# New Highly Efficient Non-invasive Nanoparticulate Delivery Systems for the Treatment of Chronic Diseases

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## ABSTRACT

We herein report the overall goals of the joint research project "Nano-Health", which was positive evaluated by international reviewers within the first call of the Austrian Nano Initiative. Furthermore, we report preliminary results of two subprojects within the joint research project "Nano-Health".

Nano-Health, which consists of nine single projects, aims to develop new multifunctional nanoparticles for the non-invasive targeted delivery of active substances for the treatment of chronical diseases like morbus Alzheimer and diabetes by different application routes, *e.g* nasal, oral and pulmonary. Furthermore, nanoparticles for a targeted release of magnetic resonance imaging contrast media for use in clinical diagnostics and therapeutics will be developed.

***Keywords***: nanoparticle, drug delivery, targeted drug delivery, chronical diseases, MRI

## 1 INTRODUCTION

### Significance of Nanoparticles for Drug Therapy

In recent years, significant effort has been devoted to develop nanotechnology for drug delivery since it offers a suitable means of delivering small molecular drugs, as well as macromolecules such as proteins, peptides or genes by either localized or targeted delivery to the tissue of interest. Nanotechnology focuses on formulating therapeutics in biocompatible nanocomposites such as nanoparticles, nanocapsules, micellar systems, and conjugates. Nanoparticles are submicron-sized polymeric colloidal particles with a therapeutic agent of interest encapsulated within their polymeric matrix or adsorbed or conjugated onto the surface. Since these systems are polymeric and submicron in size, they have multifaceted advantages in drug delivery. These systems in general can be used to provide targeted (cellular / tissue) delivery of drugs, to improve oral bioavailability, to sustain drug/gene effect in target tissue, to solubilize drugs for intravascular delivery, and to improve the stability of therapeutic agents against enzymatic degradation (nucleases and proteases), especially of protein, peptide, and nucleic acids drugs.

The size-ranges of these delivery systems offer certain distinct advantages for drug delivery. Due to their sub-cellular and sub-micron size, nanoparticles can penetrate deep into tissues through fine capillaries, cross the fenestration present in the lining (e.g. liver), and are generally taken efficiently by the cells. This allows efficient delivery of therapeutic agents to target sites in the body. Also, by modulating polymer characteristics one can control the release of a therapeutic agent from nanoparticles to achieve the desired therapeutic level in the target tissue for required duration for optimal therapeutic efficacy. Furthermore, nanoparticles can be delivered to distant target sites either by localized delivery using a catheter-based approach with a minimal invasive procedure or they can be conjugated to a biospecific ligand which could direct them to the target tissue or organ [1, 2].

### Nanoparticles derived from Synthetic-Polymers

Up to now nanoparticles have been manufactured from a huge variety of materials. Reviews upon this issue were published frequently [1, 3-5]. A majority of preparations have dealt with the nanoparticles of poly(,-lactide), poly(lactic acid) PLA, poly(,-glycolide) PLG, poly(lactide-co-glycolide), PLGA, and poly(alcyl-cyanoacrylate) PACA. Other synthetic polymers such like polymethylmethacrylate (PMMA), poly(epsilon)caprolacton, polyamide, polyphthalamide, polystyrene were used less frequently. New polymers such as thiomers or thiolated acrylates are potential materials with increased mucoadhesive properties and will be interesting drug delivery carriers for the future [6, 7].

### Nanoparticles derived from Biopolymers

In addition to synthetic polymers which have several advantages in comparison to natural biopolymers, such as defined chemical structure and higher purity, biopolymers from natural sources are also attractive excipients for pharmaceutical preparation.

Nanoparticles have been prepared from a huge variety of biopolymers for about 30 years. Bovine serum albumin (BSA) and later the human serum albumin (HSA) are the oldest biopolymers used first for microparticles and later also for nanoparticles. Gelatin, a widely used substance in food und drug industry, was also investigated for nanoparticle preparations. Sodium alginate was more often used for larger particles and for microcapsules. It has a very good biocompatibility leading to special applications such like the encapsulation of total cells. Chitosan, recently

reviewed in a special issue of the European Journal of Pharmaceutics and Biopharmaceutics (Vol 57,1), has a large potential in pharmaceutical industry. So far it was used as tableting excipient, however, newer applications focus on nanoparticles used for DNA and protein drug delivery [1, 3, 8-10].

One of the oldest and most studied biopolymers for controlled drug release is protamine, a polycationic peptide with a mass of approximately 4,000–6,000 Dalton. Studies on protamine started in the year 1868 by Friedrich Mierscher [15]. A long period of research work was necessary to characterize this group of arginine rich, strongly basic, aliphatic peptides present in the sperm cell nuclei of fish. However, already in 1936 Hans Christian Hagedorn developed the first particulate slow release drug delivery system for insulin based on protamine. This NPH insulin particle suspension was optimized over the years and became the most frequently used long-term insulin.

Nanoparticles prepared from protamine were first invented by the group of Zimmer and will be part of the present grant application [12, 13]. These nanoparticles, so called proticles (Protamine-Oligonucleotide-Particles) are now applied from different groups to deliver oligonucleotides into mammalian cells [14-19]. Recent developments have also shown the potential of these nanoparticles as drug delivery systems for proteins.

### Liposomal and Lipid-Based Nanoparticles

The potential pharmaceutical use of liposomes as biocompatible and biodegradable carriers for small molecules, peptides, proteins or DNA has been extensively studied over the past few decades. Improved pharmacological properties, enhanced safety and increased efficacy have been achieved for liposomal carriers compared to conventional formulations, in particular for applications in cancer chemotherapy, antimicrobial therapy and vaccination. Only recently, several liposomal drug formulations have been approved for clinical use and are now on the market.

Liposomes, in general phospholipid vesicles, are self-assembling, nanoscopic arrangements of one or more lipid bilayers surrounding a hydrophilic environment. Vesicles can be made in different sizes, compositions, surface charges or bilayer fluidities and can easily be manipulated. In combination with co-additives, ligands or by surface coating vesicles offer attractive possibilities to entrap drugs of different lipophilicity, either in the hydrophilic core or in the lipophilic lipid shell, or alternatively, drugs or targeting ligands can be coupled to the surface. This enormous versatility enables a rational design of lipid-based nanoparticles (~50 nm to 500 nm diameter) with respect to the target and to the route of administration. Due to the unique phospholipid shell structure providing a barrier, vesicles are efficient delivery vehicles for drugs, which would otherwise undergo proteolytic degradation in the biological milieu upon administration. Moreover, liposomal encapsulation reduces toxicity of drugs, while retaining or augmenting the therapeutical efficacy of the drug. In addition, by construction of tailor-made vesicles a sustained, slow drug release combined with a reduction of systemic side effects and target specificity can be achieved. Given the success of liposomal drug formulations currently available, more breakthroughs can be expected for the future [20-23].

Further, lipid based nanoparticles are currently under development. Due to a high pharmaceutical stability solid lipid nanoparticles (SLN), which have similar properties compared to liposomes, are most promising. However, they exhibit a solid lipid core and therefore can not encapsulate a water compartment with dissolved hydrophilic drugs [24, 25].

The most sophisticated but also most complicated lipid based nanoparticles are lipoproteins such like LDL and HDL. These carriers, so far used only in experimental medicine, are one of the natural nanoparticles of our own body which deliver lipids, drugs and other substances to specific targets for receptor mediated uptake [22].

## 2 JOINT RESEARCH PROJECT “NANO-HEALTH”


### 2.1 Goals

The main objective of this project is the development of new concepts in design and application of nanoparticles for drug, peptide and protein delivery. The novelty of the approach relies on the encapsulation or trapping of modified therapeutic agents in nanoparticles, which are designed on the basis of current knowledge of formulation, but imply distinct variations in composition, surface charge, hydrophobicity, size and shape. By the use of such “custom-made” nanocarriers for peptide and protein delivery, a significant improvement in the efficiency of organ-specific targeting i.e. brain, and lung by alternative administration routes i.e. nasal, pulmonary and oral is aimed at. Depending on the therapeutical agent to be delivered, a reduction in degradation propensity, toxicity, application rate or achievements concerning sustained release, enhanced efficiency and bioavailability are envisaged goals of the project.

The approach to design appropriate vesicular nanoparticles will be complemented by a comprehensive structural analysis of loaded and unloaded nanoparticles by different biophysical techniques, including light scattering, X-ray small angle scattering, MRI, zeta potential measurements, light microscopy and calorimetry. Likewise, cell and tissue culture studies up to animal models on uptake, biological activity, distribution of and cellular response to the various drugs tested will be performed.

As a future perspective, the knowledge gained on the biophysical, biological and cellular behaviour of special triggered drug formulations, might easily be transformed and adapted to other related substances.

## 2.2 Overall approach

The development of potent formulations for non-invasive peptide delivery represents one of the main challenges in modern pharmaceutical technology. Today most of these extraordinary pharmacological potential therapeutic agents have to be administered via parenteral routes, which are inconvenient because of pain, fear and risks being associated with this type of application. 'Injectable-to-non-invasive-conversions' and in particular 'injectable-to-oral-conversions' are consequently highly in demand. In order to provide a sufficient high bioavailability with non-invasive peptide delivery systems, however, various hurdles have to be overcome. They include the diffusion barrier being based on the mucus gel layer covering mucosal membranes, which has to be passed by peptides in order to reach the absorption membrane [26], and the enzymatic barrier being represented by secreted and membrane bound peptidases [27]. Moreover, having reached the absorption membrane in intact form therapeutic peptides have to permeate this membrane barrier in order to reach the systemic circulation [28]. Pharmaceutical technological attempts to overcome these barriers include the use of enzyme inhibitors [27], permeation enhancers [29] and multifunctional polymers ideally guaranteeing both enzyme inhibition and permeation enhancement [30]. In case of multifunctional polymers these effects, however, can only take place if a tight contact of the polymer with the mucosa is provided for the whole period of peptide drug release and absorption. Apart from enzyme inhibitory and permeation enhancing properties multifunctional polymers should therefore offer also strong mucoadhesive features.

The very slow progress in the efficacy of the treatment of severe diseases has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. Chronic diseases like diabetes, Alzheimer disease and chronic obstructive pulmonary disease are priority diseases to be addressed for the development of new treatments and dug delivery systems. [31]. One way to improve the delivery of drugs are nanoparticles. The benefit of nanoparticles as highly potent drug delivery systems is generally accepted and documented in numerous multicenter randomised clinical trials. Today, however, the full potential of nanoparticles in drug delivery has not been reached by far.

By optimization of three different nanoparticle technologies (proticles, thiomers and lipo-based) and by combining them new innovative types of nanoparticels will be generated. These multifunctional nanoparticles will be used for the non-invasive administration of therapeutic and diagnostic agents via the nasal, oral and pulmonary route and as a depot formulation for the long term treatment of specific organs e.g. lung and brain. These formulations will be tested in various biological models (cell and tissue culture, membrane and animal models and subsequently in humans).

These nanoparticles will be used for the delivery of insulin and calcitonin via the oral route, hGH and amyloid binding peptide via the nasal route and vasoactive intestinal peptide (VIP) via the pulmonary route. Nanoparticles delivering insulin and calcitonin will target the intestine by using the enhanced mucoadhesive and permeation properties of thiomer particles. The nasal route will be addressed by using small lipid based nanoparticles and thiomers. Pulmonary delivery will employ small unilamellar vesicles, proticles and thiomer technologies to deliver VIP into the deep lung.

An overview of the envisaged concept of "Nano-Health" is given in Figure 1.

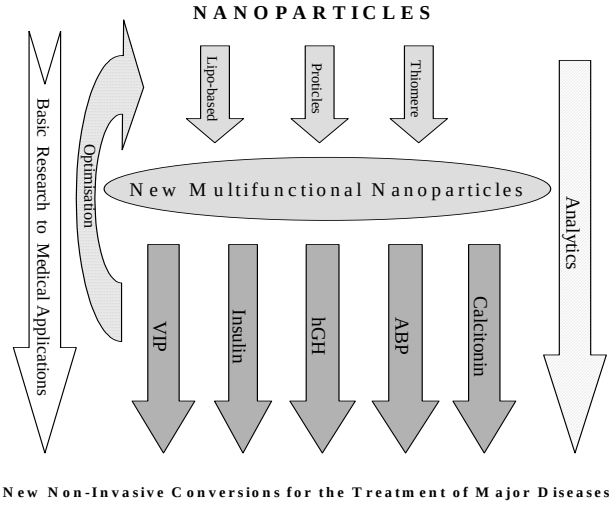


Figure 1: General approach of the project Nano-Health to develop new nanoparticle platform technologies for the treatment and diagnosis of major diseases

## 3 PRELIMINARY RESULTS

First results of this project already showed that the intestinal and nasal residence time of thiomer particles is significantly prolonged in comparison to state-of-the-art particles, as the thiol substructures of thiomer particles are capable of forming disulfide bonds with cysteine-rich subdomains of mucus glycoproteins. Due to this prolonged and intimate contact of thiomers with the mucosa a pharmacological efficacy of 7% could already be reached for orally administered insulin in diabetic mice and a relative bioavailability of 8% for nasally administered hGH in rats.

Vasoactive intestinal peptide is a potential new drug to treat severe lung diseases like primary pulmonary hypertension, (PPH) an orphan disease. PPH is a fatal disease causing progressive right heart failure within 3 years after diagnosis.

Consequently, the substitution therapy with the hormone, administered 3 times per day as a single dose inhalation, results in substantial improvement of hemodynamic and prognostic parameters of the disease without side effects.

VIP has already passed phase I and II clinical trials for PPH. The main goal of this project is now to develop new and innovative depot formulations for the non-invasive pulmonary administration by use of nanoparticulate carriers to reach the deep lung for long term treatment with improved bioavailability and biostability.

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