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Nanoformulation of Therapeutic Enzymes: A Short Review

Gergő Dániel Tóth[1], Gábor Koplányi[2], Balázs Kenéz[2], Diána Balogh-Weiser[1,2*]

[1] Department of Physical Chemistry and Materials Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Műegyetem rkp. 3., H-1111 Budapest, Hungary
[2] Department of Organic Chemistry Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Műegyetem rkp. 3., H-1111 Budapest, Hungary
[*] Corresponding author, e-mail: balogh.weiser.diana@vbk.bme.hu

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Abstract

Enzyme replacement therapy (ERT) is a therapeutic approach that involves the administration of specific enzymes to the patient in order to correct metabolic defects caused by enzyme deficiency. The formulation of ERTs involves the production, purification, and formulation of the enzyme into a stable and biologically active drug product, often using recombinant DNA technology. Non-systemic ERTs often involve the immobilization of the enzyme on a carrier, such as hydrogels, liposomes, or nanoparticles. ERT holds great promise for the treatment of a wide range of genetic disorders, and its success regarding lysosomal storage diseases, such as Fabry disease, Gaucher disease, and Pompe disease has paved the way for the development of similar therapies for other genetic disorders too.

Keywords

therapeutic enzymes, nanoformulation, nanobiocatalyst

1 Introduction

Enzymes, the biological catalysts that drive vital biochemical reactions, have long been recognized as crucial players in maintaining cellular homeostasis and orchestrating the intricate processes within living organisms. Leveraging the remarkable specificity, efficiency, and versatility of enzymes, researchers have begun exploring their immense potential in the field of therapeutic interventions. Enzyme-based therapies represent a rapidly evolving frontier in medical science, offering promising avenues for the development of targeted treatments with enhanced efficacy, reduced side effects, and improved patient outcomes [1, 2]. This scientific review elucidates the fundamental principles and emerging applications of enzyme-based therapies, highlighting their transformative impact on the landscape of modern medicine.

As the largest group of protein-based therapies, enzyme therapies can be used for the treatment of a vast range of diseases, including genetic disorders, cancer, metabolic diseases, and immune-mediated conditions, where their potential is truly remarkable [2–4]. Fig. 1 shows the real-world relevance of protein-based therapies and the types of enzymes most commonly used in enzyme therapies [5].

Fig. 1 illustrates the prevalence of enzyme drugs available on the market, with albumin-containing products emerging as the most abundant, followed closely by the three primary digestive enzymes: amylase, lipase, and pro-

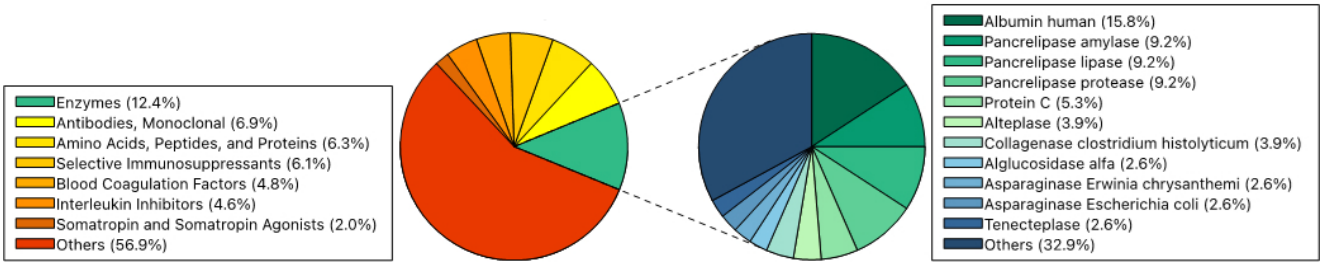

Fig. 1 The most common categories of protein-based therapeutics (a), and enzymes marketed under the most unique brand names in enzyme-based therapies (b) according to DrugBank's online database [5]

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tease. Notably, individual enzymes constitute a relatively minor proportion of the total. This observation suggests a remarkably diverse array of enzymes employed in medicine, a trend that is anticipated to grow significantly in the forthcoming years. Enzyme-based therapies can be grouped not only by the type of enzyme utilized, but also by the therapeutic sectors in which they are used. The most prominent sectors of enzyme therapies are cancer treatment, fibrinolysis, topical treatments and enzyme replacement therapies [2].

Enzyme therapies play a crucial role in cancer treatment, particularly in targeted therapies. Proteolytic enzymes, such as proteases, are employed to selectively degrade proteins that promote tumor growth and metastasis. Improving protease enzyme targeting in cancer therapy can be achieved through modulating pathways, using protease-activatable probes, pretreatment strategies, and developing low molecular weight and natural protease inhibitors, potentially leading to improved outcomes and reduced side effects compared to conventional chemotherapy [6–10].

Fibrinolysis is the natural process of breaking down blood clots. Enzyme therapies designed for fibrinolysis involve the administration of clot-dissolving enzymes, such as metalloproteases and serine proteases. These enzymes help restore blood flow in conditions like acute ischemic stroke, deep vein thrombosis, and pulmonary embolism, by dissolving the obstructing blood clots and preventing further complications [11–13].

Enzyme-based topical treatments have gained recognition in various dermatological conditions. Proteolytic enzymes, such as papain and bromelain, are used to facilitate wound healing, reduce inflammation, and remove dead tissue in conditions like chronic wounds, burns, and skin ulcers. These topical applications help promote tissue regeneration and accelerate the overall healing process [14, 15].

The most colorful and interesting sector of therapeutic enzyme use is enzyme replacement therapy (ERT), which has revolutionized the treatment of a wide range of hereditary disorders, most notably those related with lysosomal storage diseases and metabolic deficiencies [16, 17]. ERT involves the administration of specific enzymes, which are either deficient or absent in the patient's body, to correct existing defects or to prevent the development of complications. Christian de Duve and Roscoe Brady came up with the concept of ERT in 1964 after which Mark J. Poznansky and Damyanti Bhardwaj led pioneering research on this topic at the University of Alberta's Department of Physiology, where they established a rat model for enzyme therapy [18]. ERT was not utilized in clinical practice until 1991, when the FDA (U.S. Food and Drug Administration) orphan drug status to Alglucerase for the treatment of Gaucher disease [19].

Today, ERT has become an essential tool in contemporary medicine, offering hope to patients suffering from a range of genetic disorders (see Table 1). Lysosomal storage diseases, such as Fabry disease, Gaucher disease, and Pompe disease, are among the most common targets of ERT [20–22]. These diseases are caused by the deficiency of specific enzymes that are involved in the degradation of cellular waste material [23]. As a result, the waste accumulates in the lysosomes of affected cells, leading to a variety of clinical manifestations, such as hepatosplenomegaly, skeletal abnormalities, and neurological deficits [24–26].

Table 1 Examples taken from DrugBank [5] and European Medicines Agency (EMA) [44] databases of diseases that can be treated using enzyme therapies with a brand names of medicine suitable for the treatment

Disorder	Cause/Pathology
Hemophilia (Coagulation factor deficiency)	Deficiency in one of the blood clotting factors, which can lead to excessive bleeding
Cystic fibrosis	Affects the lungs, pancreas, and other organs, leading to mucus buildup and inflammation
Fabry disease	Deficiency in the enzyme α-Galactosidase A, which can lead the build up of sphingolipids in blood vessels and tissues, causes hearth, kidney or brain problems
Phenylketonuria (PKU)	Affects the metabolism of the amino acid phenylalanine, which can lead to phenylalanin accumulation, causes intellectual disability and seizures
Gaucher disease	Deficiency of the enzyme glucocerebrosidase, which can lead the build up of lipidsin spleen and liver, causes enlarged spleen, livet etc.
Pompe disease	Deficiency of the enzyme acid α-Glucosidase, which leads to the build up of complex sugars, causes the break down of muscles
Mucopolysaccharidosis (MPS)	Deficiencies in enzymes responsible for breaking down complex sugars called glycosaminoglycans, causes permanent progressive cellular damage
Hypophosphatasia (HPP)	Deficiency of the enzyme alkaline phosphatase, causes bone and teeth mineralisation problems
Acute lymphoblastic leukemia	Cancer of the blood cells

ERT has been shown to improve the symptoms of these diseases, slow down their progression, and even prolong the lifespan of affected individuals. As a therapeutic approach ERT is a valuable tool in the treatment of various metabolic diseases. In these conditions, the body lacks specific enzymes necessary for normal metabolic processes. In this case ERT involves the administration of synthetic or recombinant forms of these enzymes to supplement the deficient enzyme levels. By providing the missing enzyme, ERT aims to restore the metabolic pathway, alleviate symptoms, and prevent complications associated with metabolic diseases. Examples of metabolic disorders treated with ERT include homocystinuria, phenylketonuria and chronic pancreatitis [27–29]. ERT has shown promising results in improving patients' quality of life and reducing the progression of metabolic disorders when initiated early and combined with appropriate supportive care.

ERT can be classified into two broad categories: systemic and non-systemic. Systemic ERT involves the administration of the enzyme mostly through various injections, which allows the enzyme to reach all the affected organs and tissues [30]. Non-systemic ERT, on the other hand, involves the direct delivery of the enzyme into a specific organ or tissue, such as the joints in the case of ERT for arthritis [31], or oral formulations in case of metabolic dysfunctions [32, 33].

The formulation of ERTs is a complex process that involves the production, purification, and formulation of the enzyme into a stable and biologically active drug product [34, 35]. The manufacture of ERTs often involves the use of recombinant DNA technology [36–38], which allows for the production of large quantities of the enzyme in a relatively short time and opens up the possibility of using enzymes that would be challenging to extract naturally. The enzyme is then purified to remove impurities and formulated into a drug product that can be administered to patients. ERTs are available in various drug forms, including liquid formulations, lyophilized powders, and solid oral formulations. For non-systemic ERTs, the enzyme is often immobilized on a carrier to enhance its stability and facilitate its delivery to the target tissue. Various types of carriers, such as hydrogels [39], liposomes [40], and nanoparticles (NPs) [41–43], have been used for this purpose. Different inorganic NPs (such as, silica, iron and titan-oxide NPs), and organic NPs (for example natural and unnatural polymer NPs such as polyvinyl alcohol (PVA), polylactic acid (PLA), chitosan, polyaspatamodes) single enzyme NPs, and carbon-based NPs (such as graphene and carbon nanotubes) can all be utilized in enzyme therapy formulation [44–48].

To summarize, ERT is a fast-emerging branch of medicine that has tremendous potential for treating a wide spectrum of genetic disorders. The introduction of ERTs was a significant step forward in the treatment of lysosomal storage diseases, and its success paved the way for the development of similar medicines for other hereditary disorders. Thanks to modern recombinant DNA technology and nanotechnology, the development and uptake of ERT is expected to increase further in the future, as illustrated by Fig. 2.

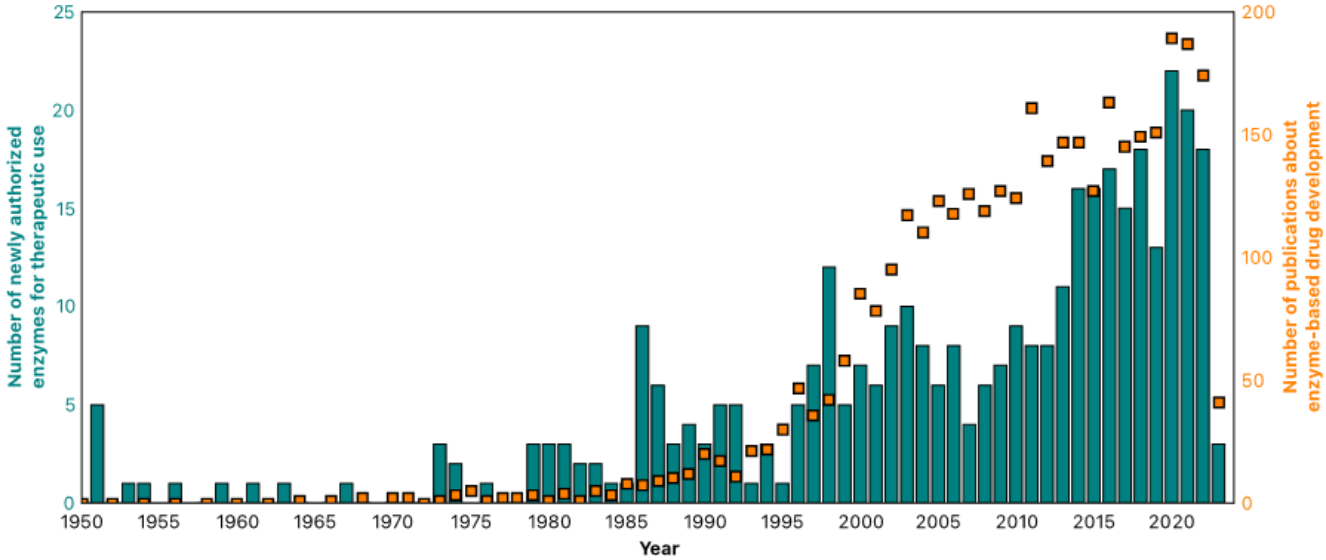

Fig. 2 The number of publications on enzyme-based drug development and the number of newly approved enzymes as active pharmaceutical ingredients by year from 1950 to 2023. In order to filter the results, publication searches were carried out by entering the subject "enzyme therapy, drug and treatment" in the PubMed database and selecting the "Title/Abstract" field [2]. To determine the number of approved enzymes, the approval dates of the drugs containing the enzymes first in time in the DrugBank Online [5] database were collected

2 Formulation of enzymes

2.1 Classic formulation of enzymes

2.1.1 Systemic enzyme replacement therapy

Systemic enzyme replacement therapy is a therapeutic approach that involves the administration of enzymes through intravenous (IV) infusion that circulate throughout the body. Systemic ERT has been licensed for clinical use in several lysosomal storage disease (LSDs), including Krabbe, Gaucher, Pompe, and Fabry diseases, as well as several mucopolysaccharidoses (MPSs) [49].

Systemic ERT is a well-established method for treating a variety of diseases. It offers efficient and widespread delivery of therapeutic enzymes directly into the bloodstream, allowing distribution to different organs and tissues throughout the body. This method also enables consistent and controlled dosing, ensuring predictable enzyme delivery. However, there are some drawbacks to consider. Systemic ERT often requires frequent administration, which can be time-consuming for patients and caregivers. Infusions for Fabry and Gaucher diseases must be administered every two weeks, with each infusion lasting two hours [50, 51]. Additionally, infusion-related reactions, ranging from mild to severe, may occur, and patients must depend on healthcare facilities for intravenous administration [52]. Also delivery or targeting of the therapeutic enzymes in treating certain organs such as bone, cartilage, heart valves, and brain poses serious challenges. This suggests the need for alternative, non-systemic delivery approaches [53]. Despite the limitations listed above, the vast majority of the most commonly used ERT medicines are currently systemic formulations [2], and they will certainly remain valuable treatment options for many diseases with established safety and efficacy profiles. Table 2 presents prominent examples of currently applied systemic ERT formulations.

2.1.2 Non-systemic enzyme replacement therapy

Non-systemic enzyme replacement therapy has emerged as a promising treatment modality for various diseases, offering targeted delivery to specific organs or tissues affected by the disorder. Various formulations, including oral pills, tablets and capsules (see Table 3), have been developed to achieve this goal. By focusing on the affected areas, non-systemic ERT minimizes the need for widespread distribution, optimizing therapeutic efficacy [54].

Another notable advantage of non-systemic ERT is that the patient is able to administer the therapeutic enzyme on their own, without the need for professional hospital staff. Treatment frequency is also reduced compared to intravenous ERT [52]. This reduced frequency and ease of administration may enhance convenience for patients and improve overall treatment compliance.

However, this approach also does not come without its limitations. One major limitation of non-systemic ERT is the limited availability of treatment options for diseases. Some disorders lack well-developed non-systemic formulations,

Table 2 Examples taken from DrugBank [5] and European Medicines Agency (EMA) [44] databases of systemic enzyme therapies and target diseases

Name of ERT drug	Formulated enzyme	Pharmaceutical form	Target disease
Cerezyme	Imiglucerase	Lyophilized powder for reconstitution and intravenous infusion	Gaucher disease
Krystexxa	Pegloticase	Sterile liquid for intravenous infusion	Chronic gout
Lumizyme	Alglucosidase alfa	Lyophilized powder for reconstitution and intravenous infusion	Pompe disease
Myozyme	Alglucosidase alfa	Lyophilized powder for reconstitution and intravenous infusion	Pompe disease
Strensiq	Asfotase alfa	Sterile liquid for subcutaneous injection	Hypophosphatasia
VPRIV	Velaglucerase alfa	Lyophilized powder for reconstitution and intravenous infusion	Gaucher disease
Pulmozyme	Dornase alfa	Sterile liquid for inhalation	Lung cystic fibrosis

Table 3 Examples taken from DrugBank [5] and European Medicines Agency (EMA) [44] databases of non-systemic enzyme therapies and target diseases

Name of ERT drug	Formulated enzyme	Pharmaceutical form	Target disease
Cerdelga	Eliglustat		Gaucher disease
DAOfood Plus	Diamine oxidase		Histamine intolerance
Creon	Pancreatic lipase, protease and amylase	Oral capsule	Exocrine pancreatic insufficiency (EPI)
Pertzye			
Ultresa			
Viokace		Oral tablet	
Lactaid Fast Act	Lactase	Oral chewing tablet	Lactose intolerance

restricting the treatment choices for patients with these specific conditions. Additionally, some of the main challenges encountered in the development of non-systemic ERTs is the short in vivo half-life of the therapeutic enzyme, along with the difficulties in achieving targeted action and addressing potential immune system reactions against the enzyme in patients [2]. These factors can severely impact the overall effectiveness of non-systemic ERT.

Despite these challenges, the discovery and use of targeted non-systemic ERTs is becoming increasingly widespread. Furthermore, modern nanoformulation approaches have the potential to significantly improve the development and effectiveness of ERTs. These methods allow for precise control of enzyme properties, targeted delivery, improved stability, extended-release time, and the potential to overcome challenges such as short in vivo half-life and immune system reactions, revolutionizing ERTs and opening new avenues for effective and targeted treatments.

3 Methods and carriers for enzyme nanoformulation

In order to achieve enhanced performance, stability, and convenient handling for the desired applications, it is essential to utilize an appropriate supporting system. For this purpose, nanomaterials, including nanoparticles, nanofibers, and nanotubes, have garnered significant attention due to their finely tunable physical-chemical properties, large specific surface area, and biocompatibility [55]. The methodologies for immobilization can be broadly classified based on enzyme-carrier interactions, primarily involving physical adsorption, entrapment, and chemical attachments through covalent bonds, ionic or coordinative interactions. Each of these approaches offers distinct advantages, allowing researchers and practitioners to tailor the immobilization process to suit specific enzyme applications [56].

3.1 Nanoparticles (for enzyme attaching, capsules etc.)

Over the years, significant advancements in nanotechnology have facilitated the application of a diverse range of NPs for immobilizing numerous enzymes. In the existing body of literature, numerous articles have delved into the topic of enzyme immobilization using various types of NPs. These include metal nanoparticles like gold (Au) and silver (Ag), metal oxide nanoparticles such as zinc oxide (ZnO) and titanium dioxide (TiO_2), as well as magnetic nanoparticles (MNPs), which are a subset of metal oxide NPs. Additionally, researchers have explored the use of spherical or porous silica nanoparticles (SNPs) and polymeric nanoparticles (PNPs), which consist of organic macromolecules like chitosan, poly(lactic-co-glycolic acid) (PLGA), and polyethylene glycol (PEG)-based nanoparticles [57–60]. Due to their exceptional properties, such as finely tunable surfaces, high chemical and mechanical resistance, and suspension stability within the appropriate size range, NPs serve as highly efficient solid support materials [61, 62]. Furthermore, immobilizing enzymes using NPs not only stabilizes them but also reduces protein unfolding, ultimately enhancing their performance. However, despite these advantages, the use of NPs does come with some drawbacks, including the costs associated with synthesis and surface functionalization processes, challenges related to large-scale applications, and difficulties in isolating NPs from the reaction media (except for MNPs) [63]. When considering some of the most commonly observed ERT-related enzymes, the immobilization process often involves functionalized SNPs, coated MNPs, or mesoporous resins. These methods have been extensively described in the case of α-Amylases and lipases from different strains [64, 65].

3.2 Nanotubes

Enzyme immobilization can utilize various types of nanotubes as inorganic supports [57]. Carbon nanotubes (CNTs) are carbon allotropes with a nanostructure that are made up of graphite sheets coiled up into a tubular form with lengths in the micrometer range and diameters of up to 100 nm. There are two forms of CNTs: single-walled carbon nanotubes (SWNTs) with a core tubule and multiwalled carbon nanotubes (MWNTs) with many layers of graphite around the central tubule [66]. Both ones are considered to be attractive supports for enzyme immobilization because of their high specific surface area and in case of MWNTs the really good dispersibility too [67]. Halloysite is another widely used material consisting of a natural kaolinite mineral. Its aluminosilicate layers form a particularly hollow tubular structure, as a result halloysite nanotubes (HNTs) with diameters of 50–70 nm could be prepared and can be used for the adsorption of proteins. Furthermore, the density of siloxane (Si-O-Si) groups on both the outer and inner surfaces of HNTs is rather high, allowing for further surface functionalization [68]. Up to now the application of surface functionalized HNTs have been described in case of lipase, laccase, and horseradish peroxidase enzymes [69]. By combining NPs and CNTs, composite supports with improved mechanical characteristics and handling may be created [70].

3.3 Nanofibers

Among all the nanomaterials used for enzyme immobilization, nanofibers are perhaps one of the most extensively

employed. The advent of electrospinning technology has simplified and accelerated the fabrication of nanofibers, resulting in uniform nanostructured supports with a significant specific surface area, high porosity, and excellent interconnectivity [71]. The entrapment of enzymes within nanofibrous matrices involves a straightforward process wherein a well-mixed, homogeneous precursor mixture containing both the enzyme and the corresponding polymer is used. During the immobilization process, the enzymes are rapidly embedded *in situ* inside the polymer nanofibers through secondary forces, all under mild conditions [72]. For this purpose, natural (chitin, chitosan, gelatine and cellulose etc.) and several synthetic (PVA, polyvinylpyrrolidone (PVP), polycaprolactone (PCL), PLA etc.) linear polymers are used [73, 74]. Among them the water-soluble synthetic polymers are commonly preferred thanks to the compatibility between the enzyme containing buffer solution and the water-based polymer sol. Moreover, the polymer chains themselves can have a stabilizing effect on the enzymes in the precursor mixture, as well [33]. PLA is a biologically inert, water insoluble polymer, which has proven to be a romising material for enzyme carrier systems over the years [75]. In case of the entrapment of lipases from different strains and a phenylalanine ammonia lyase enzymes in PLA nanofibrous matrices was described, which resulted water insoluble, stable biocatalysts [76, 77].

4 Future perspectives

The development and production of biological medical products, or biologics, is becoming increasingly important in the pharmaceutical industry. In 2020, for example, five of the top ten highest-earning drugs were biologics; the biggest earner's brand name is Humira, which contains Adalimumab as the active pharmaceutical ingredient, a monoclonal antibody [78]. The biologics can be divided into 3 main categories, monoclonal antibodies, receptor modifiers and enzymes. The research into the 3 main categories is progressing at the same rate [79]. Most of the attention is in the research of new enzymes, but the development of new drug delivery systems is also important, more specifically the development of non-invasive delivery systems [80]. Enzyme therapies hold great promise as treatments for various medical conditions, but they encounter several challenges [2]. The primary difficulties include the delivery of enzymes to specific target sites in the body, the stability of these fragile molecules during storage and administration, their potential to trigger immune responses, their short half-life requiring frequent dosing, and the high production costs limiting accessibility for patients. To address these challenges, researchers are exploring various potential solutions (see Fig. 3). These include using nanoparticle-based drug delivery systems to protect enzymes and enhance targeted delivery, employing encapsulation and stabilization techniques to extend enzyme lifespan, and utilizing PEGylation [81] to reduce immunogenicity and increase circulation time. Gene therapy [82] is being investigated as a means to introduce genetic instructions for producing required enzymes within the patient's body, while protein engineering aims to improve enzyme stability and specificity. Advances in bioreactor technology [83] could lead to more cost-effective large-scale enzyme production. Additionally, combining enzyme therapies with other treatment modalities and implementing continuous monitoring for personalized dosing adjustments offer promising ways to optimize therapeutic outcomes. Through ongoing research and innovation, these potential solutions may pave the way for more effective and accessible enzyme therapies in the future.

4.1 Development of novel nanomaterials

As mentioned earlier, in ERT, enzymes are often administered via IV or subcutaneous injection. While these delivery methods are effective, they are often painful and in the case of IV their administration requires medical personnel [80].

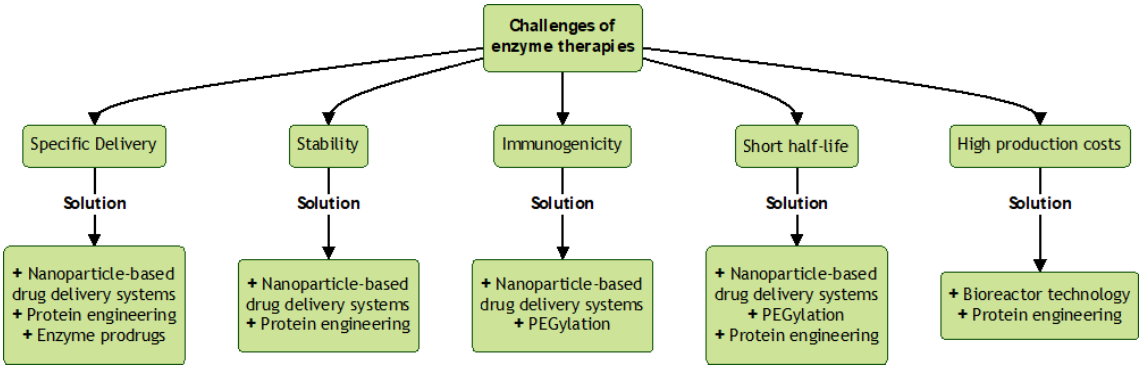

Fig. 3 The main challenges of modern enzyme therapies and possible solutions for overcoming them

To avoid these problems new non-invasive delivery methods are researched for use in the enzyme replacement therapies. The issues of the non-invasive treatments emerge from the different barriers to the circulatory system, or in the case of the oral route, the digestive system, which can inactivate the proteins. To achieve a functioning, non-invasive biologic, new developments of carrier systems, must be achieved. As a carrier system, many different nanomaterials, for example nanoparticles, liposomes and virosomes can be used. A list of older and novel nanomaterials with the bound enzymes can be found in the Table 4 [84–97].

4.2 New enzymes

Many different enzymes are currently employed for replacement therapy, but research for new and unique biologics is always progressing. There are several FDA-approved and used enzymes, such as α-glucosidase, which is used to treat Pompe's disease, a glycogen storage type 2 disease [98], or β-galactosidase, which is used to treat Fabry's disease, a neurological disorder [20]. Non-human enzymes can also be employed in enzyme therapy. For example, the plant-based enzyme phenylalanin ammonia lyase (PAL) can be used to treat phenylketonuria, which is caused by an inadequate supply of the phenylalanine hydroxylase enzyme (PAH). Normally, the PAH enzyme converts L-phenylalanine to L-tyrosine, but in phenylketonuria, this conversion is hindered, and L-phenylalanine accumulates. L-phenylalanine is converted by the PAL enzyme into trans-cinnamic acid and ammonia. In comparison to PAH, PAL enzyme treatment has some advantages. PAL does not require cofactors, and the trans-cinnamic acid produced has low toxicity. The PAL enzyme is also relatively stable throughout a wide temperature range [99, 100]. Recently high amount of research is being done on human tyrosine hydroxylase enzyme (hTH). Its function is to convert L-tyrosine to L-dihidroxiphenylalanine (L-DOPA), which is the common precursor of adrenaline, noradrenaline, and dopamine in the human body and the limiting step in their formation. A defect in the hTH enzyme can cause a variety of central nervous system diseases, such as Parkinson's disease in cases of underactivity [101].

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Table 4 Nanomaterials used for enzyme-based therapies

Enzyme	Used nanomaterial, method	Method of binding	Reference
α-glucosidase	Magnetic nanospheres	Covalent bond with glutaraldehyde	[84]
α-glucosidase	Polymethyl methacrylate/chitosan nanoparticles	Covalent bond with glutaraldehyde	[85]
α-amylase	Silica nanoparticles	Adsorption	[86]
α-amylase	Amino functionalized silica nanoparticles	Covalent bond with glutaraldehyde	[87]
L-asparaginase	Polyelectrolyte microcapsules	Encapsulation	[88]
L-asparaginase	PEGylated nanoliposomes	Encapsulation	[89]
L-asparaginase	Gold nanoparticles	Adsorption	[90]
β-galactosidase	Protein nanoparticles	Covalent bond with glutaraldehyde between the enzyme and other protein	[91]
β-galactosidase	Tannic acid stabilized silver nanoparticles	Covalent bond with cyanogen bromide (CNBr)	[92]
Catalase	Nanocapsules	Encapsulation	[93]
Superoxide dimutase	Halloysite nanotubes	Adsorption	[94]
Human tyrosine hydroxylase	Maltodextrin nanoparticles	Encapsulation	[95]
Phenylalanin ammonia lyase	PEG copolymer micelles	Encapsulation	[96]
Phenylalanin ammonia lyase	Magnetic nanospheres	Adsorption, then covalent crosslinking with glutaraldehyde	[97]

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