Novel Delivery Systems for Improving the Clinical Use of Peptides

Miia Kovalainen, Juha Mönkäre, Joakim Riikonen, UllamariPesonen, Maria Vlasova, Jarno Salonen, Vesa-Pekka Lehto, Kristiina Jarvinen, and Karl-Heinz Herzig

Institute of Biomedicine and Biocenter of Oulu, Faculty of Medicine (M.K., K.-H.H.) and Medical Research Center Oulu and Oulu University Hospital (K.-H.H.), Oulu, Finland; Department of Applied Physics, Faculty of Science and Forestry (J.R.), Department of Applied Physics, Faculty of Science and Forestry (V.-P.L.), and School of Pharmacy, Faculty of Health Sciences (M.V., K.J.), University of Eastern Finland, Kuopio, Finland; Department of Pharmacology, Drug Development and Therapeutics (U.P.), and Department of Physics and Astronomy, Faculty of Mathematics and Natural Sciences (J.S.), University of Turku, Finland; and Leiden Academic Center for Drug Research, Leiden University, Leiden, The Netherlands (J.M.)

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Acknowledgments

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Address correspondence to: Dr. Miia Kovalainen or Prof. Karl-Heinz Herzig, Institute of Biomedicine and Biocenter of Oulu, Faculty of Medicine, University of Oulu, Aapistie 5; 90014 University of Oulu, Finland. E-mail: Miia.Kovalainen@oulu.fi or karl-heinz.herzig@oulu.fi dx.doi.org/10.1124/pr.113.008367.
Peptides have long been recognized as a promising group of therapeutic substances to treat various diseases. Delivery systems for peptides have been under development since the discovery of insulin for the treatment of diabetes. The challenge of using peptides as drugs arises from their poor bioavailability resulting from the low permeability of biological membranes and their instability. Currently, subcutaneous injection is clinically the most common administration route for peptides. This route is cost-effective and suitable for self-administration, and the development of appropriate dosing equipment has made performing the repeated injections relatively easy; however, only few clinical subcutaneous peptide delivery systems provide sustained peptide release. As a result, frequent injections are needed, which may cause discomfort and additional risks resulting from a poor administration technique. Controlled peptide delivery systems, able to provide required therapeutic plasma concentrations over an extended period, are needed to increase peptide safety and patient compliance. In this review, we summarize the current peptidergic drugs, future developments, and parenteral peptide delivery systems. Special emphasis is given to porous silicon, a novel material in peptide delivery. Biodegradable and biocompatible porous silicon possesses some unique properties, such as the ability to carry exceptional high peptide payloads and to modify peptide release extensively. We have successfully developed porous silicon as a carrier material for improved parenteral peptide delivery. Nanotechnology, with its different delivery systems, will enable better use of peptides in several therapeutic applications in the near future. 

I. Introduction

Current typical drugs in clinical use have a small-molecular-weight compound (molecular mass <500 g/mol) that is absorbed into systemic circulation from the gastrointestinal tract after administration as an oral formulation. The number of approved biologic drugs (including vaccines and diagnostic proteins) is steadily increasing, however, from nine in 2010 to 18 in 2013. In 2014, there were 11 approvals [Center of Biologics Evaluation and Research, U.S. Food and Drug Administration (FDA)].

Peptides comprise amino acids connected by amide bonds. The shortest natural peptide, thyrotropin-releasing hormone, is only three amino acids long. Oligopeptides or peptides are usually shorter than 50 amino acids, but sometimes even 100-amino-acid-long chains are considered peptides (Latham, 1999; Sato et al., 2006; McGregor, 2008). In this review, peptides with a maximum of 100 amino acids are considered.

Physicochemical properties of macromolecules include high molecular weight, varying aqueous solubility, and rapid degradation in the body, which inherently hinder their efficient oral absorption. As a result, they are administered frequently as parenteral injections, the most common example being the subcutaneous injection of insulin. This review covers the current state-of-the-art of clinical peptides, with an outlook on peptide drugs in the pipeline and parenteral peptide delivery systems. Special interest focuses on the novel, biodegradable, and biocompatible drug carrier material, porous silicon, which has shown potential for clinical applications.

Peptides can be used in the treatment of various diseases, including endocrine dysfunctions, infectious diseases, cancer, central nervous system disorders, and gastroenterologic diseases (Stevenson, 2009; Malavolta and Cabral, 2011). Already more than 100 peptide-based drugs have reached the market, and hundreds of peptidergic compounds are in clinical or preclinical studies (Lien and Lowman, 2003; Bellmann-Sickert and Beck-Sickinger, 2010; Vlieghe et al., 2010; Craik et al., 2013). The advantages and disadvantages of peptides as drugs are summarized in Table 1. Compared with low-molecular-weight drugs, peptides can be more potent, more efficient, and more target specific (Vlieghe et al., 2010). Peptides are often better tolerated and may enable efficient replacement treatment; the adverse effects are related to their excessive pharmacodynamics Leader et al., 2008; Bellmann-Sickert and Beck-Sickinger, 2010). With their gentler side effect profile and greater selectivity and efficiency, peptides can easily be used in different pathologic states, including cancer. In the United States, peptides have reached the market faster compared with other compounds because the clinical and approval phases have been shorter (Reichert, 2003; Leader et al., 2008; Albericio and Kruger, 2012).

To enable the efficient use of peptides as drugs, various methods have been developed (Fig. 1). The sizes of the different peptide delivery systems vary but could extend to nanosized systems and peptide conjugates. Nanotechnology can be used to influence drug characteristics, such as solubility, distribution, elimination, or drug release. Some therapeutics exploiting nanotechnology are already on the market (Zhang et al., 2008; Malam et al., 2011).

II. Peptide Drugs Approved for Clinical Use

A. Background of Clinical Peptides

The FDA has approved 61 new biologic drugs since 2000, which constitute nearly 20% of the total approvals (Mullard, 2014). Some biologic drugs have

ABBREVIATIONS: ADME, absorption, distribution, metabolism, and excretion; AUC, area under the curve; FDA, U.S. Food and Drug Administration; GLP-1, glucagon-like peptide-1; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PSi, porous silicon; PTH, parathyroid hormone; RBC, red blood cell.
been financially successful, and several reached the blockbuster category, including insulin glargine ($6.510 million in 2012) and several antibody products (Craik et al., 2013). The most studied and well known example of a peptide in therapeutic use is the parenteral formulation of insulin. Several other peptide drugs are in clinical use and have a similar sequence as the endogenous hormones or neurotransmitters. The most well known are oxytocin for labor induction, somatotropin for growth control, and vasopressin to increase water retention. One possibility to improve pharmacokinetic or pharmacodynamics properties is to modify the natural peptide sequence. Examples of this strategy are the glucagon-like peptide-1 (GLP-1) analog liraglutide and growth hormone analog octreotide for acromegaly (Stevenson, 2009). These peptides mimic the activity and structure of the endogenous ligand but have modified amino acid backbone or include amino acids to decrease their degradation. Many of the peptidergic drugs entering the market at the moment contain modified amino acids or cyclic structures for improved ADME (absorption, distribution, metabolism, and excretion) properties.

Recombinant protein expression, protein purification, and chemical peptide synthesis techniques have significantly boosted the development of peptide drugs. The manufacturing of full-length human peptides overcame economic and immunologic restraints by the extracted animal peptides. Manufacturing of peptide agonists is more feasible and cheaper than manufacturing of antibodies or antagonists, seen in the 21st century in peptidergic drug launches (Table 2).

The major therapeutic area of peptides is metabolic diseases (25%) (Albericio and Kruger, 2012). Insulin was the first peptide drug introduced for use in patients in 1922 (Banting et al., 1922). The first product for therapeutic use was bovine insulin, followed by protamine and zinc insulin (Grunberger, 2013), and the first recombinant human insulin produced reached the market

<table>
<thead>
<tr>
<th>Pros</th>
<th>Cons</th>
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<tr>
<td>Various therapeutic targets</td>
<td>Unstable</td>
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<tr>
<td>Good selectivity</td>
<td>Poor permeability through biologic membranes</td>
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<td>Good potency</td>
<td>Very low oral bioavailability</td>
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<tr>
<td>Well tolerated</td>
<td>Variable solubility</td>
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<tr>
<td>Lower toxicity</td>
<td>High molecular size</td>
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<tr>
<td>Low accumulation</td>
<td>Often need for invasive administration</td>
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<tr>
<td>No toxic metabolites</td>
<td>Possible immunogenicity</td>
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<td>Lower manufacturing costs compared with proteins</td>
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in the 1980s (Keen et al., 1980; Clark et al., 1982). Insulin lispro was the first insulin analog created in 1996, followed by insulin aspart and glulisine in 2000 and 2004, respectively (Grunberger, 2013). Long-acting insulin products glargine and detemir were then launched simultaneously. The first noninvasive insulin administration technique was introduced by the launch of Exubera (Pfizer and Nektar Therapeutics, San Francisco, CA), the inhalable insulin, in 2006, but it was available on the market for less than a year because of low sales (Kling, 2008; Antosova et al., 2009). In addition, the FDA raised a serious concern related to the significant risk of lung cancer in Exubera users with a history of cigarette smoking (http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyRelatedDrugLabelingChanges/ucm122978.htm). In 2015, a new inhalable insulin, Afrezza, was launched in the United States by Sanofi and Mannkind Corporation. Other examples of successful peptidergic drugs are glucagon and GLP-1 agonists, like exenatide, liraglutide, pramlintide, and lixisenatide. Another peptide, related to energy metabolism, is a recombinant form of leptin, metreleptin, and was recently approved for clinical use for generalized lipodystrophy and type 1 diabetes (Chou and Perry, 2013; Sinha, 2014).

As mentioned earlier, peptide drugs are usually delivered via parenteral routes, but there are some exceptions. Cyclosporine, a cyclic decapeptide with poor aqueous solubility belonging to class IV in the biopharmaceutical classification, is one of these exceptions, and formulations with improved bioavailability have been developed using microemulsion technologies (Talegaonkar et al., 2008). In addition to metabolic diseases, peptides are also used as therapeutics in disease in other organ systems as follows.

1. **Osteoporosis.** Osteoporosis is an important area for peptide therapeutics. Previously, salmon calcitonin was available as an intranasal spray, but chronic use of calcitonin was associated with an increased risk of cancer; hence, the formulation was withdrawn in 2012, leaving only injectable preparations for short-term use (http://www.emea.europa.eu/docs/en_GB/document_library/Press_release/2012/07/WC500130122.pdf). In addition, parathyroid hormone is used for treating osteoporotic patients with a high risk of bone fractures. Besides the native hormone, the recombinant human parathyroid hormone analog teriparatide can be used as subcutaneous treatment daily (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Summary_for_the_public/human/000425/WC500027996.pdf).

2. **Gastrointestinal System.** Two peptide-based drugs have been recently approved to treat gastrointestinal diseases. Linaclotide, a 14-amino-acid peptide agonist of the guanylate cyclase 2C has been licensed for treating chronic constipation. The GLP-2 analog teduglutide is licensed for treating short-bowel syndrome and was the

<table>
<thead>
<tr>
<th>Peptide Therapeutics Approved in 2012–2014 in the European Union or United States or Both</th>
<th>Mode of Action</th>
<th>AA</th>
<th>Made of Action</th>
<th>Peptide AA</th>
<th>Mode of Administration</th>
<th>Indication</th>
<th>Formulation</th>
<th>Approval</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucinactant (Sinapultide)</td>
<td>Surfactant</td>
<td>21</td>
<td>Respiratory distress syndrome in premature infants</td>
<td>s.c., i.v.</td>
<td>Inhalation</td>
<td>Peptide AA</td>
<td>2012 U.S. Orphan indication</td>
<td>Peptide mimic of surfactant-associated protein B</td>
<td></td>
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<tr>
<td>Pegtesastide</td>
<td>Somatostatin receptor-agonist</td>
<td>21</td>
<td>Anemia in patients with chronic kidney disease</td>
<td>s.c., i.v.</td>
<td>s.c., i.v.</td>
<td>Multiple myeloma</td>
<td>2012 U.S. Orphan indication</td>
<td>Orphan indication</td>
<td></td>
</tr>
<tr>
<td>Pasireotide</td>
<td>Somatostatin receptor-agonist</td>
<td>6</td>
<td>Cushing disease</td>
<td>s.c., i.v.</td>
<td>s.c., i.v.</td>
<td>Cushing disease, chronic idiopathic constipation</td>
<td>2012 U.S., EU</td>
<td>Orphan indication</td>
<td></td>
</tr>
<tr>
<td>Carfilzomib</td>
<td>Chymotrypsin-like protease inhibitor</td>
<td>14</td>
<td>Multiple myeloma</td>
<td>s.c., i.v.</td>
<td>s.c., i.v.</td>
<td>Multiple myeloma</td>
<td>2012 U.S., EU</td>
<td>Orphan indication</td>
<td></td>
</tr>
<tr>
<td>Linaclotide</td>
<td>Guanylate cyclase-C agonist</td>
<td>14</td>
<td>Constipation-predominant irritable bowel syndrome, chronic idiopathic constipation</td>
<td>Oral</td>
<td>Oral</td>
<td>Constipation-predominant irritable bowel syndrome, chronic idiopathic constipation</td>
<td>2012 EU, U.S.</td>
<td>3 Cys-bridges</td>
<td></td>
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<tr>
<td>Teduglutide</td>
<td>GLP-2 receptor-agonist</td>
<td>33</td>
<td>Short bowel syndrome</td>
<td>s.c.</td>
<td>s.c.</td>
<td>Short bowel syndrome</td>
<td>2012 EU, U.S.</td>
<td>Orphan indication</td>
<td></td>
</tr>
<tr>
<td>Lixisenatide</td>
<td>GLP-1 receptor-agonist</td>
<td>44</td>
<td>Type 2 diabetes</td>
<td>s.c.</td>
<td>s.c.</td>
<td>Type 2 diabetes</td>
<td>2013 EU</td>
<td>Glycine modified GLP-1 fused to human albumin fragment</td>
<td></td>
</tr>
<tr>
<td>Afamelanotide</td>
<td>MC1R agonist</td>
<td>13</td>
<td>EPO protoporphyria (photoprotectant)</td>
<td>s.c. implant</td>
<td>s.c. implant</td>
<td>EPO protoporphyria (photoprotectant)</td>
<td>2014 EU, U.S.</td>
<td>Orphan indication</td>
<td></td>
</tr>
<tr>
<td>Albiglutide</td>
<td>GLP-1 receptor-agonist</td>
<td>30</td>
<td>Type 1 diabetes</td>
<td>s.c.</td>
<td>s.c.</td>
<td>Type 1 diabetes</td>
<td>2014 EU, U.S.</td>
<td>GLP-1R agonist</td>
<td></td>
</tr>
<tr>
<td>Metreleptin</td>
<td>Leptin analog</td>
<td>6</td>
<td>Generalized lipodystrophy</td>
<td>s.c.</td>
<td>s.c.</td>
<td>Generalized lipodystrophy</td>
<td>2014 EU, U.S.</td>
<td>Metreleptin</td>
<td></td>
</tr>
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</table>

AA, number of amino acids; EPO, erythropoietin; GLP-1R, glucagon-like peptide 1; MC1R, melanocortin-1 receptor; MSH, melanocyte-stimulating hormone.
first long-term therapy for treating patients dependent on parenteral nutrition (Burness and McCormack, 2013).

3. Infection: Antimicrobial Peptides. Antibiotic resistance is a growing threat in health care. The endogenously released antimicrobial peptides with effects against bacteria, fungi, protozoa, and viruses are promising pathways to combat nosocomial infections (Anglin et al., 2004; Fox, 2013; Gaspar et al., 2013). Daptomycin was introduced to European and US markets in 2006 and 2003, respectively (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Summary_for_the_public/human/000637/WC500036050.pdf). Gramicidin and bacitracin are antibacterial polypeptides that are used locally, for example, locally in topical and eye infections. Caspofungin is available for treating invasive Candida infection as an intravenous infusion.

4. Cardiovascular System. Several peptide-based drugs have been developed for cardiovascular diseases, including anticoagulants like bivalirudin and epifibatide. Bradykinin B2 receptor competitive antagonist icatibant is a decapeptide used as subcutaneous treatment of hereditary angioedema. There are also several recombinant forms of human endogenous peptides on the market as intravenous preparations, such as atrial natriuretic peptide carperitide and B-type natriuretic peptide nesiritide for different types of heart failure.

5. Endocrine System. Somatostatin and analogs such as depreotide, lanreotide, pentetreotide, and octreotide are used for treating acromegaly and variceal bleeding, as well as for diagnostic purposes. Previously, an erythropoietin analog, peginesatide, was available for treatment of chronic kidney disease patients with anemia, but it was withdrawn in 2013 for safety issues. Vasopressin and modified desmopressin, as well as terlipressin, are available for treating diabetes insipidus and hepatorenal syndromes.

6. Cancer. Anticancer drugs are the second largest group of peptide therapeutics (16%) in clinical use, most for prostate cancer. Among the newest approved oncologic drugs are brentuximab vedotin for Hodgkin lymphoma and romidepsin for cutaneous T-cell lymphoma (Albericio and Kruger, 2012). Leuprolrelin, goserelin, triptorelin, and buserelin have been on the market longer for treating prostate cancer as implantable delivery systems. Another recently approved peptide for prostate cancer treatment is degarelix. Bortezomib has been available since 2004 and 2008 in Europe and the United States, respectively, and carfilzomib was approved in 2012 by the FDA for treating patients with multiple myeloma (http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm094633.htm; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Summary_for_the_public/human/000539/WC500048136.pdf; http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/202714lbl.pdf).

B. Therapeutic Targets of Peptide Drugs

Already more than 400 peptide-based drugs (defined here as <100 amino acids) were reported to be in the pipelines at the beginning of the year 2012 (Kaspar and Reichert, 2013). Pipeline analyses show that the top therapeutic areas are metabolic diseases, oncology, and infectious diseases (Kaspar and Reichert, 2013). Approximately 10% of the peptidergic pipeline is in the area of antimicrobial peptides. The most frequent single-protein target for peptide therapeutics in clinical studies is GLP-1R, which has five drugs launched, two in preregistration, 19 in clinical studies, and an additional 19 in preclinical studies (Citeline, Jan-14; https://citeline.com/products/pharmaprojects/).

Unlike with traditional drug compounds, the pharmacologic effects of peptides often regulate complex endogenous physiologic pathways. The responses may be indirect and involve tolerance, rebound phenomena, and negative feedback controls, which have an increased interest in using physiologically-based pharmacokinetic modeling for peptide therapeutics (Diao and Meibohm, 2013). Many of the current drugs that target membrane proteins, such as G protein–coupled receptors, suffer from poor specificity causing inadequate signaling effects. The natural and synthetic therapeutic peptides are often potent receptor agonists, requiring lower concentrations for receptor activation (Hruby, 2002; Lien and Lowman, 2003). Peptides can also be used as replacement therapy when the endogenous peptide system is deficient (Leader et al., 2008; Bellmann-Sickert and Beck-Sickinger, 2010).

GLP-1 analogs exenatide, liraglutide, and the newest, lixisenatide, are examples of peptide drugs that enhance the effects of existing functional pathways to achieve the desired therapeutic effects. Those peptide drugs produce incretin-like effects, including the induction of glucose-dependent insulin secretion and the inhibition of glucagon release, slowing of gastric emptying, and induction of a sensation of fullness, all of which result in improved glucose balance and weight loss in type 2 diabetic patients (Elkinson and Keating, 2013). They act via G protein–coupled GLP-1 receptors in the pancreatic islet cells, causing protein kinase B activation and insulin secretion (Ahren, 2009). A recently approved peptide drug, teduglutide, is used in short-bowel syndrome by activating the GLP-2 receptor. It increases the release of various mediators, for example, nitric oxide, keratinocyte growth factor, and insulin-like growth factor-1 (Burness and McCormack, 2013). Peptides used for in vitro fertilization, such as human follicle-stimulating hormone or teriparatide for the treatment of osteoporosis, also stimulate the action of existing pathways. Parathyroid hormone (PTH) is the endogenous regulator of calcium and phosphate. Teriparatide is a 34-amino acid fragment of the PTH, consisting of 84 amino acids, that simulates the physiologic action of PTH, resulting in increased intestinal absorption and tubular reabsorption of calcium and
improvement in bone formation (http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021318s015lbl.pdf).

The mechanisms of actions of peptides are not always receptor mediated, for example, lucinactant, which is used as a surfactant in prematurely born infants to prevent respiratory distress syndrome (Table 2). Anticancer peptides have oncolytic, antiproliferative, and proapoptotic functions in tumor cells, which may be membranolytic or nonmembranolytic mechanisms (Gaspar et al., 2013). Many of the anticancer peptides in clinical use bind to the 20S proteasome, inhibiting its activity.

C. Peptide Pharmacokinetics

Pharmacokinetic properties of peptides are setting a major challenge for the clinical use of many peptides. Recently, some excellent reviews focusing solely on pharmacokinetics of peptides (Lin, 2009; Diao and Meibohm, 2013) have been published; hence, only the general aspects of peptide pharmacokinetics are reviewed here.

1. Absorption. Transport of peptides across biologic membranes is severely limited by their large molecular size (>500 Da) and hydrophilicity. The absorption step is avoided only in intravenous administration, but in other administration routes, peptides need to cross several biologic barriers, such as epithelia or the mucosal layer. The structural and metabolic differences in biologic barriers result in substantial differences in peptide absorption.

The subcutaneous administration route is the most commonly used way of delivering peptides (Lin, 2009) and is more convenient for administration than the intravenous route, primarily for safety reasons; however, local blood flow, injection depth and volume, peptide degradation in the subcutaneous tissue, and the molecular weight of the peptide all can affect the absorption of the peptide (Tang et al., 2004; Richter et al., 2012). Although subcutaneous administration is the most common route, many parameters affecting subcutaneous absorption of peptides or proteins are not completely understood.

In sheep, small peptides (<1 kDa) are absorbed into the systemic circulation, whereas a significant portion (20%–40%) of larger peptides and small proteins (5–12 kDa) are absorbed via the lymphatic system (Supersaxo et al., 1990; Charman et al., 2000; Lin, 2009). Lymphatic vessels have a higher permeability than blood capillaries, thus allowing better penetration for larger molecules. In rats, Kagan et al. (2007) found practically no insulin absorption (<0.1%) into the lymphatic system after subcutaneous administration. Therefore, more information is needed to understand the differences between animal models and experimental settings (Lin, 2009; Richter et al., 2012).

In other parenteral but noninvasive administration routes, peptides need to cross the physical barriers presented by epithelia cells, dynamic and steric barriers by mucus, or metabolizing enzymes on or inside epithelia. Three routes are available for peptide absorption through epithelia, similar to other drugs or xenobiotics, namely, 1) through the cells (transcellular), 2) through intercellular junctions (paracellular), or 3) by active transport or receptor-mediated mechanisms (Burton et al., 1996; Boguslavsky et al., 2003). It is typically proposed that most peptides are absorbed via the paracellular route (Patton, 1996; Veuilléz et al., 2001; Lin, 2009; Ozsoy et al., 2009).

The most important factors limiting the permeability of peptides through the epithelia are an increased number of hydrogen bonds, hydrophilicity, and molecular size greater than 700 Da (Burton et al., 1996; Ramaswami et al., 1996; Lin, 2009; Ozsoy et al., 2009; Diao and Meibohm, 2013). The transcellular transport across the lipophilic cell membranes is limited by the peptide's polarity, and paracellular permeation is restricted by their large size (He et al., 1998; Lin, 2009). Paracellular permeation has been estimated to be limited to the molecules with molecular mass =3.5 kDa in intestinal epithelia (Madara and Dharmathaphorn, 1985; Rubas et al., 1996). Absorption enhancers are usually needed for efficient peptide absorption when the size of the peptide exceeds 0.5–1 kDa (Borchardt et al., 1997; Veuilléz et al., 2001; Ozsoy et al., 2009); however, the intranasal route can allow absorption of larger peptides (>2 kDa) without absorption enhancers (Tang et al., 2004), and the pulmonary route via endothelial junctions can allow absorption molecules in size of 4–6 nm (Sayani and Chien, 1996). Besides the size, the shape of the peptide has an effect on its ability to permeate through barriers. The permeability of rigid cyclic peptides through biologic membranes is lower than that of more flexible linear peptides (Boguslavsky et al., 2003; Kwon and Kodadek, 2007).

Epithelia are significant absorption barriers for peptide absorption, but there are significant differences between administration routes. Barrier function is the weakest in lung alveoli, where epithelia are only 0.2 μm thick. The large surface area (100 m²), efficient vascularization of the lungs, and bypassing of the intestinal epithelium and liver metabolism enable good pulmonary bioavailability of peptides (Patton, 1996). In contrast, the top layer of the skin, the stratum corneum (thickness, 10–20 μm) limits the transdermal absorption of polar and large peptides (Benson and Namjoshi, 2008; Praussnitz and Langer, 2008). Peptide permeability can also vary within administration routes, such as in the oral cavity, where permeability is better in sublingual and buccal sites, which are nonkeratinized, compared with the keratinized sites of the oral cavity (Veuilléz et al., 2001).

Another factor limiting peptide absorption is the metabolic barrier caused by degrading enzymes on or inside epithelia. Enzymatic degradation also occurs in subcutaneous tissue, but the mechanisms are not completely understood (Richter et al., 2012). The advantage of
Peptide Delivery Systems

III. Peptide Delivery Systems

When using peptides as medication, it is usually necessary to adjust their pharmacokinetic character, for example, to enhance their absorption or to prolong their time of action. The objective can be achieved by 1) stabilizing the peptide structure, 2) inhibiting the degradation by suitable compounds, 3) improving the absorption with help of aiding substances, or 4) generating controlled peptide delivery systems protecting the peptide (Frokjaer and Otzen, 2005).

Oral delivery is certainly the most desired but challenging route and has been recently reviewed by Renukuntla et al. (2013); thus, it will not be discussed further here. Oral formulations of peptidergic drugs tend to have limited bioavailability. If oral formulations are not feasible, the alternative administration techniques should be as convenient as possible to ensure high patient compliance. Since poor physical chemical and ADME properties are immanent to peptides, effective delivery with controlled release and less invasive techniques are needed.
The use and fabrication of traditional peptide delivery systems have been recently reviewed elsewhere (Oak et al., 2012; Jain et al., 2013; Du and Stenzel, 2014; Mitragotri et al., 2014); therefore, we will give a more general introduction to most commonly used (nano) materials in peptide delivery. There are certain challenges or disadvantages related to the fabrication and stability of the peptides and delivery systems; these are discussed later (Frokjaer and Otzen, 2005; Jain et al., 2013; Mitragotri et al., 2014). Examples of traditional peptide delivery systems are summarized in Table 3.

Various polymer-based delivery systems have been applied for peptides using both synthetic and natural materials, including gelatin, hyaluronic acid, cellulose, chitosan, poly(lactic-co-glycolic) acid (PLGA), polycapro lactones, polyanhydrides, and cyclodextrins (Jain et al., 2013; Du and Stenzel, 2014; Mitragotri et al., 2014; Patel et al., 2014). The fabrication of polymer-based peptide delivery systems can be performed by several techniques, such as phase separation, solvent evaporation, or spraying. The drug is equally distributed in the polymer matrix (Fig. 1). Drug release mechanisms vary according to the polymer type and structure and can be controlled by different external stimuli (Oak et al., 2012).

Microemulsions are surfactant-based drug carriers that are considered “water-in-oil” or “oil-in-water” stable emulsions in which the particle size is usually few nanometers. They have a hydrophilic part outside to achieve solubility and a hydrophobic core that allows dissolution of hydrophobic compounds (Du and Stenzel, 2014). Solid lipid nanoparticles consist of a lipid core for peptide loading and a hydrophilic surfactant layer stabilizing the particle in an aqueous environment. The fabrication of solid lipid nanoparticles is not considered as stressful for the loaded compound in comparison with the most often used polymeric production techniques (Almeida and Souto, 2007); however, the technology may set limitations for hydrophilic peptides (Du and Stenzel, 2014). In contrast to previously described lipid-based delivery systems, which consist of hydrophobic core and hydrophilic shell, liposomes have an opposite structure. Liposomes are vesicles, which have a bilayer phospholipid membrane allowing entrapping hydrophilic peptides inside the protective shell. As in the other delivery systems, liposomes protect the peptide from degradation, prolonging circulation time, and increase bioavailability (Patel et al., 2014). Liposomes have been demonstrated to serve as a carrier for various peptides, such as insulin, vasoactive intestinal polypeptide, and calcitonin (Du and Stenzel, 2014); however, some major issues related to their stability need to be resolved (Patel et al., 2014).

In conclusion, different delivery systems and materials have been investigated for peptides; however, each carrier has its own advantages and disadvantages, and a suitable carrier is often determined by the physicochemical characters of the peptide, the desired route of administration, and clinical aspects of therapy.

### A. Development Challenges of Peptide Delivery Formulations

Developing peptide delivery systems is often less straightforward compared with the formulation of small-molecule compounds, and bioactivity is often jeopardized during the formulation process (Shire, 2009; Ye et al., 2010). The overall stability and the peptide structure may be affected by various factors during the formulation process, e.g., heat, pH, strong solvents, contaminations, shaking, or storing (Wang, 2005; Ye et al., 2010; Jiskoot et al., 2012). As an example, lysozyme lost almost completely its bioactivity in biodegradable microspheres as a result of the fabrication conditions (Ghaderi and Carlfors, 1997). Variable conditions in the preparation of GLP-1 solutions significantly influenced the onset of response after subcutaneous administration absorption rate and bioavailability by formed aggregates (Clodfelter et al., 1998). Furthermore, instability in the aqueous environment can be encountered as glucagon formed cytotoxic fibrillates, when stored for long periods in concentrated solutions, and at high temperature (>37°C) (Onoue et al., 2004).

Challenges are also related to the use of different kinds of particles to improve the peptide delivery. The in vitro release of calcitonin from poly(ethylene glycol)-terephthalate and poly(butylene terephthalate) delivery systems was incomplete as a result of peptide aggregation caused by sodium that was used in the in vitro release test (van Dijkhuizen-Radersma et al., 2002). Three chitosan-based oral delivery systems could demonstrate prolonged release over 4 hours in vitro, but when tested in rats, only one was able to decrease significantly plasma calcium levels in a sustained manner (12 hours) (Guggi et al.,

<table>
<thead>
<tr>
<th>Drug Delivery System (Route)</th>
<th>Peptide</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilamellar liposomes (inhalation)</td>
<td>Vasoactive intestinal peptide</td>
<td>Sustained release and extended pharmacologic effect ex vivo</td>
<td>Hajs et al., 2008</td>
</tr>
<tr>
<td>Poly(lactide-co-glycolide) microspheres (i.m.)</td>
<td>Vapreotide</td>
<td>Erosion-controlled release up to 4 wk in vivo</td>
<td>Blanco-Prieto et al., 2000</td>
</tr>
<tr>
<td>Water in oil microemulsion (intranasal)</td>
<td>Insulin</td>
<td>Relative bioavailability 21.5% to s.c. administration</td>
<td>Sintov et al., 2010</td>
</tr>
<tr>
<td>Solid lipid nanoparticle flocculates (pulmonary)</td>
<td>Insulin</td>
<td>Relative bioavailability 35.6% to s.c. administration</td>
<td>Yang et al., 2012a</td>
</tr>
</tbody>
</table>
2003). PLGA is very commonly used polymer with peptides, but its drawback is the potential of peptide degradation reactions in the delivery system (Houchin and Topp, 2008). It is important to note that many of the earlier developed carrier materials often have a restricted capacity to carry cargo as the commonly investigated microparticles have only approximately 7% peptide/protein content (Ye et al., 2010). In addition, the delivery systems are often accompanied with significant burst release of the peptide (Ye et al., 2010).

B. Parenteral Peptide Delivery Systems In Vivo

1. Peptide Delivery Formulations for Intravenous Delivery. Intravenous injection is a commonly used administration route for peptide therapeutics (Fig. 2; Table 4). The advantages include bypassing of the first-pass metabolism and 100% bioavailability as there is no drug loss at the injection site as well as fast onset of action; however, it is invasive and requires health care professionals for its administration.

To use the therapeutic effects of peptides, the aim is to prolong their circulation time after intravenous administration. In general, after intravenous injection, peptide drugs are widely distributed and rapidly eliminated. Attempts have been made to address the challenge of their short plasma half-life by use of different drug delivery systems. As a common approach, the peptide is conjugated with a carrier, which releases the drug by different mechanisms and thus protects the peptide from rapid degradation. The effects of GLP-1 and its half-life were improved by a delivery system in which protease activity releases albumin conjugated GLP-1 (Li et al., 2010). Another example is polyethylene glycol (PEG) conjugation of salmon calcitonin, which resulted in prolonged half-life but affected its potency negatively (Ryan et al., 2009), suggesting that the improved circulation time may not always improve the therapeutic effects.

2. Peptide Delivery Formulations for Subcutaneous Delivery. Table 5 presents a wide variety of different technologies that have been investigated for subcutaneous peptide delivery. The bioavailability of peptide drugs is variable after subcutaneous injection (Table 5), and neither the route nor how peptides are absorbed from the subcutaneous tissue to bloodstream are entirely understood and may be species dependent (Vugmeyster et al., 2012). As an illustration, two compounds showed low bioavailability after subcutaneous administration: less than 20% for PYY3-36 and 40% for PEGylated form of erythropoietin in rats, whereas the bioavailability of buserelin has been reported to be 70% after subcutaneous delivery to humans (Mönkäre et al., 2012; Wang et al., 2012). The absorption of some biomolecules from subcutaneous injection site is very efficient; as an example, the marketed insulin-like growth factor-1 product mecsacermin (Increlex) achieves almost 100% absolute bioavailability (Vugmeyster et al., 2012). Flip-flop kinetics, where the drug is released from its carrier, regulates the plasma concentration, thereby prolonging the presence of peptides in circulation (Diao and Meibohm, 2013).

3. Peptide Delivery Formulations for Intranasal, Pulmonary, and Transdermal Delivery. Pulmonary, intranasal, and transdermal are other promising routes for noninvasive drug delivery. Each of these routes has their distinct advantages and disadvantages based on their anatomic and physiologic features (Table 6). Recent examples of different peptide delivery systems developed for these routes and studied in vivo are summarized in Table 7.

a. Intranasal. The intranasal route has a highly vascularized subepithelia layer and lower enzymatic activity compared with the oral route, making it a more desired administration route; however, the systemic bioavailability is often limited to 1%, and absorption enhancers can be used to improve the efficiency of intranasal peptide delivery (Illum, 2012). Intranasally-administered peptide formulations that are or have been on the market (calcitonin, buserelin, desmopressin, nafarelin, and oxytocin) do not contain any absorption enhancers, most probably owing to the poor tolerability of the enhancers in the nasal cavity (Ozsoy et al., 2009;
In addition, the intranasal route suffers from large interindividual variability in absorption resulting from frequent pathologic nasal conditions (e.g., hay fever) (Lochhead and Thorne, 2012). Nasal absorption of peptides can be improved by different strategies: 1) the promotion of the absorption process by interacting with membrane, 2) prolonging the residence time of the peptide formulation in the nasal cavity, or 3) inhibiting enzyme activity. Peptide penetration through the nasal mucosa is the key factor for the nasal absorption of peptides, and it can be improved by using mucoadhesion strategies. The residence time of the formulation on mucosa is important because the half-life of the clearance for nonadhesive formulations is only 15–20 minutes. Many penetration enhancers have been studied clinically for peptide delivery, for example, cyclopenta delactone (insulin) (Leary et al., 2006), hydroxyl fatty acid esters of polyethylene glycol (CriticalSorb, insulin, teriparatide) (Lewis et al., 2009), alkylsaccharides (cyclic PTH1-31) (Illum, 2012), and chitosan (Chisys, goserelin) (Illum, 2012). However, the development of many of these enhancers has been terminated or data have not been published (Illum, 2012). CPEX Pharmaceuticals (Exeter, NH) developed Nasulin spray for intranasal insulin administration using cyclopenta delactone to enhance its absorption (Leary et al., 2006). The relative bioavailability to subcutaneous insulin in type 1 diabetes patients was 17%–20% in the first 2 hours (Leary et al., 2005, 2006), but further development was halted in 2010.

Because penetration enhancers can be toxic, microemulsions have been studied to improve intranasal absorption of insulin because they have been suggested to be better tolerated (Sintov et al., 2010). This approach was successfully applied for delivery of insulin in rabbits. The relative bioavailability of insulin was 21.5% using microemulsion resulting from enhanced intramucosal transport (Sintov et al., 2010). Another

<table>
<thead>
<tr>
<th>Technique</th>
<th>Peptide Drug</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide drug is linked by a protease-sensitive compound to albumin binding peptide</td>
<td>Glucagon-like peptide 1 (GLP-1, 3.4 kDa)</td>
<td>Sustained the pharmacologic effect and prolonged half-life of elimination</td>
<td>Li et al., 2010</td>
</tr>
<tr>
<td>Peptide is conjugated using a poly(PEG) methyl ether methacrylate with a comb shape</td>
<td>Salmon calcitonin (3.5 kDa)</td>
<td>Significantly prolonged half-life of elimination (even 15×) but a decrease in potency</td>
<td>Ryan et al., 2009</td>
</tr>
<tr>
<td>Adsorption of peptide into porous silicon nanocarriers</td>
<td>Human peptide YY3-36</td>
<td>Successful delivery of an active peptide but no improvement in circulation time or pharmacokinetic parameters compared with peptide solution</td>
<td>Kovalainen et al., 2013</td>
</tr>
<tr>
<td>Immobilization of cysteine including peptide thiolated carboxymethyl dextran-cysteine conjugate</td>
<td>DALCE</td>
<td>Five-fold improvement in elimination half-life and 6.7-fold decreased plasma clearance rate</td>
<td>Shahnaz et al., 2012b</td>
</tr>
</tbody>
</table>

**TABLE 4**

Differential in vivo investigated peptide delivery systems for subcutaneous delivery

<table>
<thead>
<tr>
<th>Technique</th>
<th>Peptide Drug</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPEG-PLGA-mPEG-based thermosensitive in situ forming gel</td>
<td>Salmon calcitonin (mol. mass 3.5 kDa)</td>
<td>Controlled delivery and pharmacologic activity for 20–40 d</td>
<td>Tang and Singh, 2010</td>
</tr>
<tr>
<td>Noncovalent Zn-peptide adduct (spray dried)</td>
<td>BMS-686117 (mol. mass 1.5 kDa)</td>
<td>Terminal half-life prolonged by 6 h and $C_{\text{max}}$ values reduced 6- to 8-fold compared with solution</td>
<td>Qian et al., 2009</td>
</tr>
<tr>
<td>Thermoresponsive poly lactic-acid-polyethylene glycol-poly lactic acid triblock copolymer</td>
<td>Insulin</td>
<td>Controlled release of bioactive molecule up to 3 mo</td>
<td>Al-Tahami et al., 2011</td>
</tr>
<tr>
<td>Aminocid modification</td>
<td>Insulin lispro</td>
<td>55–77% absolute bioavailability</td>
<td>Vugmeyster et al., 2012</td>
</tr>
<tr>
<td>Solution</td>
<td>Liraglutide</td>
<td>55% absolute bioavailability</td>
<td>Stevenson, 2009; Perry, 2011</td>
</tr>
<tr>
<td>PLGA microspheres</td>
<td>LXT-101 (LHRH antagonist)</td>
<td>Sustained-delivery system, efficacy</td>
<td>Du et al., 2006</td>
</tr>
<tr>
<td>Porous silicon microparticles and nanoparticles</td>
<td>PYY3-36</td>
<td>Controlled-delivery system, increased bioavailability</td>
<td>Kovalainen et al., 2012, 2013</td>
</tr>
</tbody>
</table>

DALCE, [D-Ala2, Leu5, Cys6]-enkephalin; LHRH, luteinizing hormone–releasing hormone.
recent alternative to improve nasal absorption are unsaturated glycoceramides, which use endogenous sphingolipid trafficking (te Welscher et al., 2014). Absorption of GLP-1 linked to glycosphingolipid GM1 was enhanced, demonstrating therapeutic potential; yet there are challenges in the efficient cleavage of the linkage between the peptide and glycosphingolipid after absorption.

Thiolated polymers have been used as potential excipients for the nasal delivery since they are safe, improve residence time on the nasal mucosa, and act as penetration enhancers and enzyme inhibitors (Vetter and Bernkop-Schnurch, 2010; Vetter et al., 2010; Palmberger et al., 2011). In rats, leuprolide-loaded thiolated chitosan nanoparticles increased the area under the curve (AUC), $C_{max}$, and elimination half-life after nasal application by 6.9-, 3.8-, and 4-fold, respectively, compared with the solution administration (Shahnaz et al., 2012a). Relative bioavailability was reported to be 19.6%. In the case of nonthiolated chitosan nanoparticles, such an improved delivery in comparison with the leuprolide solution was not seen. Earlier, thiolated chitosan microparticles have been also shown to improve intranasal delivery of insulin (Krauland et al., 2006).

Intranasal administration can offer enhanced central nervous system absorption via the olfactory region. This has been shown for insulin (Renner et al., 2012),

<p>| TABLE 6 | Key characteristics of different nonparenteral peptide administration routes |</p>
<table>
<thead>
<tr>
<th>Nonparenteral Administration Routes</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Efficient systemic delivery</td>
<td>Self-administration is not possible</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Relatively efficient systemic delivery. Clinical accepted</td>
<td>Mechanisms affecting absorption are not well known</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>First-pass metabolism avoided</td>
<td>Injections are needed</td>
</tr>
<tr>
<td>Intranasal</td>
<td>High surface area and vascularization, rapid absorption</td>
<td>Failure of inhaled insulin</td>
</tr>
<tr>
<td></td>
<td>First-pass metabolism avoided</td>
<td>Ensuring efficient inhalation</td>
</tr>
<tr>
<td></td>
<td>Intranasal peptide drugs on market.</td>
<td>Variability of absorption (from, e.g., hay fever or common cold)</td>
</tr>
<tr>
<td>Transdermal</td>
<td>Easy to access for patient</td>
<td>Stratum corneum creates a strong absorption barrier</td>
</tr>
</tbody>
</table>

<p>| TABLE 7 | Examples of in vivo studies of peptide delivery systems for intranasal, pulmonary, and transdermal administration |</p>
<table>
<thead>
<tr>
<th>Technology</th>
<th>Peptide (mol. mass)</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intranasal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without microemulsion to enhance absorption without using irritating excipients</td>
<td>Insulin (5.8 kDa)</td>
<td>Relatively bioavailability 21.5% to s.c. injection. Microemulsion-accelerated intramucosal transport</td>
<td>Sintov et al., 2010</td>
</tr>
<tr>
<td>Thiolated chitosan nanoparticles for improved mucoadhesion, enzyme inhibition, and penetration enhancement</td>
<td>Leuprolin (1.2 kDa)</td>
<td>Relative bioavailability 19.6%, AUC, $C_{max}$, and elimination half-life improved by 6.9-, 3.8-, and 4-fold, respectively</td>
<td>Shahnaz et al., 2012a</td>
</tr>
<tr>
<td>Cosolvent formulation with n-tridecyl-$\beta$-maltoside as permeation enhancer in brain delivery</td>
<td>Hexarelin (0.9 kDa)</td>
<td>1.6-fold higher peptide concentration in brains compared with i.v. administration despite lower plasma concentrations after intranasal administration</td>
<td>Yu and Kim 2009</td>
</tr>
<tr>
<td>Pulmonary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP-$\beta$-cyclodextrin and linear or branched PEG chains spray dried to microparticles</td>
<td>Salmon calcitonin (3.5 kDa)</td>
<td>1.5- and 2.3-fold increase of bioavailability with branched and linear PEG, respectively, compared with nebulized solution</td>
<td>Tewes et al., 2011</td>
</tr>
<tr>
<td>Palmityl-acylation of peptide to increase adsorption on porous PLGA microparticles and albumin binding in blood circulation</td>
<td>Exendin-4 (4.2 kDa)</td>
<td>Native and palmityl-acylated peptide administered in microparticles induced hypoglycemia for 36 h and over 5 days, respectively</td>
<td>Kim et al., 2011</td>
</tr>
<tr>
<td>Micellar formulation for improved peptide stability and transepithelial absorption</td>
<td>Salmon calcitonin (3.5 kDa)</td>
<td>1.6-fold increase in bioavailability after administration of micelles than in a plain solution</td>
<td>Baginski et al., 2012</td>
</tr>
<tr>
<td>Transdermal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two-layered dissolving microneedles prepared from water-soluble biopolymers</td>
<td>Desmopressin (1.1 kDa)</td>
<td>Absolute bioavailability $\geq$90% and maximum plasma concentration reached in 30 min</td>
<td>Fukushima et al., 2011</td>
</tr>
<tr>
<td>Insulin encapsulated nanovesicles delivered with iontophoresis through microneedle-induced microchannels</td>
<td>Insulin (5.8 kDa)</td>
<td>Comparable hypoglycemic effect on s.c. insulin by using 80-fold higher dose</td>
<td>Chen et al., 2009</td>
</tr>
<tr>
<td>Removal surface layers of skin with microdermabrasion technique</td>
<td>Insulin (5.8 kDa)</td>
<td>Hypoglycemic effect similar to 160-fold lower s.c. insulin dose after removal of epidermis. No hypoglycemic effect after removal of stratum corneum</td>
<td>Andrews et al., 2011</td>
</tr>
</tbody>
</table>
orexin-A (Dhuria et al., 2009), and exendin (Banks et al., 2004). Hexarelin, a growth hormone–releasing peptide, was delivered to the brain using a cosolvent and n-tridecyl-β-D-maltoside for improving the peptide permeation and enabling 1.6-fold greater plasma concentrations compared with intravenous administration (Yu and Kim, 2009).

b. Pulmonary. Lungs are an attractive administration route for peptides because of their high surface area (100–140 m²), high vascularization, and lower enzymatic activity (Patton, 1996; Dombu and Betbeder, 2013). Alveoli also have a thin epithelium layer (0.1–0.5 μm) and no additional mucosal layer, which can allow fast absorption and high bioavailability (10- to 200-fold greater than in other noninvasive administration routes) of pulmonary administered peptides (Patton and Byron, 2007). Epithelia lining fluid, epithelia cell layer, and endothelia membrane are still forming significant absorption barriers, however.

Peptides can be administered either as liquid (nebulizer) or as a dry powder, but in both cases, inhalator devices are needed for efficient pulmonary peptide delivery. For efficient pulmonary drug delivery, the formulations should have optimal aerodynamic characteristics to reach the alveoli, enhance the permeability, prolong the retention time, and control the release of the peptide and prevent its degradation (Wan et al., 2012). The optimal particle size (mass median aerodynamic diameter) for alveoli deposition would be 1 to 5 μm, whereas smaller particles (<1 μm) are exhaled, and large particles (>10 μm) are deposited in the upper airways (Carvalho et al., 2011). Small nanoparticles (<100 nm) might be also deposited in the alveoli (Möller et al., 2008; Yang et al., 2008; Geiser and Kreyling 2010).

Improved pulmonary bioavailability was seen in salmon calcitonin microparticles formed by spray drying HP-β-cyclodextrin with either linear or branched PEG chains (Tewes et al., 2011). Microparticles with linear PEG had lower surface energy and better aerodynamic properties, whereas branched PEG salmon calcitonin was more protected from the chemical degradation. The pulmonary bioavailability of salmon calcitonin microparticles with branched-PEG was 1.5-fold, and microparticles with linear-PEG 2.3-fold higher than that of nebulized salmon calcitonin solution. Absolute bioavailabilities were 26.3% and 17.0% for linear and branched-PEG, respectively. Another proposed approach for pulmonary delivery of salmon calcitonin was the adsorption of the peptide on the surface of PLGA nanospheres (Yang et al., 2012b). Lyophilized nanospheres were composed on inhalable lactose to have micron-sized particles to improve inhalation properties. Intratracheal administration in rats demonstrated that micron-sized particles and nanospheres had similar pharmacologic effects. The third approach for pulmonary delivery of salmon calcitonin is micellar formulations that improve the peptide stability against enzymatic degradation and increase transepithelial absorption (Baginski et al., 2012). In vivo studies showed 1.6-fold greater bioavailability after the administration of salmon calcitonin in micelles than in plain solution. In 2014, the FDA approved another inhaled insulin formulation: MannKind’s Afrezza (Valencia, CA), an inhalable short-acting insulin powder (http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm403122.htm). Another commercially investigated option for inhalable insulin is based on nebulization (Heinemann 2014; http://dancebiopharm.com/dance-501/).

Inhaled delivery can be also used for local delivery of peptides needed for treatment of pulmonary diseases. At the moment, aviptadil, vasoactive intestinal polypeptide, is in phase 2 clinical studies for treatment of respiratory distress syndrome and pulmonary hypertension.

c. Transdermal. The easy accessibility of the skin makes it a desired route for peptide administration, but the skin barrier needs to be overcome by various chemical and physical means (Kalluri and Banga 2011; Alexander et al., 2012; Schoellhammer et al., 2014). The recent approaches have focused mostly on the iontophoresis and microneedle techniques or a combination of the two.

Microneedles are micron-sized, needle-shaped structures that are long enough to penetrate the stratum corneum but short enough not to evoke pain sensation (van der Maaden et al., 2012). Microneedles offer four different approaches, all of which have been applied to peptides: 1) pretreatment with microneedles followed by application of the formulation (Zhou et al., 2010; Mohammed et al., 2014); 2) dissolving microneedles that release the peptide rapidly and simultaneously to the microneedle dissolution (Fukushima et al., 2011; Ling and Chen 2013); 3) coated microneedles releasing their peptide coating (Cormier et al., 2004; Tas et al., 2012); and 4) hollow microneedles enabling microinjections (Davis et al., 2005; Wang et al., 2006; Norman et al., 2013). When commercially available microneedle rollers were used as pretreatment to create micro pores into rat skin followed by the application of insulin solution, the microneedles rollers could increase skin permeability for insulin-lowering blood glucose levels (Zhou et al., 2010).

Tas et al. (2012) used coated steel microneedles with salmon calcitonin together with trehalose (for improved protein stability) and compared the microneedle administration with subcutaneous injection in rats. The salmon calcitonin AUC values of microneedle administration and subcutaneous injection were not significantly different (Tas et al., 2012). Compared with intranasal administration, the AUC after microneedle administration was 13-fold higher than after intranasal administration. Two clinical studies have been performed with parathyroid hormone (1–34)-coated titanium microneedles, and those studies showed that transdermal microneedle administration was able to deliver consistent and therapeutically relevant concentrations (Daddona et al., 2011). The phase 2 study even suggested that microneedles could provide
a better plasma profile than subcutaneous injection because of faster absorption. In accordance with these findings, Prausnitz and colleagues have shown that microinjections of insulin with hollow microneedles into the depth of 0.9–1 mm induced a faster onset of insulin action than subcutaneous injections (Gupta et al., 2009, 2011; Norman et al., 2013). This result suggests that the microneedle injection of insulin is delivered to the papillary dermal region, which has rich capillary and lymphatic networks, allowing faster absorption than after subcutaneous injection.

Dissolving microneedles can be prepared from various polymers, and they are usually designed to dissolve in the skin within minutes to an hour after their application. In the case of insulin-loaded microneedles, no significant differences in the blood glucose levels of rats were found between microneedle and subcutaneous administrations (Ito et al., 2012; Liu et al., 2012). Hyaluronic acid–based microneedles had a relative physiologic activity (RPA) of 94%–98% (Liu et al., 2012), whereas chondroitin sulfate microneedles had a RPA of 91%–100% compared with subcutaneous insulin solution (Ito et al., 2012). In dogs, for similar types of chondroitin sulfate microneedles, the RPA value was 59%–71% of subcutaneous insulin solution (Fukushima et al., 2010). Dissolving microneedles also have been studied for delivery of other peptides such as desmopressin with fast absorption (absorption half-life 14 minutes) and high bioavailability (90%–93%) (Fukushima et al., 2011). Peptides were chemically stable for at least 1 month of storage at +4°C (Fukushima et al., 2010, 2011) or at room temperature (Ito et al., 2012; Liu et al., 2012).

Iontophoresis uses small currents to drive charged molecules through the skin and has been studied for peptide delivery (Herwadkar and Banga, 2012). Peptides for iontophoteric delivery must be charged and ideally have their isoelectric point either below 4 or above 7.4. Chen et al. (2009) circumvented this requirement by encapsulating insulin (isoelectric point 5.4) into charged nanovesicles that masked the charge of insulin. According to the authors, nanovesicles themselves were able to penetrate through the skin, but iontophoresis increased penetration by 3.3- to 5.3-fold. The combination of iontophoresis with micropores created by microneedles increased penetration rate by 93- to 145-fold. The synergistic effects of iontophoresis and microneedle-created micropores are based on the ability of iontophoresis to increase transport and micropores to decrease the skin barrier properties. Similarly, the combination of microneedles and iontophoresis increased maximum plasma concentration of salmon calcitonin 9-fold in comparison with iontophoresis alone, and therapeutic levels were achieved within 5 minutes (Vemulapalli et al., 2012).

Other techniques for transdermal peptide delivery in vivo include cell-penetrating peptides (Chang et al., 2013), microdermabrasion (Andrews et al., 2011), and electroporation (Wong et al., 2011). Chang et al. (2013) synthesized and screened 20 cationic cyclopeptides based on highly hydrophilic cyclic peptides (named TD-1; ACSSSPKHC). Cyclopeptide, a cell-penetrating peptide with bisubstituted lysine, was the most effective in lowering blood glucose levels and loosening of epidermal tight junctions. In microdermabrasion, the top skin layers, namely, the stratum corneum and epidermis, can be locally removed to enhance the delivery of peptides. In the case of insulin, removal of both stratum corneum and epidermis had similar glucose-lowering effects than subcutaneous injection (Andrews et al., 2011). The removal of only the stratum corneum had no effect on insulin absorption, and it was concluded that not only stratum corneum but also epidermis form an absorption barrier for insulin. Electroporation creates temporary structural perturbation of lipid membrane bilayers by using very short high-voltage pulses (Alexander et al., 2012). Electroporation of insulin, particularly when combined with local hyperthermia, has been shown to lower blood glucose levels, although no subcutaneous insulin injection was used as a control (Wong et al., 2011).

IV. Porous Silicon as a Novel Material for Peptide Delivery

The interest in investigating porous silicon (PSi) as a drug carrier has arisen from promising features of this biocompatible material, which include the ability to 1) carry large drug amounts; 2) enhance the dissolution of drugs with low solubility; and, most important, and 3) have its properties modified (Anglin et al., 2008; Salonen et al., 2008; Jaganathan and Godin, 2012). PSi differs from many of the commonly used drug carriers since the loading of peptides is much easier compared with, for example, typically used polymer materials, and there is no need to use conditions that can be harmful for peptides such as strong solvents or high temperatures. Our research group demonstrated the promising features of PSi for sustained delivery of peptides (Kilpeläinen et al., 2009; Kovalainen et al., 2012; Huotari et al., 2013). In the following sections, we give an overview on the PSi material and its use in peptide delivery where the subcutaneous route has shown to be the preferable administration route.

A. Fabrication and Properties of Porous Silicon

PSi consists of pore walls of elemental silicon with highly tunable morphologies (Fig. 3). PSi has been known for almost six decades, and during this time, the perception of the material has evolved from an unwanted side product to a scientific curiosity, a potential material in electronics, and, more recently, in drug delivery systems. This fascinating material combines the properties of nanomaterials, semiconductors, and
biomaterials, and it has therefore many beneficial qualities to be exploited in various applications.

The oldest and still most commonly used method to produce PSi is electrochemical anodization. Already in 1956, Uhlir (1956) had observed that the surface of silicon wafer changed its color if it was anodized with an appropriate current density in an electrolyte solution containing hydrofluoric acid. In anodization, silicon serves as an anode electrode, and the cathode is usually made of platinum. When a certain etching current is conducted through the electrodes, a porous layer is formed on the surface of the silicon substrate. In the film, the pores typically pass perpendicularly through the film, which can be detached from the substrate and processed for further use. Several different types of pore structures can be produced by electrochemical etching: from randomly orientated, spongelike pore structures to highly ordered cylindrical pores with smooth pore walls and pore sizes from a few-nanometers-wide micropores to micrometer-scale macropores (Heinrich et al., 1992; Salonen et al., 2005; Bimbo et al., 2010). The control over particle size is especially important when considering different administration routes.

Crystalline silicon has long been considered as bioinert material, until, in 1995, PSi was found to be bioactive (Canham, 1995), with its biologic behavior dependent on its porosity (Bowditch et al., 1999; Canham et al., 2000; Anderson et al., 2003). PSi dissolves to orthosilicic acid, a natural and biologically important form of silicon, for example, for optimal bone and collagen growth (Anderson et al., 2003; Salonen et al., 2008). Another beneficial property of PSi is the possibility to make it photoluminescent (Canham, 1990). In biomedical applications, the particles can be tracked by fluorescent microscopy without additional fluorescent labels that can cause undesired changes in the surface chemistry (Gu et al., 2013).

### B. Porous Silicon as a Peptide Delivery System

Controlling surface chemistry of PSi is highly important in the drug delivery application. Surface chemistry has three basic functions for the material: 1) it determines the rate of degradation, together with porosity; 2) it controls interactions between PSi and the loaded molecules; and 3) it determines the interactions of PSi with biologic systems.

PSi surface is typically modified in two steps. First, the native unstable hydrogen terminated surface of PSi is replaced with a passivation layer to improve its stability in biologic environments. Second, the stabilized surface is functionalized with molecules that typically have functional carboxyl or amine groups. These functional groups determine the particle surface charge, which may be further modified, for example, with biologically active molecules.

The most frequently used method of stabilizing PSi is oxidation (Riikonen et al., 2012). This can be performed in gas phase at elevated temperatures (250–1000°C) or in liquid phase with oxidants such as hydrogen peroxide. The formed silicon oxide surface is hydrophilic with an intermediate stability and has –OH groups on the surface for further functionalization.

Carbonization is an effective stabilization method that creates surfaces more stable than the oxide surfaces. It can be performed by thermal decomposition of carbon-containing molecules such as acetylene or poly(furfuryl) alcohol on hydrogen terminated PSi surface (Salonen et al., 2002, 2004; Tsang et al., 2012). At relatively low temperatures (i.e., around 500°C), acetylene gas can partially decompose and form a hydrocarbon terminated surface on PSi. This thermal hydrocarbonization process produces relatively stable surfaces with hydrophobic characteristics. Even more stable surfaces can be formed by thermal carbonization at higher temperatures (i.e., around 800°C), at which the carbon atoms can enter the crystal structure of PSi and form a silicon carbide surface. These surfaces are more hydrophilic and highly resistant to various chemical environments.

Hydrogen terminated PSi surfaces can be directly functionalized by hydrosilylation (Buriak, 2002). In this method, a molecule is grafted on the PSi surface by a reaction between terminal carbon-carbon double bond of the grafted molecule and a hydride groups on the PSi surface. Various alkene molecules can be grafted on PSi surface by this method. It also stabilizes the surface to some extent compared with unmodified PSi surface, especially if the grafted molecules are highly hydrophobic (Buriak and Allen, 1998). A similar method has recently been used to graft undecylenic acid on thermally hydrocarbonized PSi (Jalkanen et al., 2012).

Oxidized or carbonized surfaces can be functionalized by silanization, in which alkoxysilanes react with –OH groups on the stabilized surface (Mäkilä et al., 2012; Xu et al., 2012). This is the most commonly used functionalization method of silica-based materials, and it is most often used to attach amine terminated molecules on the surface. These surfaces have a positive charge in physiologically relevant solutions and may need to be further modified for biomedical use. Because of the positive charge and reactivity of amine groups, these amine-terminated
surface modifications are the only surface chemistry that has shown some cytotoxic effects during in vitro studies. All the other stabilized surface chemistries have been found to be nontoxic at the clinically relevant concentrations (Lehto et al., 2013).

To provide more advanced biologic properties, PSi needs to be further functionalized. Targeting peptides can be grafted on the surface to enhance accumulation of PSi nanoparticles into target tissue (Kinnari et al., 2013; Yokoi et al., 2013). The pores can also be capped with proteins or cyclodextrin, which can reduce the release of the loaded molecules at certain pH (Perelman et al., 2008; Xue et al., 2011). Furthermore, PSi particles can be encapsulated by, for example, solid lipids to improve their behavior in vivo (Liu et al., 2013b). In a recent study, PSi particles were covered with cellular membranes of leukocytes, giving the particles advanced cell-like functions (Parodi et al., 2013).

When considering peptide loading in a drug carrier, it is important that the loading method be as simple and as gentle as possible to preserve the bioactivity of the peptides. This is a clear advantage of PSi compared with many other drug delivery systems in which the payload has to be added into the fabrication process of the delivery system. In the case of PSi, it is possible to load the drug afterward, at room temperature, using mild solvents.

The drug-loading methods of PSi can be divided into two categories. In immersion loading, PSi is immersed in a loading solution, the volume of which is clearly greater than the pore volume of the PSi. In many cases, this requires excess of drug to obtain high payload to carrier mass ratio in the system. Usually, the method also requires removal of PSi from the loading solution. This can be done by filtration; or, in the case of nanoparticles, centrifugation is preferred. The other category of loading methods is so-called impregnation (incipient wetness method). In this type of method, the amount of drug solution corresponding to the pore volume of PSi is added to the sample and allowed to infuse into the pores by capillary action. The advantage of this method is the high loading efficiency, minimizing the wasted drug. On the other hand, because of the low volume of loading solution, the payload (loading degree) obtained can be rather low. Crystallization of the drug on the external surface of PSi particles (outside the pores) can occur; however, both methods are performed at room temperature, and virtually any solvent suitable for the peptide can be used.

The loading is simple to perform, but the interactions involved in the loading are more difficult to control. In most cases, chemical reactions between the drug molecules and the pore wall are undesired and can be avoided with the proper choice of the surface chemistry of PSi. These interactions (e.g., drug-solvent, drug-pore wall, and solvent-pore) affect the loading degree. Determination of the drug-loading degree is determined by liquid extraction of the drug and measurement is by high-performance liquid chromatography; the amount of drug in PSi can be determined using thermogravimeter (Lehto et al., 2005) (Fig. 4).

Kovalainen et al. (2012) studied the loading efficiency of PYY3-36 in PSi with three different surface chemistries (Fig. 5). Interestingly, loading efficiency (i.e., what percentage of the drug from the solution is loaded into the pores) close to 100% was observed in the case of thermally hydrocarbonized (hydrophobic) PSi with greater than 7 w-% loading degree. Similar behavior was observed with splice correction oligonucleotides where greater than 14 w-% loading degree was obtained with positively charged PSi nanoparticles, the loading efficiency being 100% (Rytkönen et al., 2014). This highlights the ability of PSi to adsorb all the drug molecules from the solution in suitable conditions. The surface chemistry plays an important role in the release and bioavailability of subcutaneously administrated peptide (Kovalainen et al., 2012).

The first study in which PSi silicon has been used in vitro with peptides or proteins was reported by Foraker et al. (2003). In their article, oxidized PSi was loaded with insulin, and the permeability of insulin through Caco-2 cell membrane was observed to increase due to the PSi microparticles. Prestidge and coworkers have systematically studied interactions between peptides/proteins and PSi (Prestidge et al., 2008; Jarvis et al., 2010, 2012), and how various parameters like surface modifications, together with pore diameters, affect the loading degree. They have also shown that surface affects how peptides are adsorbed on PSi surfaces. The first demonstration of using PSi for peptide delivery in vivo showed that PSi microparticles prolong the pharmacodynamics response of a food intake regulating peptides in mice (Kilpeläinen et al., 2009). Later investigation of

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Fig. 4. Results of thermogravimetric measurements of peptide ghrelin antagonist (GhA) and loaded into PSi microparticles. The results show near complete decomposition of GhA as such. The GhA-loaded PSi microparticles show a mass loss of 18.5%, which can also be assumed as the loading degree of the particles.
peptide pharmacokinetics proved that controlled release over several days can be achieved (Kovalainen et al., 2012).

In addition, PSi microparticles were used to carry antigenic peptides into human monocyte–derived dendritic cells to enhance the generation of antiviral cytotoxic T-lymphocyte response (Jimenez-Perianez et al., 2013). PSi appears to be a potential peptide delivery system as a result of its several advantageous properties, like biodegradability, nontoxicity, high peptide-loading capacity, and easy physicochemical modification of the constituent. PSi has also been shown to be capable to carry different molecules simultaneously (Liu et al., 2013a). Loading capacity determining factors from previous studies are summarized in Table 8.

Biodegradation of PSi has not been evaluated systematically. PEGylation of the chemically oxidized PSi particles was recognized to prolong the degradation time of PSi by 3 days (Godin et al., 2010). Biodegradation in vivo has been evaluated for oxidized PSi particles when delivered intravenously, but a corresponding study has not been conducted for subcutaneously injected particles (Park et al., 2009). It is known that the injected particles do not migrate from the deposit site for 4 hours (Bimbo et al., 2010), but after 4 weeks, thermally hydrocarbonized microparticles have mostly dissolved from the injection site. As the peptide release can be modulated by changing the biodegradation rate of the carrier, and as the in vivo–in vitro correlation is known to be poor in this regard, knowledge about the real degradation rates after subcutaneous delivery would be critical for the development of PSi formulation for sustained peptide delivery.

V. Immunogenicity and Adverse Effects of Parenteral Peptide Delivery Systems

A. Immunogenicity

Risk of immunogenic reactions exists when biologic compounds are used as drugs, and the severity of the reaction may vary from negligible events to serious anaphylactic shock (Vugmeyster et al., 2012). Particularly, compounds that are originated from other species than humans tend to stimulate formation of antibodies. As an example, the original insulin formulations from bovine and porcine pancreas induced more allergic reactions compared with insulins with human amino acid sequence. Formation of antidrug antibodies may alter the ADME and pharmacodynamics, which may result in enhanced clearance or altered biologic activity of the drug (Vugmeyster et al., 2012).

B. Adverse Effects

The use of particulate delivery systems in medicine results in direct exposures of body cells and tissues to foreign materials. Physicochemical properties (size, shape, surface chemistry, and charge), along with the site of the particle's administration, define their protein interactions with the body. The surface area is an essential parameter to obtaining high loading capacity/degree. The ratio of the pore diameter to the peptide molecule size needs to be sufficiently high to achieve effective penetration of the molecules in the pores. Hydrophobic surfaces can facilitate peptide loading in aqueous solutions but might be hazardous to peptide stability if the surface of PSi is not passivated before the peptide loading. Electrostatic attraction is an effective way to promote loading of the peptides in PSi carriers. The charges of the peptide and PSi particles depend on the properties of the medium (e.g., solvent, pH, and excipients), which need to be considered in planning the loading procedure.

### Table 8

Factors determining the drug-loading capacity of PSi

<table>
<thead>
<tr>
<th>Surface area</th>
<th>Essential parameter to obtaining high loading capacity/degree.</th>
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<tbody>
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<td>Ratio of the pore diameter to the peptide molecule size</td>
<td>Needs to be sufficiently high to achieve effective penetration of the molecules in the pores.</td>
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<tr>
<td>Electrostatic attraction</td>
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</tr>
<tr>
<td>Charges of the peptide and PSi particles</td>
<td>Depend on the properties of the medium (e.g., solvent, pH, and excipients), which need to be considered in planning the loading procedure.</td>
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</table>
and cellular interactions (Nel et al., 2009; Arora et al., 2012; Treuel et al., 2013). Changing the PSI particle surface characteristics offers unique advantages to design a carrier that can efficiently deliver therapeutic molecules to diseased tissue (Limmell et al., 2007; Jarvis et al., 2011). Yet changing of the particle’s very same properties can result in their unexpected bio-interactions and adverse effects. Understanding whether the drug carriers themselves may induce adverse effects is important for engineering safe-in-use delivery systems.

Size is an important property for the development of parenteral and especially for intravenous delivery systems. The recommended size of the particles for intravenous administration should not exceed the diameter of the fine capillaries (9 \( \mu m \)), preferentially staying in submicron range to avoid their possible blockage, which could lead to a fat embolism and result in death (Mehnert and Mader, 2001). This cutoff is the most critical for the particles with limited deformability or particles tending to aggregate, such as lipid-based drug carriers (Elgart et al., 2012).

Reduction of the particle size to nanoscale generates extremely high surface area to volume ratio, which determines an increase in the potential number of the reactive groups on the particles surface and creates the opportunity for their increased uptake, intense interaction with and translocation through the biologic tissues. Therefore, a smaller range size can become a key factor of nanoparticle’s safety. Attachment of nanoparticles to the surface of the red blood cells (RBCs) has been suggested as a tool for extending circulation time and sustained release of therapeutics (Hall et al., 2007). It has recently been found that this surface modification of nanoparticles can size-dependently decrease membrane deformability of RBCs, which is critical for effective blood flow (Zhao et al., 2011). This study revealed that among several surface types and sizes of PSI nanoparticles, only small MCM-41-type nanoparticles in a range of 100–200 nm can adsorb to the RBC surface without affecting their membrane or morphology; however, the interactions of these nanoparticles with other blood cells should be evaluated before the final conclusion about their hemocompatibility is made (Semberova et al., 2009).

Design of charged nanoparticles is gaining popularity in drug delivery applications (Malik et al., 2000; Elgart et al., 2012; Lin et al., 2013). Modulation of the surface charge is used to stabilize colloidal systems of nanoparticles (Elgart et al., 2012), to prolong the circulation time (Malik et al., 2000), and to increase cellular uptake (Lin et al., 2013). At the same time, the charge can be the critical factor for electrically excitable cardiac tissue. Recently, it has been shown that polystyrene latex nanoparticles, which were amine-modified and have a positive charge, induced large-scale damage to cardiomyocytes, leading to cell death (Miragoli et al., 2013). By contrast, negatively charged nanoparticles of the same nature (carboxyl-modified polystyrene latex nanoparticles) were not cytotoxic; however, exposure to negatively charged nanoparticles changed electrophysiologic characteristics of cardiomyocytes, sensitizing them toward arrhythmias (Miragoli et al., 2013).

In conclusion, it is not possible to define any new chemical material as nontoxic without testing it in the final application in vivo. The diversity of the particles, in particular nanosized particles, being developed as advanced delivery systems makes prediction of their possible adverse effects according to physicochemical characteristics quite difficult. The increasing volume in data on nanotoxicology, entailing physicochemical determinants, biodistribution, and biologic effects of nanoparticles defines the key points for testing their toxicity. Thus, the risk of in vivo use of nanoparticle delivery systems should be thoroughly assessed by combination of the test procedures on case-by-case basis.

VI. Future Potential of Peptide Drugs

The predicted forecast for the future drug discovery is that more and more potential drug targets involve protein-protein interactions. An additional challenge in the future will be cell-penetrating peptides, which target intracellular proteins.

The market for protein- and peptide-based drugs is currently estimated at greater than $40 billion per year, or 10% of the total pharmaceutical market. This market share is growing much faster than that of other pharmaceutical fields, and success rates for bringing biologics to market are now about twice that of small-molecule drugs (Craik et al., 2013). Although the figures presented for biologics refer mainly refer to blockbuster monoclonal antibodies, it can be predicted that the share of peptidergic drugs will increase as well. Kaspar and Reichert (2013) analyzed the sales of the 25 top peptide therapeutics and found that the global sales accumulated to $14.7 billion in 2011. This will put pressure on manufacturing technology as well as on innovative delivery and transport technologies.

The list of bioactive peptides with potential therapeutic value is huge and is growing each year (Kaspar and Reichert, 2013). More and more potential therapeutics lead peptides can be found by proteomics and screening of natural sources, like animal venoms, which are known to be potent and fast acting. In the future, as the biologic therapeutic segment grows, peptides may appear not only as prescribed drugs, but as functional foods and nutraceuticals. There is also great potential in peptide antigens in vaccines and diagnostics. It can be predicted that peptidergic drugs will be emerging as an important therapeutic alternative to large biologic and small-molecule drugs.

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