5 days).[50]

Calcitonin is a peptide consisting of 32 amino acids and is used as a treatment for postmenopausal osteoporosis. Attempts have been made to conjugate it to a polymeric delivery system to overcome peptide degradation once it has been introduced into the body. The N-terminus was successfully attached to a comb-shaped copolymer system where short PEG chains protruded from a poly(methacrylate) backbone (Table 1, **R17**).[51] The ends of the PEG branches were initially converted into aldehyde groups before the polymer was mixed with calcitonin and sodium cyanoborohydride (to catalyze the reductive animation required).

3.2.2. Lysine. Calcitonin conjugation to polymers has also been attempted at the lysine residue present at position 18 on the chain. Again, PEG-NHS was used, but initiation of the reaction was conducted using triethylamine[52] (Table 1, **R8**). To inhibit the reaction of the polymer at the N-terminus as well as with the lysine at position 11, Youn et al. protected the two amino acid residues using fluorenylmethyloxycarbonyl chloride before conjugation to the polymer at the desired lysine site. Overall, they found that PEG_{2000} was the most promising conjugate as the polymer−peptide conjugate possessed a half-life three times greater than free calcitonin (100 min vs 35 min) while retaining more than 80% of the bioactivity of free calcitonin.

3.2.3. Glycine. As with the N-terminus, glycine residues also offer a primary amine as a functional group for conjugation. A simple conjugation method would include the direct reaction between the amine and a polymer containing carboxyl-functionalized monomers. Carboxymethyl dextran (CMD) is a biocompatible glycopolymer which fits this criterion and was used as a polymer substrate for insulin attachment. Baudyš et al. activated the polymeric carboxylic acids with tributylamine and isobutylchlorofomrate before adding insulin, but low carboxylic acid activation meant that only ∼3.5 units of insulin was attached to each CMD_{50000} chain (Table 1, **R28**).[53] However, with the presence of other amine groups (from a lysine residue and the terminal amine), the authors were not targeting glycine in particular as much as they were targeting a free amine group. Their results seemed to indicate that the glycine amine was the most reactive, hence attachment at that site.

Another class of common activating agents is carbodiimides, in particular, *N*,*N*-dicyclohexylcarbodiimide (DCC) and EDC. A copolymer comprising *N*-isopropylacrylamide (NIPAAm) and acrylic acid (AA) had its carboxylic acids activated with EDC for reaction with insulin at the glycine amine (Table 1, **R29**).[54] Again, the authors were not targeting the glycine residue, but the conjugation appeared to occur selectively at that site with this system as well.

3.2.4. Cysteine. DALCE, a derivative based on leu-enkephalin and with structure [D-Ala^2, Leu^5, Cys^6]-enkephalin, is a peptide that has been identified as a possible treatment for ischemia. Shahnaz et al. aimed to attach copies of this peptide to a CMD chain via the cysteine reside on DALCE to form a disulfide bond between it and the polymer in hopes that it will increase the half-life of DALCE in the bloodstream, as well as achieve a more sustained release profile due to the natural thiol/disulfide exchange reaction facilitated by glutathione in the body.[55] Following on from their previous work,[56] the group initially functionalized the carboxylic acid groups on CMD with a cysteine residue, to give thiol-functionality to the polymer, before adding DALCE (Table 1, **R24**). The CMD-DALCE contained 22.6 wt % DALCE and had a mean residence time of 256 min (compared to 53 min for DALCE alone), demonstrating the benefits of polymer conjugation to a peptide drug.[55]

Calcitonin, as previously discussed, contains 32 amino acids, two of which are cysteine residues (positions 1 and 7). Cheng et al. attempted to use these cysteine residues for conjugation to a novel carrier system consisting of both a PEG chain and a lipid molecule. They accomplished this via an ε-maleimido-lysine linker which contained the lipid and PEG chain. The thiol-maleimido reaction allowed addition to calcitonin at both cysteine residues (Table 1, **R25**).[57] Even though the calcitonin-lipid-PEG structure did increase the stability of calcitonin against intestinal degradation, the increase may not be enough to be of pharmaceutical significance (<10% remaining after 7 min compared to after 2 min for free calcitonin).

Due to the reactivity of cysteine, it is a residue that is often artificially included in peptides in an attempt to introduce functionality to the compound. This is especially the case when other functional groups do not exist or the functional groups form the site of activity for the peptide. Dharap et al. took this approach when they introduced a cysteine at the end of the BH3 peptide chain and subsequently conjugated it with vinylsulfone-functionalized PEG to give the product shown in Table 1, **R26**.[58]

3.2.5. Threonine. The hydroxyl group on a threonine residue is often too unreactive without modification, and like serine, oxidation of the alcohol to an aldehyde is possible with sodium periodate. In an effort to conjugate YIGSR to a PEG-based liposome, where each PEG chain was end-functionalized with a hydrazide group, Zalipsky et al. introduced a threonine residue

at the N-terminus of YIGSR to form TYIGSR.[59] Oxidized TYIGSR was incubated together with the liposome and without the addition of other catalysts and approximately 200 peptides were conjugated to each vesicle (Table 1, **R30**).

3.2.6. Other. Despite the focus of the review on reactions pertaining to the 20 naturally occurring amino acids found in peptides, it has to be noted that not all peptide-based drugs are strictly composed of natural amino acids only. In addition, peptides that are combined with carbohydrates are common. Inclusion of non-natural amino acids or amino acids that are modified with a functional groups that is not naturally occurring allow sometimes a more targeted conjugation approach.

The chimeric B-cell epitope (J14, KQAEDKVKASREAKK-QVEKALEQLEDKVK), derived from the M protein expressing *Streptococcus pyogenes*, has been developed as a vaccine against this widespread human pathogen. Modification of this peptide on the N-terminus using azidoacetic acid led to a reactive peptide unit that could be reacted with various reactive scaffolds. For example, four copies were conjugated via click reaction to a small hydrophobic molecules resulting in the formation of an amphiphilic structure, which could self-assemble into particles. The nanoparticles caused a high systemic IgG antibody concentration against the epitope without help of an adjuvant.[60] The same peptide was further clicked to a four-arm star poly(*t*-butyl)acrylate (PtBA)[61] or a polyacrylate dendrimer.[62] Click chemistry was also employed to attached a human papillomavirus (HPV) E7 protein-derived peptide antigen to star polymers.[63]

Compounds such as glycopeptides have also been found to be potentially useful in treating illnesses and diseases. Vancomycin is a glycopeptide used as a "last-line-of-defense" antibiotic for the treatment of methicillin-resistant *Staphylococcus aureus*. As it can be seen from Figure 5, there are

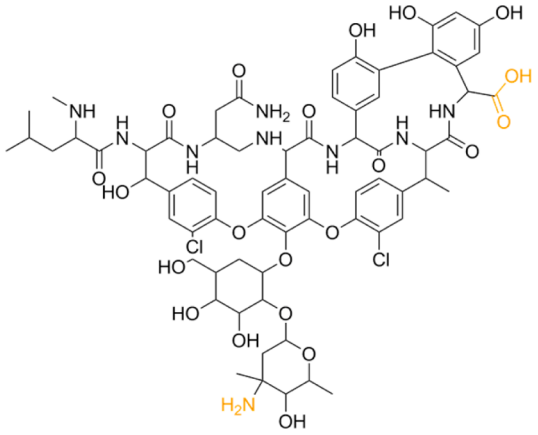

Figure 5. Structure of vancomycin.

many functional groups that can be targeted for conjugation, where the two most useful groups are the primary amine and a carboxylic acid. Despite the size of vancomycin, the primary amine is reactive enough to undergo reactions with compounds containing aldehyde functionality. Arimoto et al. took advantage of this and synthesized a ring-opening metathesis polymerization (ROMP) monomer in a divergent approach to peptide conjugation, where they obtained 60% conversion after 1.5 days at room temperature.[64] In this way, they overcame the main disadvantage of the approach as they introduced more than one copy of the peptide per polymer chain. In fact, by incorporating the peptide into the monomer, they ensured that every monomer unit contained a peptide molecule. Thereby, by controlling the degree of polymerization, the group was given direct control over the loading efficiency of the drug. The scheme for this reaction is shown in Figure 6.

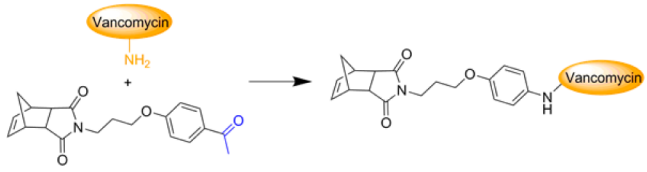

Figure 6. Formation of ROMP monomer containing vancomycin via reaction between amine (orange) and aldehyde.

As mentioned previously, the carboxylic acid of vancomycin can also be targeted for reaction. Solid phase peptide synthesis (SPPS) reagents are a new class of such compounds that activate carboxylic acids toward reactions with nucleophiles, specifically amines. Metallo et al. wanted to conjugate vancomycin to poly(*N*-acryloyloxysuccinimide) (PNAS), but theorized that the primary amine on vancomycin was too hindered for a convergent approach, even though amines react readily with NHS groups. Hence, they activated the carboxylic acid with HBTU and attached diaminobutane as a linker molecule to give a relatively unhindered primary amine at the end of the butane chain. They added 5 mol % of this modified vancomycin and achieved complete conjugation onto the polymer (Figure 7).[65]

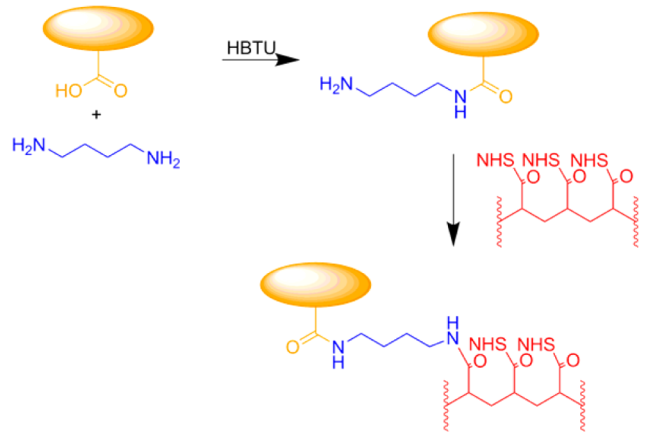

Figure 7. Conjugation of vancomycin at the carboxylic acid (orange) using diaminobutane (blue) as a linker molecule onto PNAS (red).

4. PHYSICAL ENCAPSULATION OF PEPTIDES IN NANOPARTICLES

It becomes evident that although many conjugation techniques are available, the chemical conjugation of clinically used peptides to polymers has not been commonly employed. This may be due to the absence of sufficiently reactive functional groups on the peptide, but could also be due to some concerns about the bioavailability of the drug after conjugation.

In contrast, formulations of proteins or peptides are in contrast widely available and two peptides worthy of being singled out here are Cyclosporine A and insulin. The former can be considered a true peptide while the latter with its molecular weight of 5808 Da composed from 51 amino acids is already at the interface of what is typically considered a protein.

Both peptides have attracted significant attention by researchers in the pharmaceutical area and numerous formulations are available, some of which are described in detail below. It could even be argued that these two drugs often act like default testing peptides, used by the material chemist wanting to test their innovative idea for a peptide drug delivery system on clinically relevant peptides.

The delivery of proteins and peptides by physically encapsulating the drug has been widely applied for most administrative routes.[66] Very commonly, the protein or peptide is processed into a formulation with various sugars or other nonbioactive proteins with the purpose to increase the stability of the drug and to facilitate administration. Formulations with BSA were used to enhance pulmonary delivery[67] while mucoadhesive polymers such as polysaccharides like amylose, cellulose, or hyaluronan were used for nasal delivery. Although biopolymers are most commonly used, synthetic polymers such as poly(acrylic acid) and poloxamer are often employed to deliver drugs via the nasal route. While an array of polymers is available, only a few have been tested for protein delivery, most notably insulin, via this route.[68] Insulin has been delivered using cellulose,[69] gelatin,[70] poly-L-arginine[71] and hyaluronic acid.[72] Surfactants, assembled into liposomes[73] or solid-lipid nanoparticles[74] were the vehicle of choice for ocular delivery of peptides while the types of drug carriers employed to enhance the oral delivery route appear to be limitless. Just the types of polymers tested for oral delivery of peptides alone span the whole catalogue of available structures including polyalkylcyanoacrylates polymethacrylic acid based polymers, polysebacicanhydride-*co*-polyfumaric anhydride, PLGA, chitosan derivatives and alginate.[75] The reader is referred to an excellent book edited by van der Walle,[66] which discusses the different administration pathways and the biological activity associated with the this route.

In the following, the focus will not be on the biological activity or administrative route, but on the type of drug carriers that have been developed for this purpose.

4.1. Carriers Based on Surfactants. *4.1.1. Microemulsions.* Microemulsion are defined as "a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution".[76] Depending on the amount of water and oil, either the formation of oil-in-water (o/w) or water-in-oil (w/o) microemulsions is prevalent. The type of microemulsion, the particle size, the stability, and other parameters are underpinned by various theories. Central to all these theories is the interfacial energy between the oil and the water phase. The interplay between concentrations and nature of all three components led to the establishment of phase diagrams.[77] The phase boundaries can easily be disturbed by external factors such as temperature and pressure, but other molecules such as drugs and cosurfactants can initiate morphology changes and disaggregation. The surfactant, which can be either nonionic, zwitterionic, cationic, or anionic, is the key to a stable system. Combinations of surfactants are often employed to increase the region in which microemulsions exist. The reader is referred to some excellent review articles on the background of microemulsions.[77−79]

Despite their dynamic properties, microemulsion systems are attractive drug delivery vehicles. The hydrophilic part of the surfactants ensures high water solubility of the carrier, while the hydrophobic oil pool in the center creates an environment suitable for the dissolution of hydrophobic drugs (Figure 8). One set-back is the nature of the oil-core that, typically based on hydrocarbons, is often too hydrophobic to be able to hold many guest molecules.[80] The water-solubility of many peptide drugs does not allow the direct use of o/w microemsulsion system. Peptides such as luteinizing hormone-releasing hormone (LHRH) were delivered in a w/o (lecithin (surfactant)/ethyl oleate (oil)/hexanoic acid (cosurfactant)) emulsion for intramuscular delivery, an administration route where the water-solubility of the carrier does significantly impact its delivery.[81] Oral administration is another area that benefits from the use of w/o microemulsion. The HIV transactivator protein TAT as a model peptide was delivered orally and its microemulsion formulation was shown to have a longer half-life than the free drug.[82] The poster child of peptide drug delivery using microemulsions is the hydrophobic cyclosporine A. The hydrophobicity of cyclosporine A facilitates the use of o/w microemulsion, which leads to an easily administered aqueous solution. A range of commercial products are already available using various formulations. All these formulations are based on self-microemulsifying drug delivery systems (SMEDDS), which are o/w systems that form with only mild agitation in the presence of water.[83,84] Neoral uses an isotropic concentrated blend of surfactants based on partial glycerides of medium chain length, a triglyceride oil of medium chain length and the drug.

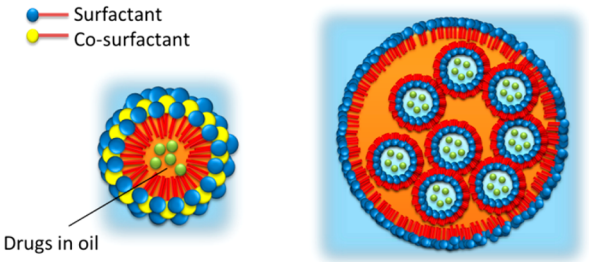

Figure 8. Delivery of peptide drugs using microemulsions (left) and w/o/w emulsion (middle) and an example of peptide drugs delivered using both routes [Examples: LHRH,[81] cyclosporin A,[88−90] insulin,[78,83−85] HIV transactivator protein TAT,[82] carnosine,[91] calcitonin,[92] D-Lys6−GnRH,[93] and vancomycin[94]].

Insulin, in contrast, is a water-soluble large peptide and requires more optimization to achieve high bioavailability. The different challenges were recently summarized in a review article.[85] Traditional w/o microemulsions were reported due to the hydrophilic nature of insulin. Addition of chitosan to the water droplets can additionally protect insulin in the aqueous core.[86] However, the use of w/o/w is more prevalent in order to be able to create formulations in water (Figure 8). Insulin was dissolved in an aqueous solution and added to an oil solution, which was then again emulsified in water.[85]

To conclude, the success of Neoral highlights that microemulsion offer an efficient solution for the safe delivery of peptides. According to a recent opinion article, microemulsions are promising drug carriers, but more aspects, such as their mechanical stability, might have to be addressed in the future.[87]

4.1.2. Solid Lipid Nanoparticles (SLN). A solid lipid nanoparticle (SLN) is a spherical core−shell particle, which is built from a lipid core and a surfactant shell for stabilization. The lipid core is often based on naturally occurring lipid substances such triglycerides, fatty acids, steroids, and waxes. The lipid core is suitable for the encapsulation of drugs while the particle is stabilized by a surfactant shell, whose role is the stabilization of the structure against aggregation. First reported

by Müller et al.,[71] this technique is now well-established as a means to deliver drugs including proteins and peptides.[72,73] Several pathways are available to prepare these formulations. They involve the mixing of drug, lipid, and surfactant (emulsifier) using either heat or solvent, followed by the formation of small particles using a high pressure homogenizer, ultrasonicator, needle injection, cooling the solution under stirring (microemulsion technique) or simply evaporation of the solvent (Figure 9).[72−74]

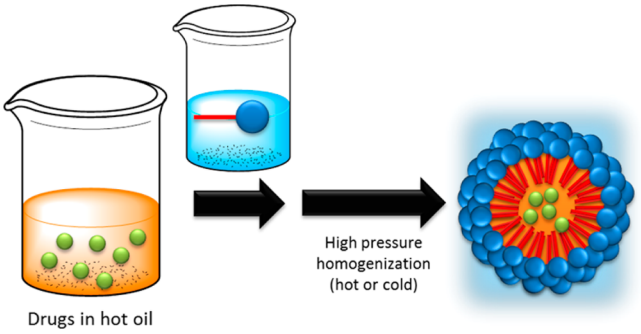

Figure 9. Delivery of peptide drugs using solid-lipid nanoparticles (SLN) including a table listing examples of peptide drugs delivered using this route [Examples: cyclosporin A,[96,97] insulin,[50,52,54,55] calcitonin,[101,102] somatostatin,[103] luteinizing-hormone-releasing hormone (LHRH),[95,99] thymocartin,[103] and melittin[104]].

SLN's are typically used for the delivery of hydrophobic drugs due to the hydrophobic nature of the core materials. The hydrophilicity of some peptides may present an obstacle to this approach, although some success was made to adjust the system to achieve high loading of clinically relevant peptides. Some peptides such as cyclosporine A are sufficiently hydrophobic to allow encapsulation into the lipid core without any alterations. Initial attempts to encapsulate peptides using w/o/w microemulsion-based technique only led to a low drug loading efficiency, but it could prove that SLNs are, in principle, suitable to deliver these drugs.[95]

High pressure homogenization was successfully applied to deliver hydrophobic cyclosporine A,[96,97] while emulsion techniques were more suitable for more hydrophilic drugs. Not only does the double-emulsion (w/o/w) technique enhance solubility of hydrophilic peptides, it also allows for experimental conditions which are less aggressive toward potentially sensitive peptides. It is therefore not surprising that, in addition to successful delivery of cyclosporin A (which is relatively small for a biomolecule), delivery of larger peptides such as calcitonin would also benefit. Although the approach is promising in the delivery of insulin, the nanoparticles required a polymer coating to avoid burst release.[98]

The delivery of insulin was improved using an o/w emulsion−diffusion method, which was also successfully used for encapsulation of luteinizing-hormone-releasing hormone (LHRH; Gonadotropin-releasing hormone (GnRH) or luliberin).[99] However, one drawback to these techniques is the use of toxic organic solvents. As a result, the use of super critical fluid has been proposed as an attractive alternative, but small amounts of solvent are sometimes still necessary to dissolve the peptide.[100]

The use of heat, organic solvents, or other conditions that might be stressful for the peptide during preparation can be avoided by absorbing the peptide drug onto preformed SLNs. This pathway has been applied more commonly to proteins than peptides, although some selected examples, such as the absorption of calcitonin, have been reported.[101]

SLNs have already matured into a well-established drug delivery technique for peptides and proteins. The reader is referred to more detailed review articles that summarize the intricacies of each approach when attempting to deliver peptides or proteins.[73]

4.1.3. Liposomes. Liposomes, in contrast to microemulsions and SLNs, carry an interior water pool surrounded by a lipid bilayer, which facilitates high solubility of hydrophilic peptides while the membrane protects the drug from destructive forces (Figure 10).[105,106] Liposomes have indeed been shown to be a more successful delivery vehicle for hydrophilic drugs since hydrophobic drugs tend to permeate very fast through the membrane. Liposomes are usually prepared from a combination of surfactants, often mixed with cholesterol for added stability and to prevent drug leakage.[105]

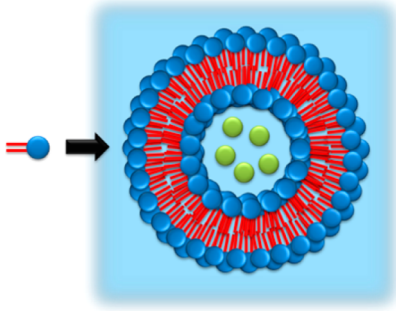

Figure 10. Delivery of peptide drugs using liposomes, including a table listing examples of peptide drugs delivered using this route [Examples: calcitonin,[112,114,117,119] insulin,[109] VIP,[110,111,113] cetrorelix,[120] LHRH,[121] carnosine,[122] and vancomycin[123,124]].

Liposomal delivery of peptides and proteins is therefore a research area of significant activity that has led to the design of formulations which are suitable for parenteral, oral, buccal, pulmonary, intranasal, transdermal, and ocular delivery.[107,108] These pathways of delivery have been recently summarized in an interesting opinion article on the liposomal delivery of proteins and peptides.[107] Although encapsulation of proteins into liposomes dominates the literature, the discussion regarding liposomal peptide formations is dominated by peptides such as vasoactive intestinal peptides (VIP), insulin, and calcitonin. In general, the delivery of peptides using liposomes has significant effects such as prolonged hypoglycaemic effect of insulin[109] and prolonged hypotensive effect and better uptake of VIP.[110,111] Although the peptide should, in theory, be located within the water pool, the charged, lipophilic, or amphiphilic nature of many peptides result in interaction with the membrane surfactants, as has been demonstrated with calcitonin[112] and VIP.[113] The type of charge present in the surfactant and peptide drug is of substantial influence. For example, positively charged calcitonin binds favorable to negatively charged surfactants, thereby increasing its loading efficiency.[114]

The resemblance of liposomes to the cell membrane can, however, represent some limitations when attempting to deliver peptides whose activity is to interfere with such a membrane. This can occur to such as extent that the bilayer structure is destroyed. Melittin, a peptide that consists of 26 amino acids, is

effective in destroying the HIV virus by depleting the viral envelope. This behavior however is not only limited to viruses, but also to liposomes resulting in melittin-induced pore formation and leakage.[115] The nature of the surfactant and cosurfactant not only affects its interaction with the peptide, but will also determine the nature of the overall vesicle such as the surface charge, size, and stability. For example, a positively charged surface in a liposomal system for insulin could provide better protection against digestion by trypsin.[116] Moreover, the flexibility of a drug carrier for calcitonin was affected by the surfactant formation where slight changes to the composition were observed to affect the softness, potentially influencing the penetration of a liposomal drug carrier.[117]

Liposomal drug delivery systems are generally commercially successful, with several formulations currently on the market.[118] Although many systems for peptides have shown promising results in the lab (Figure 10), none of them have made it to the market yet. Stimuvax, a peptide-based therapeutic cancer vaccine administered in liposomes, has only now entered phase III in clinical trials.[118]

4.2. Carriers Based on Polymers. *4.2.1. Liposomes.* As discussed above, traditional liposomes are successful in delivering various drugs. However, it has been proposed that coating liposomes with polymers can improve these carriers by enhancing the circulation time and cellular uptake. In addition, polymers on the surface can affect and further control the drug release while the altered surface characteristics can influence the interaction with the body.[105]

Liposomes are typically coated with polymers by utilizing the electrostatic interactions between polymers and surfactant (Figure 11). The polymers are titrated into the preformed liposome solution, leading to an interaction between the polymer and the charged liposome surface, which can be monitored using isothermal titration calorimetry (ITC) and differential scanning calorimetry (DSC).[125] Moreover, the preparation of polymer-coated liposomes can be directly achieved by mixing low-molecular weight surfactants with polymeric surfactants. Reports on polymeric surfactants are mainly limited to PEG, probably due to the success of PEG in drug delivery systems in general. By tethering PEG chains to the surface of liposomes, circulation half-life was increased by reducing the uptake by the mononuclear phagocyte system.[126] Although PEG incorporation using PEG-based surfactants has been widely reported, other techniques such as physical absorption of PEG or the chemical attachment of PEG onto already formed liposomes by employing reactive surfactants have also been explored.[127] PEG coating of liposomes was reported to have several positive effects on the delivery of peptides. Insulin, administered in such a liposome, led to a low plasma glucose level in rats[128] while VIP, packaged into PEG stabilized liposomes, could normalize arterial pressure in hypertensive hamsters.[129] In particular, coating with mucoadhesive polymers enhanced oral delivery. The holey grail of peptide delivery is undoubtedly oral administration of insulin and it is therefore not surprising that several efforts have been undertaken to enhance formulations for this route. Chitosan has been the most popular polymer in that regard. Coating of insulin-loaded liposomes with chitosan led to better drug absorption and lower glucose levels in the blood.[130−132] Other coatings had similar effects by increasing the residence time of the drug carrier in the stomach as demonstrated using liposomes coated with mucin.[133] The coatings described for liposomal peptide delivery were nontargeting. Although liposomes, coated with polymers with attached targeting moieties such as folates,[134] were reported for other drugs, the field of active targeting of peptides using liposomes has potential to grow. Focus for future investigations may include altering the size of liposomes as it is often in the micrometer range which make the particles subject of opsonisation.[135]

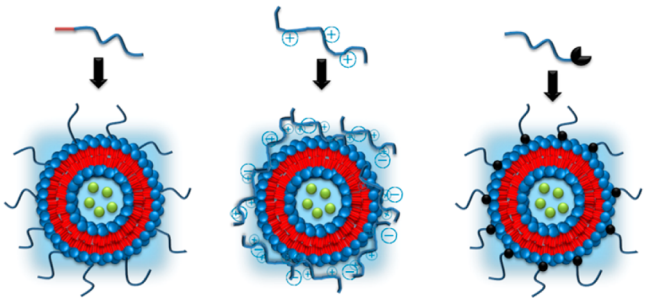

Figure 11. Left: pathways to liposome stabilization using polymers by (a) employing a polymeric cosurfactant; (b) absorption of polymer by electrostatic forces; (c) conjugation of polymers using a reactive surfactant and polymer. Right: examples of polymers used to stabilize liposomes and the respective peptide delivered [Calcitonin (chitosan,[136] pectin,[137,138] cross-linked poly(acrylic acid) (carbopol) conjugates with lectin[139]); vancomycin (PEG[140]); insulin (glycoprotein,[141] PEG,[128,133] chitosan,[130,131] polyvinyl alcohol,[130] poly(acrylic acid),[130] alginate-chitosan,[132] cross-linked poly(acrylic acid) (carbopol)[142]); VIP (PEG[129,143])].

4.2.2. Microspheres. Encapsulation of proteins and peptides into polymeric microparticles is widely described in literature. The polymer is usually dissolved in an appropriate solvent and emulsified with proteins, which can either be in the dry state or dissolved in an aqueous solution. This is followed by fabrication into microspheres by a variety of techniques including combinations of phase separation or precipitation, emulsion/solvent evaporation, and/or spraying methods (Figure 12).[144,145] The drug is then released by an erosion mechanism, but diffusion also plays a part. Erosion can be divided into bulk and surface erosion. The former allows fast water penetration into the polymer matrix, which is often associated with a burst release.[144] The type of release is determined by the nature and structure of the polymer used, which can range from biopolymers such as starch and chitosan, to synthetic polymers with PLA playing a major role in this technique among other degradable polymers.[145,146] The largest challenge of this technology, when attempting to encapsulate proteins and peptides, is the inherent low stability of these drugs. The physical forces during processing, contact with organic solvent and loss of water result in protein inactivation, predominantly through protein aggregation and denaturation.[147] Despite the challenges, many protein- or vaccine-loaded particles are under investigation,[144] while several products are already on the market.[145]

Some peptides offer the advantage of potentially being more robust during processing due to the lack of a vital tertiary structure. Vapreotide, a somatostatin analogue, was delivered using poly(lactide-*co*-glycolide) (PLGA) microspheres without loss of activity of the peptide.[148] The slow erosion of the polymer allowed sustained release for up to 4 weeks, which contrasts other techniques discussed above. An extended release within a similar time frame was achieved using various compositions of PLA and poly(D,L-lactide-*co*-glycolide) (PLGA) with orntide acetate, a luteinizing hormone-releasing

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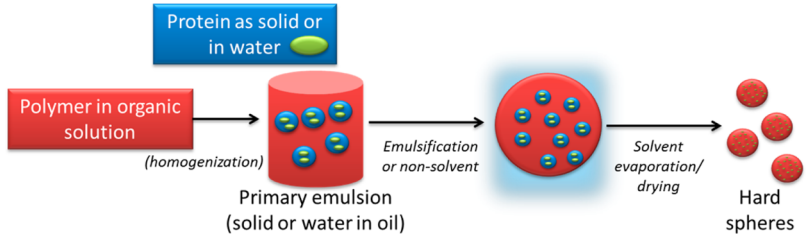

Figure 12. Synthesis of hard polymer microspheres including examples of peptides delivered using the listed polymer [calcitonin (PLA[150]); orntide acetate (PLA/PLGA[149]); vapreotide (PLGA[148]); TAT-HSP27 (PVA/PLGA[158]); insulin (polyesteramides,[151] eudragit (R) L100,[160] starch,[156] hyaluronic acid, [157] alginate[155]); adrenomedullin (chitosan[152]); serpin-derived peptide (poly(*β*-amino ester)[159]); antimicrobial KSL-W (PLGA[161]); major T cell epitope of Ole e 1 (PLGA)[162]].

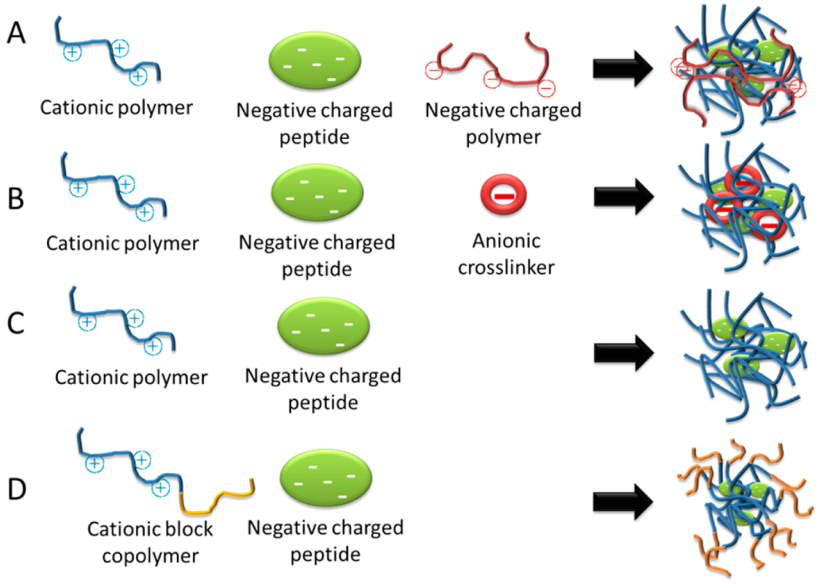

Figure 13. Polyelectrolyte complex prepared (A) using addition charged polymer as cross-linker; (B) using a low molecular weight charged cross-linker; (C) in the absence of any added cross-linker; (D) using charged block copolymers to create polyion complex micelles.

hormone (LHRH) analogue. Drug loading and release could then be fine-tuned by the hydrophilicity of the polymer.[149] Similar results in terms of release characteristics could be obtained with calcitonin in PLA microspheres.[150] Microspheres are particularly attractive materials for the oral delivery of insulin. The described poly(ester amide) (PEA) microspheres, which contains additional arginine functionalities, could lower the blood glucose level for 10 h.[151] Apart from synthetic degradable polymers, a range of biopolymers exists, with examples being chitosan,[152,153] alginate,[154,155] starch,[156] and hyaluronic acid.[157] However, to accommodate the specific properties of the peptide, the preparation of hybrid materials is often necessary. An injectable hybrid system was created using an W/O/W double emulsion system to accommodate the hydrophobic heat shock protein 27 (TAT-HSP27). The drug was loaded into PLGA microspheres, which were then dispersed in PVA[158] hydrogel microspheres or alginate.[154] In contrast, an ionic serpin-derived peptide was first complexed with a degradable cationic polymer and then processed into PLGA microspheres.[159]

Hence, the delivery of peptides seems a mature field, as evidenced by various review articles.[135,145,146] In most reports, the polymers used are established, often FDA approved materials. More tailored-made polymers have been emerging in the past few years providing features such as cell-penetrating functionalities.[151] However, most conventional polymers were shown to be sufficient and successful for their purposes.

4.2.3. Nanoparticles. Both liposomes and microspheres produce particles that are often too big for certain administrative routes. Nanoparticles, in particular particles below 100 nm, can potentially evade detection by the mononuclear phagocyte system (MPS) and therefore increase circulation time of the drug. In addition, noninvasive administration routes benefit from smaller particle sizes as discussed in a recent review article.[163]

Polyelectrolyte Complex. The most popular route for the delivery of protein and peptides is based on polyelectrolyte complex formation between chitosan and a negatively charged polymer (Figure 13). Insulin, which has an overall negative charge at pH7, was added to a negatively charged polymer such as hydroxypropyl methylcellulose phthalate,[164] tripolyphosphate,[164] dextran sulfate,[165,166] hydropropylmethylcellulose,[167] alginate,[168] or a mixture of ionic polymers.[169] This is followed by the addition of chitosan, a positively charged biopolymer. Other low molecular weight anionic cross-linkers which have been tested include sulfobutylether-*β*-cyclodextrin.[170] The three components interlocked with each other leading to the formation of insulin-loaded nanoparticles cross-linked by electrostatic forces. However, the particle sizes were around 200 nm or higher, but seldom below 100 nm.[163] Occasionally,

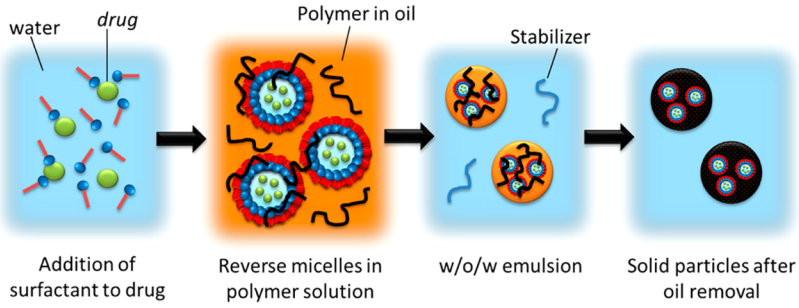

Figure 14. Process of loading polymer particles with peptides using w/o/w technique.

chitosan was modified with functional groups, such as cysteine, to alter the properties of the nanocarriers.[171,172]

Although chitosan, as a naturally positively charged carrier is an attractive material, a range of various synthetic polymers were prepared and applied for peptide delivery. The advantage of synthetic polymers clearly lies in the ability to tailor their properties and this was nicely demonstrated on modified poly(allyl amine). Quarterization of the amines increased the strength of the charge and consequently better binding with insulin was obtained. In addition, functionalization of this polymer with various hydrophobic groups offered better protection against enzymatic degradation of the peptide.[173,174] The possibility to fine-tune polymers made the addition of negatively charged polymers, as it was done in many chitosan systems, obsolete and insulin itself was able to act as a cross-linker. Other synthetic graft polymers employed for this approach were based on poly(vinyl alcohol).[175,176]

Most reports on polyelectrolyte complexes describe the encapsulation of insulin as the peptide of choice. This stems not only from the importance of insulin as a drug, but also from the size of insulin, which lies on the borderline between a protein and a peptide. Insulin is retained into the charged network by its own negative charges, whereas smaller peptides, in contrast, have less charged functionalities, leading to less effective binding to the polymer matrix. It is therefore not surprising that this approach has been mainly applied to larger peptides such as calcitonin. The positively charged calcitonin was combined with spermine and poly(acrylic acid) to create polyelectrolyte complex nanoparticles.[177] Alternatively, polystyrene that was grafted with hydrophilic polymers such as poly(*N*-isopropyl acrylamide) (PNIPAAm), among others, and the copolymer was used to form nanoparticles with calcitonin. Depending on the structure of the polymer the peptide incorporation ranged from 9 to 100% with charged polymers showing better drug loading efficiencies compared to neutral PNIPAAm.[178]

Small peptides, however, have rarely been delivered using this approach due to the absence of sufficient amount of charges that would help to retain the drug in the carrier. The peptide Arg−Gly−Asp (RGD) was loaded onto chitosan modified with cholanic acid to create an amphiphilic structure with an antiangiogenic effect. The particles showed a burst release of the peptide, most likely due to weak interactions between the cationic chitosan and RGD.[179,180]

For insulin and other large peptides, however, this approach is a research area full of exciting potential. The biological activity of these nanoparticles have been discussed in detail elsewhere.[163]

Polyion Complex Micelles. The size of the particles obtained during the polyelectrolyte complex formation is usually well above 200 nm. Block copolymers composed of a charged block and a neutral block that does not undergo any interaction with the drug, such as PEG, can prevent the uncontrolled growth of the particle through stabilization via the PEG shell (Figure 12). As a result, nanoparticles with sizes well below 100 nm are obtained. These polyion complex micelles have been tested on proteins,[14] but to our knowledge not yet on peptides. Similar to polyelectrolyte complexes, the peptide drug needs to be sufficiently charged to act as cross-linker for the network.

It should be noted here that noncharged micelles, which are one of the most popular drug carriers for hydrophobic drugs, are often not suitable for the delivery of many peptides due to the charge and amphiphilic nature often found with peptide drugs.

Emulsion Techniques. The efficient loading of smaller peptides seems to be more successful with other techniques such as emulsion techniques. Although insulin has seen the highest research activity, smaller peptides are just as suitable for this approach. Among all emulsion techniques, a double emulsion-solvent evaporation technique has been described most frequently (Figure 14). This process enabled the loading of insulin into noncharged polymers such as PLGA. To aid the lipophilicity of insulin, the peptide was incubated in an organic solvent together with the degradable polymer composed of amphiphilic molecules such as phospholipids,[181] hypromellose phthalate,[182] eudragit,[183] or pluronics.[184] The emulsion was then poured into an aqueous solution, stabilized with PVA, followed by the evaporation of the solvent. Even though PVA was the preferred stabilizer, addition of other polymers enabled additional coating of the nanoparticles. For example, the addition of Eudragit L30D created core−shell nanoparticles with modulated release profiles for insulin.[185] Helodermin, a vasoactive intestinal peptide-like peptide with 34 amino acids, was loaded into PEG-*b*-PLA/PLGA nanoparticles via this pathway,[186] while the small peptide thymopentin was loaded into PLGA.[187] Interestingly, the w/o/w emulsion technique can also employ more complex polymers.[188] PLGA coupled to the lectin wheat germ agglutinin (WGA) was employed to generate peptide-loaded particles with a lectin surface.[188] Particles sizes obtained using w/o/w emulsions typically range between 100 to 200 nm and, similar to other particles, provide an attractive vehicle to increase the activity of their payload.[163]

Inverse emulsion polymerization was alternatively applied to obtain nanoparticles. Starch was cross-linked within the aqueous nanoreactor using either epichlorohydrin or $POCl_3$ as cross-linker. This gave hydrophilic particles of 200−300 nm, which were then loaded with insulin by immersing the nanoparticles into a peptide solution.[189]

An interesting approach is the loading of the drug prior to the inverse polymerization. Although the presence of radicals

during radical polymerization may compromise the drug, the advantage lies clearly in the better loading efficiency. This technique has been tested with proteins using acid degradable cross-linkers that were copolymerized with a hydrophilic monomer in the presence of the drug in an inverse emulsion system.[190] Similarly, the addition of the protein cytochrome C during the inverse emulsion RAFT polymerization of *N*-(2-hydroxypropyl)methacrylamide and *N*,*N*′-bis(acryloyl)-cystamine) also showed promising loading efficiencies of the protein (73 wt % of added material) in the resulting nanogel,[191] but to our knowledge, the technique has not been applied to peptides.

4.2.4. Hydrogels. Hydrogels are network polymers prepared from hydrophilic polymer chains, which are either chemically or physically cross-linked. Hydrogels can hold large amounts of water and therefore represent an ideal carrier for the hydrophilic peptide drugs. In contrast to most carriers discussed above, hydrogels are frequently used as macroscopic materials such as films, but this does not mean that hydrogel nanoparticles have been neglected. Hydrogels can be prepared from any water-soluble polymer and hydrogel nanoparticles are subject to intensive research activity.[192] Literature is dominated by HEMA copolymers, synthetic polymers such as polyacrylamide, *N*-vinylpyrrolidone, *N*-isopropylacrylamide, and polyvinylalcohol and polysaccharides such as alginates, chitosan, and hyaluronic acid.[193−196] Network formation can take place during the polymerization or afterward, where the polymers are cross-linked. Recent advances in the field include cross-linking based on peptide self-assembly among others.[194] Due to the aqueous nature of the material, the polymer can respond quickly to environmental changes. For that reason, hydrogel research often has a strong focus on the development of materials that respond to changes in temperature, pH, light and glucose levels.[195]

Hydrogels have frequently been employed for the delivery of proteins.[193,197] Hydrogels containing charged active ingredients are frequently used for tissue engineering where the slow release of bioactive molecules is aimed toward the cell growth process. These bioactive molecules are often proteins such as growth factors and are therefore not covered here. The reader is referred to review articles covering this area.[198] Another area that raises significant interest is the use of hydrogels for the treatment of skin ulcers although, again, the delivery of proteins such as growth factors is prevalent.[199] Peptides are also often tethered to the hydrogel materials to enhance the wound healing[200] possibly due to the fast diffusion of free peptides in the hydrogel leading to fast leaching of the drug.[201] Various hydrogels were developed for the oral delivery of peptide drugs, but upon closer inspection, these polymers were typically cross-linked via the formation of polyelectrolyte complexation, which has been discussed earlier. Very few reports use hydrogels for peptide delivery that are not stabilized via electrostatic forces. An example is the cross-linked polymer poly((meth)acrylic acid) grafted with poly(ethylene glycol) for insulin delivery. The hydrogel particles are protonated at low pH values, thus collapsing the hydrogel particle together with the drug. The pH value found in the intestines deprotonates the polymer and creates a swollen network that releases insulin.[202] A recent review on the oral insulin delivery highlights that most hydrogels for this purpose are indeed based on ionic interactions for stability and retention of the drug.[203]

4.2.5. Injectable Implants. The discussion on the delivery of peptides would not be complete without mentioning injectable implants. Injectable implants are often employed when the site of action is easily accessible via injections. Advantage is the targeted delivery of therapeutics while systemic loss of the drug is reduced. Injectable implants are liquid formulations that are injected intramuscularly or subcutaneously, followed by solidification which is often triggered by the environment (Figure 15). Injectable implants are often based on hydrogels, but

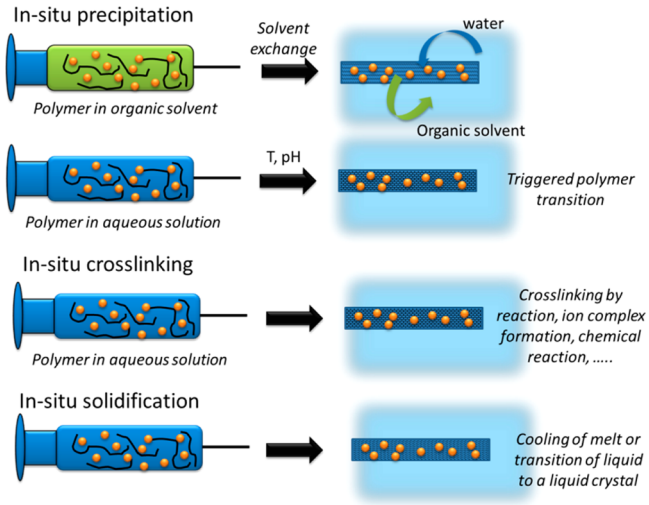

Figure 15. Potential pathways for peptide-loaded injectable implants.

hydrophobic polymers are also common. Implant formation is usually achieved in situ either by precipitation of the polymer, by cross-linking or by solidification. Precipitation occurs when hydrophobic polymers are injected in organic solvents, which is followed by spreading of the organic solvent in the tissue while water penetrates into the injection site. Since the use of organic solvents can cause some concerns in regards to toxicity, other systems have been developed that use the external stimuli to cause transitions in the polymer behavior. For example, polymer solutions in water that undergo an LCST is in solution at room temperature, but rapid precipitation occurs at body temperature. In-situ cross-linking can be achieved using polymers with polymerizable groups. The subsequent polymerization is then frequently initiated with the help of a photoinitiator. Moreover, reactions such as click reactions or Michael additions have been employed to harden the gel after injection. Injection of polymers that can undergo polyelectrolyte complex formation is also popular. Solidification can also be achieved by injecting polymer melts that are liquefied above body temperature or by the use of liquid crystalline polymers. The reader is referred to review articles on injectable implants.[203−205]

Insulin has been delivered by dissolving the peptide together with PLA in benzyl benzoate and benzyl alcohol. Upon injection and depending on the initial polymer and solvent concentration, insulin was slowly released over a period of 90 days.[206] A further study using a similar system could show that the activity of the insulin was maintained under these conditions.[207] This common system has been employed for other peptide drugs such as leuprolide acetate. The rate of release in this case was tailored by changing the molecular weight of the polymer.[208] A further study showed that the release profile can differ noticeably between in vitro and in vivo studies.[209] The stability of the encapsulated peptide is not always warranted. It has been noted that a hydrophobic

environment can lead to the aggregation of proteins and peptides while the degradation of polymer such as PLA and PLGA resulted in significant lowering of the pH value which can affect the stability of the peptide. Addition of PEG not only aids the stability of the peptide, but also allows switching from organic solvents to aqueous solutions. The PLGA-PEG-PLGA triblock copolymer with a low molecular weight is water-soluble at room temperature. Increasing the temperature above the LCST, which coincides with the injection of the polymer solution into the tissue, leads to hydrogel formation with hydrophobic PLGA domains.[210] Delivery of insulin from such a system almost eliminates the burst release known from other systems as insulin was released over a time frame of up to 2 weeks. However, random aggregation of the peptide prevents the full release of insulin. To combat the loss of active drug, Zn^{2+} was added to initiate the formation of a hexameric state of insulin. As a result, 100% of insulin could be released.[210] A block copolymer that has similar properties is PEG-poly-(alanine-*co*-phenyl alanine) (PEG-PAF). An aqueous solution of this polymer was found to have a sol−gel transition temperature between approximately 10 and 20 °C, depending on concentration and salt content. Proteolytic enzymes were found to degrade the polymer, liberating insulin over a period of more than 2 weeks.[211] In general, the introduction of hydrophilic blocks and the formation of hydrogel-like structures were found to be beneficial for the full release of the drug. When poly(L-lactic acid)-*graft*-poly(ethylene glycol) (PLLA-*g*-PEG) was employed as injectable implant material for the delivery of somatotropin, the peptide drug was released almost to completion.[212] In contrast, PLGA alone prevented full release of the drug.[212] It is notable that most reports describe the use of hydrophobic or partly hydrophobic materials for the delivery of peptides. However, fully water-soluble polymers that form highly swollen hydrogels have been tested with success. Chitosan, blended with poly(vinyl alcohol) was cross-linked via electrolyte complex formation to create an injectable system for cyclosporine A. The peptide drug was slowly released over 50 days, while the blood concentration of the drug was observed to be constant over approximately 30 days.[213]

4.2.6. Stimuli-Responsive Materials. Stimuli-responsive polymers have been attractive materials for many years now and the area has permeated widely throughout the drug delivery field. As briefly touched on previously, stimuli-responsive polymers change its properties once the environment changes. This can be either temperature, pH changes, the presence of certain chemicals, or a range of other stimuli.[214,215]

Stimuli-responsive materials, however, have not widely found their way into the delivery of peptide drugs. Most carriers discussed above may undergo slight changes in response to external stimuli, but they do not undergo substantial changes in response to demand or upon reaching the site of action. As with many other systems, insulin is an exception and has been subject to the development of various responsive materials. The response is based on the increased concentration of glucose. The presence of glucose can be felt by enzymes (glucose oxidase) or glucose-binding proteins that are embedded into a polymer matrix. Upon sensing changes in glucose centration, the polymer responds in ways such as swelling of the material and release of insulin. Boronic acid side groups on polymers, on the other hand, are also capable of responding to glucose without the need to incorporate sensitive proteins or enzymes, making such materials robust and attractive as drug carriers.[216] Boronic acid reacts rapidly with 1,2-diol functionalities present in glucose and as a result, the polymer becomes more hydrophilic, swells and releases insulin. The release of insulin using glucose-responsive materials has been reviewed recently and is therefore not discussed in detail here.[217]

5. CONCLUSIONS AND FUTURE PERSPECTIVE

The reader probably already acquired a feeling for the vastness of this field. Almost all known drug delivery carriers have been tested in the delivery of peptides; some of them with more success than others. It should be noted here that some areas such as inorganic drug carriers, microneedle, or natural carriers such as viruses have not been addressed here and only polymers and surfactants that build up large assemblies are discussed. It is probably difficult to single out a carrier that is more suitable than others for the delivery of peptides. One determining factor is the nature of the peptide. A hydrophobic peptide can be delivered using SLN, while a hydrophilic peptide should rather be delivered in a liposomal system, potentially a liposome decorated with polymers, as it was shown to enhance the delivery. Large peptides that are borderline proteins can, furthermore, be delivered using hydrogels and polyion complex, while this system is rather challenging for small peptides. The researcher may consider delivering small peptides with undefined nature, neither hydrophilic nor hydrophobic, maybe amphiphilic, as polymer−peptide conjugate. This ensures sufficient retention in the drug delivery system, while the added advantage is that the amount of loading is very well-known.

As understanding of peptide functionality and its therapeutic effects continues to grow, it is conceivable that peptide-based treatments will form an integral part of medication for the future. As with traditional drugs, the delivery mechanism of potential peptide drugs is often just as important as the peptide itself and can be the determining factor behind whether the therapeutic benefits of the peptide can be realized or not. With the growth in research concerning biological applications of polymeric materials, it is envisioned that methods of peptide conjugation or physical encapsulation, as discussed in this review, will only become more important as attention turns toward peptide delivery for disease prevention and treatment. With the large variability in function and activity found in peptides, it is logical that polymeric materials represent one of the most promising delivery vehicles due the polymers' own variance and the natural adaptability that arises. Hence, regardless of the characteristics of the peptide, a polymeric vehicle can be found, or synthesized, to meet those needs and effectively deliver the peptide.

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Notes

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REFERENCES

(1) Huang, J.; Heise, A. *Chem. Soc. Rev.* **2013**, *42*, 7373.
(2) He, C. L.; Zhuang, X. L.; Tang, Z. H.; Tian, H. Y.; Chen, X. S. *Adv. Healthcare Mater.* **2012**, *1*, 48.

(3) Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Sakellariou, G. *Chem. Rev.* **2009**, *109*, 5528.
(4) Bonduelle, C.; Lecommandoux, S. *Biomacromolecules* **2013**, *14*, 2973.
(5) Lalatsa, A.; Schatzlein, A. G.; Mazza, M.; Le, T. B.; Uchegbu, I. F. *J. Controlled Release* **2012**, *161*, 523.
(6) Law, B.; Tung, C.-H. *Bioconjugate Chem.* **2009**, *20*, 1683.
(7) Lindgren, M.; Langel, U. *Methods Mol. Biol.* **2011**, *683*, 3.
(8) Huang, Y. Z.; Jiang, Y. F.; Wang, H. Y.; Wang, J. X.; Shin, M. C.; Byun, Y.; He, H. N.; Liang, Y. Q.; Yang, V. C. *Adv. Drug Delivery Rev.* **2013**, *64*, 1299.
(9) Gooding, M.; Browne, L. P.; Quinteiro, F. M.; Selwood, D. L. *Chem. Biol. Drug Des.* **2012**, *80*, 787.
(10) Bechara, C.; Sagan, S. *FEBS Lett.* **2013**, *587*, 1693.
(11) Svensen, N.; Walton, J. G. A.; Bradley, M. *Trends Pharmacol. Sci.* **2012**, *33*, 186.
(12) Torchilin, V. P.; Lukyanov, A. N. *Drug Discovery Today* **2003**, *8*, 259.
(13) Davis, S. S. *Am. Pharm. Rev* **2002**, *5*, 29.
(14) Gu, Z.; Biswas, A.; Zhao, M.; Tang, Y. *Chem. Soc. Rev.* **2011**, *40*, 3638.
(15) Gauthier, M. A.; Klok, H. A. *Chem. Commun.* **2008**, 2591.
(16) Ban, H.; Gavrilyuk, J.; Barbas, C. F. *J. Am. Chem. Soc.* **2010**, *132*, 1523.
(17) Ban, H.; Nagano, M.; Gavrilyuk, J.; Hakamata, W.; Inokuma, T.; Barbas, C. F. *Bioconjugate Chem.* **2013**, *24*, 520.
(18) Gavrilyuk, J.; Ban, H.; Nagano, M.; Hakamata, W.; Barbas, C. F. *Bioconjugate Chem.* **2012**, *23*, 2321.
(19) Joshi, N. S.; Whitaker, L. R.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 15942.
(20) Seim, K. L.; Obermeyer, A. C.; Francis, M. B. *J. Am. Chem. Soc.* **2011**, *133*, 16970.
(21) Feng, Y.; Forsell, M. N. E.; Flynn, B.; Adams, W.; Loré, K.; Seder, R.; Wyatt, R. T.; Karlsson Hedestam, G. B. *Virology* **2013**, *446*, 56.
(22) Chen, X.; Henschke, L.; Wu, Q.; Muthoosamy, K.; Neumann, B.; Weil, T. *Org. Biomol. Chem.* **2013**, *11*, 353.
(23) Chen, X.; Muthoosamy, K.; Pfisterer, A.; Neumann, B.; Weil, T. *Bioconjugate Chem.* **2012**, *23*, 500.
(24) Tanaka, K.; Fujii, Y.; Fukase, K. *ChemBioChem* **2008**, *9*, 2392.
(25) Dingels, C.; Wurm, F.; Wagner, M.; Klok, H.-A.; Frey, H. *Chem.—Eur. J.* **2012**, *18*, 16828.
(26) Wurm, F.; Dingels, C.; Frey, H.; Klok, H.-A. *Biomacromolecules* **2012**, *13*, 1161.
(27) Wurm, F.; Steinbach, T.; Klok, H.-A. *Chem. Commun.* **2013**, *49*, 7815.
(28) Wurm, F. R.; Klok, H.-A. *Chem. Soc. Rev.* **2013**, *42*, 8220.
(29) Ruiz-Rodríguez, J.; Albericio, F.; Lavilla, R. *Chem.—Eur. J.* **2010**, *16*, 1124.
(30) Antos, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2004**, *126*, 10256.
(31) Antos, J. M.; McFarland, J. M.; Iavarone, A. T.; Francis, M. B. *J. Am. Chem. Soc.* **2009**, *131*, 6301.
(32) Foettinger, A.; Melmer, M.; Leitner, A.; Lindner, W. *Bioconjugate Chem.* **2007**, *18*, 1678.
(33) Chan, A. O.-Y.; Ho, C.-M.; Chong, H.-C.; Leung, Y.-C.; Huang, J.-S.; Wong, M.-K.; Che, C.-M. *J. Am. Chem. Soc.* **2012**, *134*, 2589.
(34) Uhlig, N.; Li, C.-J. *Org. Lett.* **2012**, *14*, 3000.
(35) Gauthier, M. A.; Klok, H.-A. *Biomacromolecules* **2010**, *12*, 482.
(36) Gauthier, M. A.; Ayer, M.; Kowal, J.; Wurm, F. R.; Klok, H.-A. *Polym. Chem.* **2011**, *2*, 1490.
(37) Espuña, G.; Arsequell, G.; Valencia, G.; Barluenga, J.; Alvarez-Gutiérrez, J. M.; Ballesteros, A.; González, J. M. *Angew. Chem., Int. Ed.* **2004**, *43*, 325.
(38) Cong, Y.; Pawlisz, E.; Bryant, P.; Balan, S.; Laurine, E.; Tommasi, R.; Singh, R.; Dubey, S.; Peciak, K.; Bird, M.; Sivasankar, A.; Swierkosz, J.; Muroni, M.; Heidelberger, S.; Farys, M.; Khayrzad, F.; Edwards, J.; Badescu, G.; Hodgson, I.; Heise, C.; Somavarapu, S.; Liddell, J.; Powell, K.; Zloh, M.; Choi, J.-w.; Godwin, A.; Brocchini, S. *Bioconjugate Chem.* **2012**, *23*, 248.
(39) Bernardes, G. J. L.; Chalker, J. M.; Errey, J. C.; Davis, B. G. *J. Am. Chem. Soc.* **2008**, *130*, 5052.
(40) Kundu, R.; Ball, Z. T. *Chem. Commun.* **2013**, *49*, 4166.
(41) On-Yee Chan, A.; Lui-Lui Tsai, J.; Kar-Yan, L. V.; Li, G.-L.; Wong, M.-K.; Che, C.-M. *Chem. Commun.* **2013**, *49*, 1428.
(42) Han, M.-J.; Xiong, D.-C.; Ye, X.-S. *Chem. Commun.* **2012**, *48*, 11079.
(43) Zhao, Y.; Perrier, S. *Chem. Commun.* **2007**, *0*, 4294.
(44) Yamamoto, Y.; Tsutsumi, Y.; Mayumi, T. *Current Drug Targets* **2002**, *3*, 123.
(45) Chandna, P.; Saad, M.; Wang, Y.; Ber, E.; Khandare, J.; Vetcher, A. A.; Soldatenkov, V. A.; Minko, T. *Mol. Pharmaceutics* **2007**, *4*, 668.
(46) Chandna, P.; Khandare, J.; Ber, E.; Rodriguez-Rodriguez, L.; Minko, T. *Pharm. Res.* **2010**, *27*, 2296.
(47) Maeda, H. *Adv. Drug Delivery Rev.* **2001**, *46*, 169.
(48) Mu, Y.; Kamada, H.; Kaneda, Y.; Yamamoto, Y.; Kodaira, H.; Tsunoda, S.-i.; Tsutsumi, Y.; Maeda, M.; Kawasaki, K.; Nomizu, M.; Yamada, Y.; Mayumi, T. *Biochem. Biophys. Res. Commun.* **1999**, *255*, 75.
(49) Ukawala, M.; Chaudhari, K.; Rajyaguru, T.; Manjappa, A. S.; Murthy, R. S. R.; Gude, R. *J. Drug Targeting* **2012**, *20*, 55.
(50) Hinds, K.; Koh, J. J.; Joss, L.; Liu, F.; Baudyš, M.; Kim, S. W. *Bioconjugate Chem.* **2000**, *11*, 195.
(51) Ryan, S. M.; Wang, X.; Mantovani, G.; Sayers, C. T.; Haddleton, D. M.; Brayden, D. J. *J. Controlled Release* **2009**, *135*, 51.
(52) Youn, Y. S.; Kwon, M. J.; Na, D. H.; Chae, S. Y.; Lee, S.; Lee, K. C. *J. Controlled Release* **2008**, *125*, 68.
(53) Baudyš, M.; Letourneur, D.; Liu, F.; Mix, D.; Jozefonvicz, J.; Kim, S. W. *Bioconjugate Chem.* **1998**, *9*, 176.
(54) Chen, G.; Ito, Y.; Imanishi, Y. *Biotechnol. Bioeng.* **1997**, *53*, 339.
(55) Shahnaz, G.; Iqbal, J.; Rahmat, D.; Perera, G.; Laffleur, F.; Rossi, D.; Bernkop-Schnürch, A. *J. Controlled Release* **2012**, *157*, 375.
(56) Shahnaz, G.; Perera, G.; Sakloetsakun, D.; Rahmat, D.; Bernkop-Schnürch, A. *J. Controlled Release* **2010**, *144*, 32.
(57) Cheng, W.; Lim, L.-Y. *J. Pharm. Pharmacol.* **2010**, *62*, 296.
(58) Dharap, S. S.; Qiu, B.; Williams, G. C.; Sinko, P.; Stein, S.; Minko, T. *J. Controlled Release* **2003**, *91*, 61.
(59) Zalipsky, S.; Puntambekar, B.; Boulikas, P.; Engbers, C. M.; Woodle, M. C. *Bioconjugate Chem.* **1995**, *6*, 705.
(60) Skwarczynski, M.; Ahmad Fuaad, A. A. H.; Rustanti, L.; Ziora, Z. M.; Aqil, M.; Batzloff, M. R.; Good, M. F.; Toth, I. *Drug Delivery Lett.* **2011**, *1*, 2.
(61) Fuaad, A. A. H. A.; Jia, Z.; Zaman, M.; Hartas, J.; Ziora, Z. M.; Lin, I. C.; Moyle, P. M.; Batzloff, M. R.; Good, M. F.; Monteiro, M. J.; Skwarczynski, M.; Toth, I. *Nanomedicine* **2014**, *9*, 35.
(62) Skwarczynski, M.; Zaman, M.; Urbani, C. N.; Lin, I. C.; Jia, Z.; Batzloff, M. R.; Good, M. F.; Monteiro, M. F.; Toth, I. *Angew. Chem., Int. Ed.* **2010**, *49*, 5742.
(63) Liu, T. Y.; Hussein, W. M.; Jia, Z. F.; Ziora, Z. M.; McMillan, N. A. J.; Monteiro, M. J.; Toth, I.; Skwarczynski, M. *Biomacromolecules* **2013**, *14*, 2798.
(64) Arimoto, H.; Nishimura, K.; Hayakawa, I.; Kinumi, T.; Uemura, D. *Chem. Commun.* **1999**, 1361.
(65) Metallo, S. J.; Kane, R. S.; Holmlin, R. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **2003**, *125*, 4534.
(66) *Peptide and Protein Delivery*; VanDerWalle, C. F. Ed.; Academic Press: Waltham, Massachusetts, 2011.
(67) Adi, S.; Adi, H.; Tang, P.; Traini, D.; Chan, H.-k.; Young, P. M. *Eur. J. Pharm. Biopharm.* **2008**, *35*, 12.
(68) Ritthidej, G. C. *Nasal Delivery of Peptides and Proteins with Chitosan and Related Mucoadhesive Polymers*; Academic Press: Waltham, Massachusetts, 2011.
(69) Teshima, D.; Yamauchi, A.; Makino, K.; Kataoka, Y.; Arita, Y.; Nawata, H.; Oishi, R. *Int. J. Pharm.* **2002**, *233*, 61.
(70) Seki, T.; Kanbayashi, H.; Chono, S.; Tabata, Y.; Morimoto, K. *Int. J. Pharm.* **2007**, *338*, 213.
(71) Müller, R. H.; Mäder, K.; Gohla, S. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 161.
(72) Das, S.; Chaudhury, A. *AAPS PharmSciTech* **2011**, *12*, 62.

(73) Almeida, A. J.; Souto, E. *Adv. Drug Delivery Rev.* **2007**, *59*, 478.
(74) Iqbal, M. A.; Md, S.; Sahni, J. K.; Baboota, S.; Dang, S.; Ali, J. *J. Drug Targeting* **2012**, *20*, 813.
(75) Rekha, M. R.; Sharma, C. P. *Nanoparticle Mediated Oral Delivery of Peptides and Proteins: Challenges and Perspectives*; Academic Press: Waltham, Massachusetts, 2011
(76) Danielsson, I.; Lindman, B. *Colloids Surf.* **1981**, *3*, 391.
(77) Lawrence, M. J.; Rees, G. D. *Adv. Drug Delivery Rev.* **2000**, *45*, 89.
(78) Spernath, A.; Aserin, A. *Adv. Colloid Interface Sci.* **2006**, *128−130*, 47.
(79) Nagarajan, R.; Ruckenstein, E. *Langmuir* **1991**, *7*, 2934.
(80) Malcolmson, C.; Satra, C.; Kantaria, S.; Sidhu, A.; Lawrence, M. J. *J. Pharm. Sci.* **1998**, *87*, 109.
(81) Constantinides, P.; Scalart, J.-P.; Lancaster, C.; Marcello, J.; Marks, G.; Ellens, H.; Smith, P. *Pharm. Res.* **1994**, *11*, 1385.
(82) Liu, D. Y.; Kobayashi, T.; Russo, S.; Li, F. L.; Plevy, S. E.; Gambling, T. M.; Carson, J. L.; Mumper, R. J. *AAPS J.* **2013**, *15*, 288.
(83) Kuentz, M. *J. Drug Delivery Sci. Technol.* **2011**, *21*, 17.
(84) Khan, A. W.; Kotta, S.; Ansari, S. H.; Sharma, R. K.; Ali, J. *Expert Opin. Drug Delivery* **2012**, *9*, 1305.
(85) Cárdenas-Bailón, F.; Osorio-Revilla, G.; Gallardo-Velázquez, T. *J. Microencapsulation* **2013**, *30*, 409.
(86) Elsayed, A.; Remawi, M. A.; Qinna, N.; Farouk, A.; Badwan, A. *Eur. J. Pharm. Biopharm.* **2009**, *73*, 269.
(87) He, C. X.; He, Z. G.; Gao, J. Q. *Expert Opin. Drug Delivery* **2010**, *7*, 445.
(88) Ritschel, W. A. *Anglais* **1991**, *13*, 205.
(89) Ritschel, W. A. *Clin. Transpl.* **1996**, *10*, 364.
(90) Liu, H. Z.; Wang, Y. J.; Lang, Y. Y.; Yao, H. M.; Dong, Y.; Li, S. M. *J. Pharm. Sci.* **2009**, *98*, 1167.
(91) Goebel, A. S. B.; Schmaus, G.; Neubert, R. H. H.; Wohlrab, J. *Skin Pharmacol. Physiol.* **2012**, *25*, 281.
(92) Fan, Y. T.; Li, X. R.; Zhou, Y. X.; Fan, C.; Wang, X. N.; Huang, Y. Q.; Liu, Y. *Int. J. Pharm.* **2011**, *416*, 323.
(93) Kafka, A. P.; Rades, T.; McDowell, A. *Biomed. Chromatogr.* **2010**, *24*, 132.
(94) Nair, R.; Chakrapani, M.; Kaza, R. *Drug Delivery Lett.* **2012**, *2*, 26.
(95) Morel, S.; Rosa Gasco, M.; Cavalli, R. *Int. J. Pharm.* **1994**, *105*, R1.
(96) Müller, R. H.; Runge, S.; Ravelli, V.; Mehnert, W.; Thünemann, A. F.; Souto, E. B. *Int. J. Pharm.* **2006**, *317*, 82.
(97) Müller, R. H.; Runge, S. A.; Ravelli, V.; Thünemann, A. F.; Mehnert, W.; Souto, E. B. *Eur. J. Pharm. Biopharm.* **2008**, *68*, 535.
(98) García-Fuentes, M.; Torres, D.; Alonso, M. J. *Colloids Surf., B* **2003**, *27*, 159.
(99) Hu, F. Q.; Hong, Y.; Yuan, H. *Int. J. Pharm.* **2004**, *273*, 29.
(100) Salmaso, S.; Elvassore, N.; Bertucco, A.; Caliceti, P. *J. Pharm. Sci.* **2009**, *98*, 640.
(101) Garcia-Fuentes, M.; Torres, D.; Alonso, M. J. *Int. J. Pharm.* **2005**, *296*, 122.
(102) Chen, C. H.; Fan, T. T.; Jin, Y.; Zhou, Z.; Yang, Y.; Zhu, X.; Zhang, Z. R.; Zhang, Q.; Huang, Y. *Nanomedicine* **2013**, *8*, 1085.
(103) Reithmeier, H.; Herrmann, J.; Göpferich, A. *J. Controlled Release* **2001**, *73*, 339.
(104) Huang, C.; Jin, H.; Qian, Y.; Qi, S.; Luo, H.; Luo, Q.; Zhang, Z. *ACS Nano* **2013**, *7*, 5791.
(105) Allen, T. M.; Cullis, P. R. *Adv. Drug Delivery Rev.* **2013**, *65*, 36.
(106) Weiner, A. L. *ImmunoMethods* **1994**, *4*, 201.
(107) Swaminathan, J.; Ehrhardt, C. *Expert Opin. Drug Delivery* **2012**, *9*, 1489.
(108) Martins, S.; Sarmento, B.; Ferreira, D. C.; Souto, E. B. *Int. J. Nanomed.* **2007**, *2*, 595.
(109) Stevenson, R. W.; Patel, H. M.; Parsons, J. A.; Ryman, B. E. *Diabetes* **1982**, *31*, 506.
(110) Gao, X.-p.; Noda, Y.; Rubinstein, I.; Paul, S. *Life Sci.* **1994**, *54*, PL247.
(111) Refai, E.; Jonsson, C.; Andersson, M.; Jacobsson, H.; Larsson, S.; Kogner, P.; Hassan, M. *Nuclear Med. Biol.* **1999**, *26*, 931.
(112) Arien, A.; Toulme-Henry, N.; Dupuy, B. *Pharm. Res.* **1995**, *12*, 1289.
(113) Stark, B.; Debbage, P.; Andreae, F.; Mosgoeller, W.; Prassl, R. *Biochim. Biophys. Acta* **2007**, *1768*, 705.
(114) Law, S. L.; Shih, C. L. *J. Microencapsulation* **2001**, *18*, 211.
(115) Rex, S.; Schwarz, G. *Biochemistry* **1998**, *37*, 2336.
(116) Kato, Y.; Hosokawa, T.; Hayakawa, E.; Ito, K. *Biol. Pharm. Bull.* **1993**, *16*, 457.
(117) Chen, M.; Li, X.-R.; Zhou, Y.-X.; Yang, K.-W.; Chen, X.-W.; Deng, Q.; Liu, Y.; Ren, L.-J. *Peptides* **2009**, *30*, 1288.
(118) Chang, H. I.; Yeh, M. K. *Int. J. Nanomed.* **2012**, *7*, 49.
(119) du Plessis, L. H.; Lubbe, J.; Strauss, T.; Kotze, A. F. *Int. J. Pharm.* **2010**, *385*, 181.
(120) Grohganz, H.; Tho, I.; Brandl, M. *Eur. J. Pharm. Biopharm.* **2005**, *59*, 439.
(121) Arunothayanun, P.; Turton, J. A.; Uchegbu, I. F.; Florence, A. T. *J. Pharm. Sci.* **1999**, *88*, 34.
(122) Maherani, B.; Arab-Tehrany, E.; Kheirolomoom, A.; Cleymand, F.; Linder, M. *Food Chem.* **2012**, *134*, 632.
(123) Sande, L.; Sanchez, M.; Montes, J.; Wolf, A. J.; Morgan, M. A.; Omri, A.; Liu, G. Y. *J. Antimicrob. Chemother.* **2012**, *67*, 2191.
(124) Nicolosi, D.; Scalia, M.; Nicolosi, V. M.; Pignatello, R. *Int. J. Antimicrob. Agents* **2010**, *35*, 553.
(125) Bai, G.; Wang, Y.; Nichifor, M.; Bastos, M. *Langmuir* **2013**, *29*, 13258.
(126) Allen, T. M.; Hansen, C.; Martin, F.; Redemann, C.; Yau-Young, A. *Biochim. Biophys. Acta* **1991**, *1066*, 29.
(127) Immordino, M. L.; Dosio, F.; Cattel, L. *Int. J. Nanomed.* **2006**, *1*, 297.
(128) Kim, A.; Yun, M. O.; Oh, Y. K.; Ahn, W. S.; Kim, C. K. *Int. J. Pharm.* **1999**, *180*, 75.
(129) Rubinstein, I.; Ikezaki, H.; Önyüksel, H. *Int. J. Pharm.* **2006**, *316*, 144.
(130) Takeuchi, H.; Yamamoto, H.; Niwa, T.; Hino, T.; Kawashima, Y. *Pharm. Res.* **1996**, *13*, 896.
(131) Wu, Z. H.; Ping, Q. N.; Wei, Y.; Lai, J. M. *Acta Pharmacol. Sin.* **2004**, *25*, 966.
(132) Ramadas, M.; Paul, W.; Dileep, K. J.; Anitha, Y.; Sharma, C. P. *J. Microencapsulation* **2000**, *17*, 405.
(133) Iwanaga, K.; Ono, S.; Narioka, K.; Kakemi, M.; Morimoto, K.; Yamashita, S.; Namba, Y.; Oku, N. *J. Pharm. Sci.* **1999**, *88*, 248.
(134) Watanabe, K.; Kaneko, M.; Maitani, Y. *Int. J. Nanomed.* **2012**, *7*, 3679.
(135) Tan, M. L.; Choong, P. F.; Dass, C. R. *Peptides* **2010**, *31*, 184.
(136) Tan, H. W.; Misran, M. *J. Liposome Res.* **2012**, *22*, 329.
(137) Smistad, G.; Boyum, S.; Alund, S. J.; Samuelsen, A. B. C.; Hiorth, M. *Carbohydr. Polym.* **2012**, *90*, 1337.
(138) Thirawong, N.; Thongborisute, J.; Takeuchi, H.; Sriamornsak, P. *J. Controlled Release* **2008**, *125*, 236.
(139) Werle, M.; Makhlof, A.; Takeuchi, H. *Chem. Pharm. Bull.* **2010**, *58*, 432.
(140) Muppidi, K.; Wang, J.; Betageri, G.; Pumerantz, A. S. *Antimicrob. Agents Chemother.* **2011**, *55*, 4537.
(141) Goto, T.; Morishita, M.; Nishimura, K.; Nakanishi, M.; Kato, A.; Ehara, J.; Takayama, K. *Pharm. Res.* **2006**, *23*, 384.
(142) Takeuchi, H.; Matsui, Y.; Yamamoto, H.; Kawashima, Y. *J. Controlled Release* **2003**, *86*, 235.
(143) Camelo, S.; Lajavardi, L.; Bochot, A.; Goldenberg, B.; Naud, M. C.; Fattal, E.; Behar-Cohen, F.; de Kozak, Y. *Mol. Vision* **2007**, *13*, 2263.
(144) Kim, K.; Pack, D. In *BioMEMS and Biomedical Nanotechnology*; Ferrari, M., Lee, A., Lee, L. J., Eds.; Springer: New York, 2006; p 19.
(145) Sinha, V. R.; Trehan, A. *J. Controlled Release* **2003**, *90*, 261.
(146) Degim, I. T.; Celebi, N. *Curr. Pharmaceutical Des.* **2007**, *13*, 99.
(147) Schwendeman, S. P. *Crit. Rev. Ther. Drug Carrier Syst.* **2002**, *19*, 73.

(148) Blanco-Prieto, M. J.; Besseghir, K.; Zerbe, O.; Andris, D.; Orsolini, P.; Heimgartner, F.; Merkle, H. P.; Gander, B. *J. Controlled Release* **2000**, *67*, 19.
(149) Kostanski, J. W.; Thanoo, B. C.; DeLuca, P. P. *Pharm. Dev. Technol.* **2000**, *5*, 585.
(150) Prabhu, S.; Sullivan, J. L.; Betageri, G. V. *Drug Delivery* **2002**, *9*, 195.
(151) He, P.; Liu, H.; Tang, Z.; Deng, M.; Yang, Y.; Pang, X.; Chen, X. *Int. J. Pharm.* **2013**, *455*, 259.
(152) Wang, L.; Zheng, L.; Li, C. Y.; Dong, S. J.; Lan, A.; Zhou, Y. M. *Biomed. Eng. Online* **2013**, *12*, 99 DOI: 10.1186/1475-925X-12-99.
(153) Wang, M. B.; Feng, Q. L.; Guo, X. D.; She, Z. D.; Tan, R. W. *Polym. Degrad. Stab.* **2011**, *96*, 107.
(154) Shin, S.-H.; Lee, J.; Lim, K. S.; Rhim, T.; Lee, S. K.; Kim, Y.-H.; Lee, K. Y. *J. Controlled Release* **2013**, *166*, 38.
(155) Builders, P. F.; Kunle, O. O.; Okpaku, L. C.; Builders, M. I.; Attama, A. A.; Adikwu, M. U. *Eur. J. Pharm. Biopharm.* **2008**, *70*, 777.
(156) Grinberg, O.; Gedanken, A. *Macromol. Chem. Phys.* **2010**, *211*, 924.
(157) Illum, L.; Farraj, N. F.; Fisher, A. N.; Gill, I.; Miglietta, M.; Benedetti, L. M. *J. Controlled Release* **1994**, *29*, 133.
(158) Lee, J.; Cha, M. J.; Lim, K. S.; Kim, J. K.; Lee, S. K.; Kim, Y. H.; Hwang, K. C.; Lee, K. Y. *J. Drug Targeting* **2013**, *21*, 822.
(159) Shmueli, R. B.; Ohnaka, M.; Miki, A.; Pandey, N. B.; Silva, R. L. E.; Koskimaki, J. E.; Kim, J.; Popel, A. S.; Campochiaro, P. A.; Green, J. J. *Biomaterials* **2013**, *34*, 7544.
(160) Marais, E.; Hamman, J.; du Plessis, L.; Lemmer, R.; Steenekamp, J. *Molecules* **2013**, *18*, 6734.
(161) Machado, H. A.; Abercrombie, J. J.; You, T.; DeLuca, P. P.; Leung, K. P. *Biomed. Res. Int.* **2013**, DOI: 10.1155/2013/387863.
(162) Marazuela, E. G.; Prado, N.; Moro, E.; Fernandez-Garcia, H.; Villalba, M.; Rodriguez, R.; Batanero, E. *Clin. Exp. Allergy* **2008**, *38*, 520.
(163) Kammona, O.; Kiparissides, C. *J. Controlled Release* **2012**, *161*, 781.
(164) Makhlof, A.; Tozuka, Y.; Takeuchi, H. *Eur. J. Pharm. Sci.* **2011**, *42*, 445.
(165) Sarmento, B.; Ribeiro, A.; Veiga, F.; Ferreira, D. *Colloids Surf., B* **2006**, *53*, 193.
(166) Sarmento, B.; Ribeiro, A.; Veiga, F.; Ferreira, D.; Neufeld, R. *Biomacromolecules* **2007**, *8*, 3054.
(167) Li, M. G.; Lu, W. L.; Wang, J. C.; Zhang, X.; Wang, X. Q.; Zheng, A. P.; Zhang, Q. *Int. J. Pharm.* **2007**, *329*, 182.
(168) Sarmento, B.; Ferreira, D. C.; Jorgensen, L.; van de Weert, M. *Eur. J. Pharm. Biopharm.* **2007**, *65*, 10.
(169) Goycoolea, F. M.; Lollo, G.; Remuñán-López, C.; Quaglia, F.; Alonso, M. a. J. *Biomacromolecules* **2009**, *10*, 1736.
(170) Teijeiro-Osorio, D.; Remuñán-López, C.; Alonso, M. J. *Biomacromolecules* **2008**, *10*, 243.
(171) Yin, L.; Ding, J.; He, C.; Cui, L.; Tang, C.; Yin, C. *Biomaterials* **2009**, *30*, 5691.
(172) Wang, X.; Zheng, C.; Wu, Z.; Teng, D.; Zhang, X.; Wang, Z.; Li, C. *J. Biomed. Mater. Res., Part B* **2009**, *88*, 150.
(173) Thompson, C. J.; Tetley, L.; Uchegbu, I. F.; Cheng, W. P. *Int. J. Pharm.* **2009**, *376*, 46.
(174) Thompson, C. J.; Tetley, L.; Cheng, W. P. *Int. J. Pharm.* **2010**, *383*, 216.
(175) Simon, M.; Behrens, I.; Dailey, L. A.; Wittmar, M.; Kissel, T. *Eur. J. Pharm. Biopharm.* **2007**, *66*, 165.
(176) Packhaeuser, C. B.; Kissel, T. *J. Controlled Release* **2007**, *123*, 131.
(177) Makhlof, A.; Werle, M.; Tozuka, Y.; Takeuchi, H. *J. Controlled Release* **2011**, *149*, 81.
(178) Sakuma, S.; Suzuki, N.; Kikuchi, H.; Hiwatari, K.-i.; Arikawa, K.; Kishida, A.; Akashi, M. *Int. J. Pharm.* **1997**, *149*, 93.
(179) Park, J. H.; Kwon, S.; Nam, J.-O.; Park, R.-W.; Chung, H.; Seo, S. B.; Kim, I.-S.; Kwon, I. C.; Jeong, S. Y. *J. Controlled Release* **2004**, *95*, 579.
(180) Kim, J.-H.; Kim, Y.-S.; Park, K.; Kang, E.; Lee, S.; Nam, H. Y.; Kim, K.; Park, J. H.; Chi, D. Y.; Park, R.-W.; Kim, I.-S.; Choi, K.; Chan Kwon, I. *Biomaterials* **2008**, *29*, 1920.
(181) Cui, F.; Shi, K.; Zhang, L.; Tao, A.; Kawashima, Y. *J. Controlled Release* **2006**, *114*, 242.
(182) Cui, F.-d.; Tao, A.-j.; Cun, D.-m.; Zhang, L.-q.; Shi, K. *J. Pharm. Sci.* **2007**, *96*, 421.
(183) Damge, C.; Maincent, P.; Ubrich, N. *J. Controlled Release* **2007**, *117*, 163.
(184) Kumar, P. S.; Saini, T. R.; Chandrasekar, D.; Yellepeddi, V. K.; Ramakrishna, S.; Diwan, P. V. *Drug Delivery* **2007**, *14*, 517.
(185) Naha, P. C.; Kanchan, V.; Manna, P. K.; Panda, A. K. *J. Microencapsulation* **2008**, *25*, 248.
(186) des Rieux, A.; Fievez, V.; Momtaz, M.; Detrembleur, C.; Alonso-Sande, M.; Van Gelder, J.; Cauvin, A.; Schneider, Y. J.; Preat, V. *J. Controlled Release* **2007**, *118*, 294.
(187) Yin, Y.; Chen, D.; Qiao, M.; Lu, Z.; Hu, H. *J. Controlled Release* **2006**, *116*, 337.
(188) Yin, Y.; Chen, D.; Qiao, M.; Wei, X.; Hu, H. *J. Controlled Release* **2007**, *123*, 27.
(189) Jain, A. K.; Khar, R. K.; Ahmed, F. J.; Diwan, P. V. *Eur. J. Pharm. Biopharm.* **2008**, *69*, 426.
(190) Murthy, N.; Xu, M.; Schuck, S.; Kunisawa, J.; Shastri, N.; Fréchet, J. M. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4995.
(191) Wutzel, H.; Richter, F. H.; Li, Y.; Sheiko, S. S.; Klok, H.-A. *Polym. Chem.* **2014**, *5*, 1711.
(192) Hamidi, M.; Azadi, A.; Rafiei, P. *Adv. Drug Delivery Rev.* **2008**, *60*, 1638.
(193) Lee, K. Y.; Yuk, S. H. *Prog. Polym. Sci.* **2007**, *32*, 669.
(194) Kopecek, J. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 5929.
(195) Qiu, Y.; Park, K. *Adv. Drug Delivery Rev.* **2001**, *53*, 321.
(196) Kirschner, C. M.; Anseth, K. S. *Acta Mater.* **2013**, *61*, 931.
(197) Vermonden, T.; Censi, R.; Hennink, W. E. *Chem. Rev.* **2012**, *112*, 2853.
(198) Pereira, P. In *Peptide and Protein Delivery*; Walle, C. V. D., Ed.; Academic Press: Boston, 2011; p 291.
(199) Moura, L. I.; Dias, A. M.; Carvalho, E.; de Sousa, H. C. *Acta Biomater.* **2013**, *9*, 7093.
(200) Clark, R. A.; Ghosh, K.; Tonnesen, M. G. *J. Invest. Dermatol.* **2007**, *127*, 1018.
(201) Domb, A.; Davidson Iii, G. W. R.; Sanders, L. M. *J. Controlled Release* **1990**, *14*, 133.
(202) Foss, A. C.; Peppas, N. A. *Eur. J. Pharm. Biopharm.* **2004**, *57*, 447.
(203) Chaturvedi, K.; Ganguly, K.; Nadagouda, M. N.; Aminabhavi, T. M. *J. Controlled Release* **2013**, *165*, 129.
(204) Li, Y.; Rodrigues, J.; Tomas, H. *Chem. Soc. Rev.* **2012**, *41*, 2193.
(205) Agarwal, P.; Rupenthal, I. D. *Drug Discovery Today* **2013**, *18*, 337.
(206) Kang, F.; Singh, J. *Int. J. Pharm.* **2005**, *304*, 83.
(207) Dhawan, S.; Kapil, R.; Kapoor, D. N. *J. Biomater. Appl.* **2011**, *25*, 699.
(208) Astaneh, R.; Moghimi, H. R.; Erfan, M.; Mobedi, H. *Daru, J. Pharm. Sci.* **2006**, *14*, 65.
(209) Mashayekhi, R.; Mobedi, H.; Najafi, J.; Enayati, M. *Daru, J. Pharm. Sci.* **2013**, *21*, 57.
(210) Kim, Y. J.; Choi, S.; Koh, J. J.; Lee, M.; Ko, K. S.; Kim, S. W. *Pharm. Res.* **2001**, *18*, 548.
(211) Jeong, Y.; Joo, M. K.; Bahk, K. H.; Choi, Y. Y.; Kim, H.-T.; Kim, W.-K.; Jeong Lee, H.; Sohn, Y. S.; Jeong, B. *J. Controlled Release* **2009**, *137*, 25.
(212) Cho, K. Y.; Lee, S.; Park, J. K. *Macromol. Biosci.* **2007**, *7*, 784.
(213) Sun, J.; Jiang, G.; Wang, Y.; Ding, F. *J. Appl. Polym. Sci.* **2012**, *125*, 2092.
(214) Liu, F.; Urban, M. W. *Prog. Polym. Sci.* **2010**, *35*, 3.
(215) Schattling, P.; Jochum, F. D.; Theato, P. *Polym. Chem* **2014**, *5*, 25.
(216) Cambre, J. N.; Sumerlin, B. S. *Polymer* **2011**, *52*, 4631.
(217) Wu, W.; Zhou, S. *Macromol. Biosci.* **2013**, *13*, 1464.