Nano- and micro-based systems for immunotolerance induction in Multiple Sclerosis

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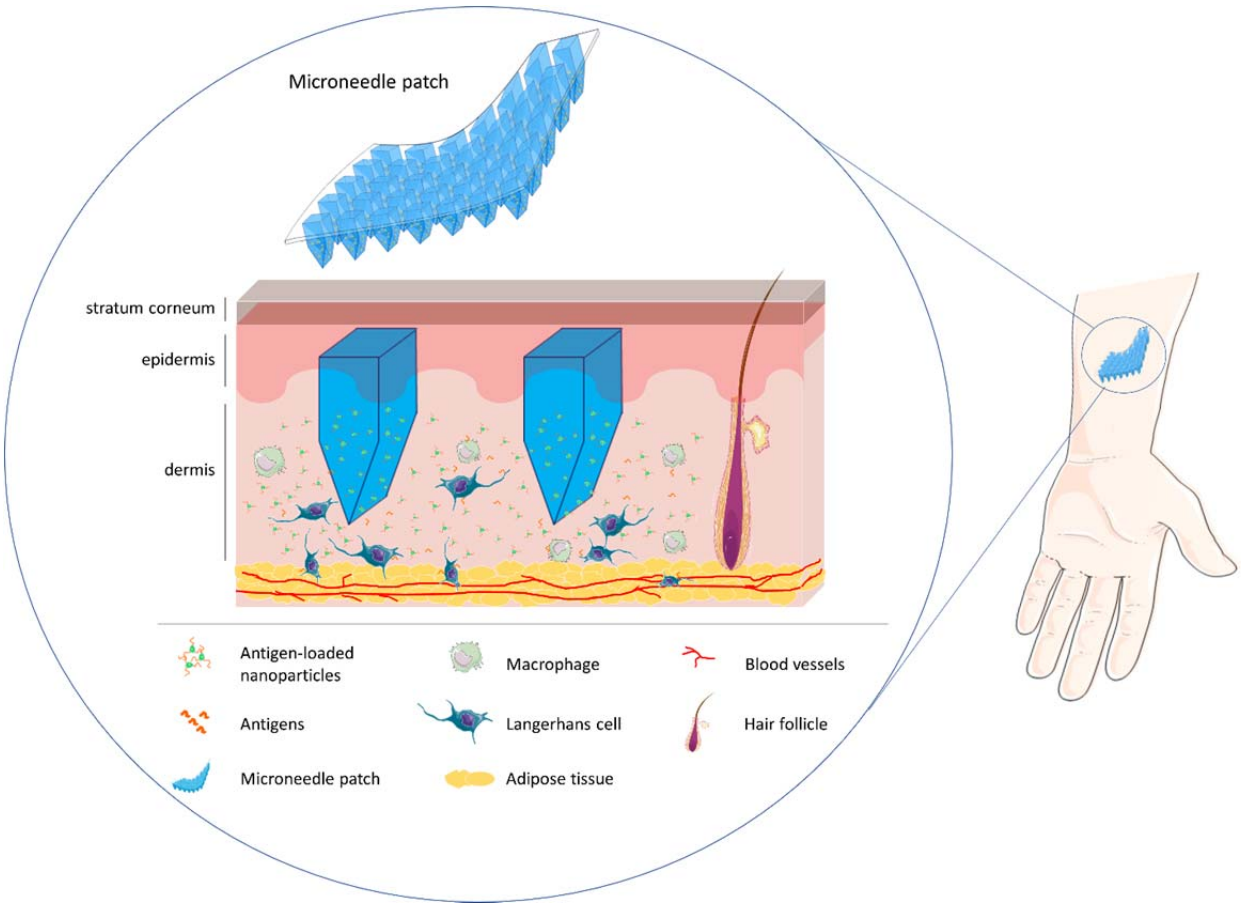
Abstract

It is estimated that more than 2.5 million individuals worldwide have multiple sclerosis (MS). MS is an autoimmune neurodegenerative disease resulting from the destruction of the myelin sheath that enwraps axons driven by an immune cell attack to the central nervous system. Current therapeutic programs for MS focus in immunosuppression and more recently in the use of immunomodulatory molecules. These therapeutic approaches provide significant improvements in the management of the disease, but are frequently associated with an increased susceptibility of opportunistic infection. In this commentary, we highlight the application of nano and micro-technologies as emerging and innovative solutions for MS therapy with the potential to restore immune homeostasis via antigen-specific interactions. Furthermore, we propose and discuss the usage of a minimally invasive approach, namely microneedle patches, as a new therapeutic route.



Microneedle patches for the delivery of specific antigens to restore immunotolerance in the context of Multiple Sclerosis.

Keywords (5-10)

Multiple Sclerosis, Microneedles, Nanoparticles, Tolerance, immunomodulation

1. Multiple Sclerosis: etiology and current therapeutics

Multiple sclerosis (MS) is a chronic immune mediated demyelinating disease of the central nervous system (CNS) caused by a strong T cell attack directed towards proteins of the myelin sheath enwrapping CNS axons; this ultimately culminates in demyelination and neuronal degeneration [1, 2]. In developed countries it is the second cause of neurological disability in young adults, with high burden for the patient, the family and the resources of the health system [3]. It is a complex disease and its underlying mechanisms are only partially understood. Most patients initially present with a clinically isolated syndrome (CIS). These CIS patients experience an acute episode, which typically affects one brain region, being the clinical symptoms variable depending on the involvement of motor, sensory, visual or autonomic systems [4]. Some CIS patients will evolve to definite MS disease, while others won't. Nowadays, the diagnosis of definite MS is based on recognized clinical criteria, with the support of magnetic resonance imaging (MRI) data and cerebrospinal fluid (CSF) analysis [2], and can only be done

when there is dissemination of neurologic dysfunction in space and time [2, 4, 5], and after differential diagnosis is excluded [5]. Concerning the MRI findings, the presence of multifocal demyelinating lesions at different timepoints involving preferentially the periventricular white matter, the brain stem, the cerebellum and the spinal cord are indicative of MS [2]. Furthermore, the presence of oligoclonal bands or increased concentration of immunoglobulin (Ig)G in the patients' CSF are widely used to support MS diagnosis, but are not MS- specific [2, 6].

Patients with definite MS can develop different profiles of the disease, being classified as relapse-remitting (RR)-MS, primary progressive (PP)-MS or secondary progressive (SP)-MS. RR-MS represents about 80-85% of MS cases [7] and is characterized by transient symptoms (relapse) that often improve within weeks (remission). However, the ability to fully recover from relapse episodes diminishes with time, and irreversible damage accumulates in the CNS, giving rise to SP-MS. The remaining 15-20% of patients has PP-MS, and does not show this relapse-remitting pattern; rather, their symptoms become gradually worst along the course of the disease.

MS is nowadays a treatable, although not curable, disease. The first proven MS treatments were approved in the nineties and consisted in different formulations of interferon-1 administered intramuscularly or subcutaneously. Although a major breakthrough at the time and still an important part of treatment options today, given their excellent safety, interferon-1 based treatments are only moderately efficacious, leading to full control of the disease in only a small percentage of patients [8]. Interferons have pleiotropic effects, including a reduced T-cell entry into the CNS [9]. Glatiramer acetate is a mixture of oligopeptides designed to mimic the aminoacid composition of myelin that induces a skew towards a regulatory response. Also administered subcutaneously, it has an efficacy similar to interferons and an excellent safety record that make it still an important player in MS treatment options [8].

Recently the portfolio of approved MS treatments was enriched by more options that encircle two major therapeutic approaches targeting either T-cells or B-cells. Concerning T-cells modulation a collection of drugs is currently being applied in the clinical setting, with moderate success, as next described. Teriflunomide is an oral medication that interferes with the fast expansion of recently activated lymphocytes, preserving the basal proliferation of memory cells. It has a moderate efficacy, similar to interferons, but some safety concerns, including teratogenic potential [10]. Dymethylfumarate is an oral treatment with a putative dual mechanism of action, including immunosuppression and neuroprotection. In clinical trials it demonstrated a good efficacy in controlling the disease but concerns regarding its long-term safety, particularly the profound lymphopenia and the risk of a serious opportunistic CNS infection, progressive multifocal leukoencephalopathy (PML), might limit its use [11]. Fingolimod, a functional antagonist of S1P receptors that blocks lymphocyte egress from lymph nodes [12], has also a good efficacy but similar concerns over lymphopenia and PML [8]. Natalizumab is a highly efficacious monoclonal antibody that blocks lymphocyte entry into the CNS; however, it is associated with a high risk of PML in patients which have antibodies against the causing organism, JC virus, which almost limits its use to seronegative patients representing less than half of MS patients [13].

While all the above-mentioned drugs interfere with T-cell function, alternatively, ocrelizumab is a monoclonal antibody that destroys B-cells and, surprisingly, was shown to have a good efficacy in MS patients [14]. Although not yet approved, it has forced a major revision of MS pathogenesis to take into account the role of B-cells, which are not only seen as antibody producers but also as antigen presenting and cytokine releasing cells, able to activate Th1 and Th17 responses and induce pathology [15]. Although data are still preliminary, ocrelizumab may increase the risk of serious infections, including PML, which might limit its use. Targeting both B and T cells, alemtuzumab is a monoclonal antibody that induces a severe depletion of circulating lymphocytes and is said to "reset" the immune system [16]. Upon immune reconstitution, alemtuzumab-treated patients are able to stay disease free for periods of up to 5 years (longer follow-ups are still scarce), in what represents a first step towards an effective cure [16]. However, such efficacy comes at the cost of an increased risk of serious infections in the first weeks,

requiring antibiotic profilaxis and precautions, and a significantly higher risk of autoimmune disturbances in the following years [16].

As can be gleaned from the above, MS treatments are in an exciting era, with several new options being approved, many more in the pipeline, and new drug targets and modes of action being available. More importantly, some treatments have been shown to allow a reset of the immune system, which might make a cure even more reachable than before. However, we weren't able, so far, of breaking the close ties between efficacy and risk. These severely limit the use of more efficacious medications in all patients and the extension of their benefits to all patients. Thus, the finding of a highly efficacious and safe therapeutic is still an unmet medical need. This paves the way for nanotechnology-based approaches.

2. Nanoengineered systems for MS therapeutics

The use of nanotechnology and in particular of nanoparticles has been actively investigated for the development of new therapies for MS. Taking advantage of their size, nanoparticles are easily internalized by the cells, being suitable carriers for drugs, immunomodulatory molecules or antigens. The use of materials at the nanoscale is expected to provide unique opportunities to improve drug solubility and bioavailability, allowing targeted delivery, controlled release and consequently more effective routes of administration and lower toxicity [17]. Interestingly, not only nanoparticles can serve as carriers of relevant molecules as they can also trigger an immunomodulatory effect. In fact, variations in the chemical composition, size and shape of nanoparticles differentially impact the immune response [18] [19], which might be even more significant in the context of autoimmune diseases [20].

In MS, the use of nanoparticles has been investigated within two major applications: 1) as drug delivery systems, and 2) as vectors for antigen-specific immunomodulation. Nanoparticles can bring new solutions for the delivery of drugs that specifically target the immune or the neurodegenerative aspects of MS. Recent reports describe the encapsulation in liposomal formulations of immunomodulatory drugs currently applied in MS therapeutics such as methylprednisolone [21] or fingolimod [22]. These nano-sized formulations showed a higher efficiency in a MS animal model due to improved pharmacokinetics and biodistribution when compared to the free drugs. Focusing in reducing neurodegeneration, polymeric nanoparticles targeted to oligodendrocyte precursor cells were applied to deliver leukaemia inhibitory factor (LIF) and to successfully promote remyelinization [23].

Importantly, nanoparticles have also been explored as vectors for antigen-specific immunomodulation. The delivery of autoantigens related with the autoimmune response in MS is expected to allow the specific blockade of the damaging effects of self-reactive immune-cell function while maintaining the ability of the immune system to clear non-self antigens, thus restoring immunotolerance. This "tolerant approach" was firstly tested by the administration of soluble autoantigens, but the massive amounts of antigen required [24] along with some reported cases of anaphylactic response [25] prompt the need for new and safer solutions. The administration of peptides crosslinked to splenic leucocytes demonstrated very promising results, inducing a robust antigen-specific tolerance [26, 27]. Nonetheless, in order to circumvent the use of cellular components and the drawbacks associated, like cost and manipulation, researchers have been focused in the development of alternative strategies based on nanoparticulate systems. The premise is that nanoparticles crosslinked with disease-associated antigens and their epitopes can target antigen-presenting cells (APCs) capable to regulate T cell function and simultaneously support the induction and/or expansion of regulatory T cells (Tregs), restoring immunological tolerance.

The intravenous administration of poly(D,L-lactide-co-glycolide) (PLGA) microparticles crosslinked with proteolipid protein $(PLP)_{139-151}$ peptide (the immunodominant T cell myelin epitope in SJL mice from the

myelin most abundant protein - PLP) showed remarkable results, being able not only to reduce the clinical score if administrated prophylactically, but also to treat ongoing disease [28]. Interestingly, $PLP_{139-151}$ administrated in the form of colloidal hydrogel demonstrated to be effective only if administrated before the disease onset [29]. The use of poly(ethylene-co-maleic acid) (PEMA) as surfactant allowed the preparation of PLGA nanoparticles and provided a reliable platform for different antigen crosslinking, as demonstrated by the relevant results in the induction of immunological tolerance both in the context of experimental autoimmune encephalomyelitis (EAE), the animal model for MS [30] and in a transplantation model [31].

Alternatively to antigen crosslinking, nanoparticles can be loaded with the antigen of interest. This concept can be extended to the development of multifunctional systems that combine the delivery of antigens with other molecules/drugs as a mean to turn the immune response more specific and/or more effective. Loading gold nanoparticles with the T-cell epitope from myelin oligodendrocyte glycoprotein (MOG_{35-55}) and ITE (2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester - a tolerogenic molecule) showed to induce functional regulatory T cells in an EAE animal model more efficiently than MOG-loaded particles [32]. PLGA nanoparticles containing MOG_{35-55} and interleukin-10 (IL-10) mediate a sustained release of the molecules [33] and, although the results in terms of regulatory T cells expansion were not as impressive as the obtained for crosslinked nanoparticles, it was shown that the severity of the disease can be reduced via subcutaneous administration of the particles. In the PLP-associated EAE model, the use of rapamycin, an immunosuppressant molecule, loaded in PLGA nanoparticles along with $PLP_{139-151}$ peptide promoted complete inhibition of disease relapses after both intravenous and subcutaneous administration [34].

The results achieved so far using nanoparticles to induce immunotolerance are promising and should soon initiate clinical trials. A further step towards the use of nanoparticles to induce immunotolerance might be achieved when combined with alternative and minimally invasive routes of administration, which will next be explored.

3. A microneedle-based immunotolerance approach for MS

Microneedles have been extensively investigated in the recent years as mean to mediate the delivery of drugs and/or antigens to the epidermal and/or intradermal space, overcoming the skin stratum corneum barrier. These devices hold the potential of allowing self-administration and painless application. Moreover, microneedle devices can be designed to dissolve in the skin, eliminating the issue of microneedle remaining and removal from the skin and allowing a safe disposal without biohazardous waste [35].

Although microneedles show great promise for the delivery of drugs [36] and also as sensing devices [37], it is in the research area of vaccines that they showed, so far, more advances. It was demonstrated that vaccination using microneedles triggers stronger immune responses comparing to conventional injection procedures, allowing sparing of antigens [38]. Indeed, the use of microneedle-based devices for influenza vaccination is currently under clinical trials.

The improved antigen immunogenicity using microneedle devices is considered to be related to the delivery of antigens at the epidermal and intradermal layer of the skin. The skin is highly rich in immunologically active APCs, which deliver antigens to the proximal lymph nodes where T and B cells are activated, triggering the immune response [39]. Also, in the skin (particularly at the epidermis and the epithelium from the hair follicles) monocytes and Langerhans cells are abundant. Langerhans cells display intrinsic tolerogenic properties *in vivo* [40]. Moreover, a small trial enrolling 14 MS patients

showed that the passive diffusion of myelin-related peptides through the skin induces some improvements in the disease, namely reducing the incidence of relapses and the area of lesion (assessed by MRI) [41]. These findings highlight the potential of this route of administration in immunotolerance-based therapies.

Overall, it is clear that therapeutics based on immunotolerance will take remarkable benefit from the application of micro and nano- technological knowledge. Nanoparticle design can assure antigen-specific response, targeted delivery and controlled dosing; whereas microneedle devices, as we propose, open the unique opportunity for sustained intradermal delivery, further contributing to an antigen-specific response and a tolerogenic effect in a minimally invasive therapeutic approach for MS.

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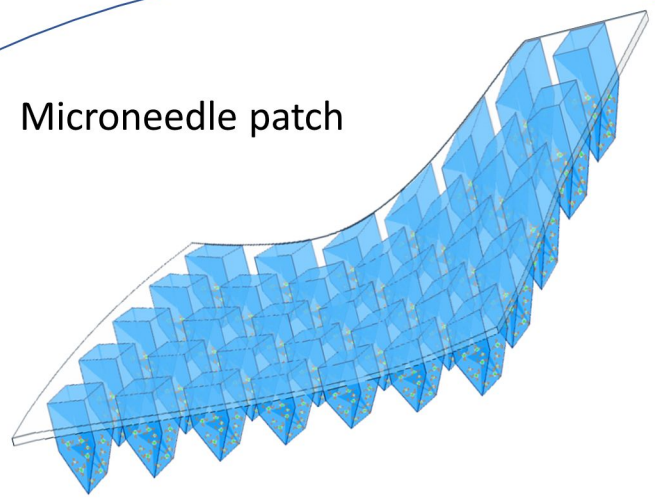
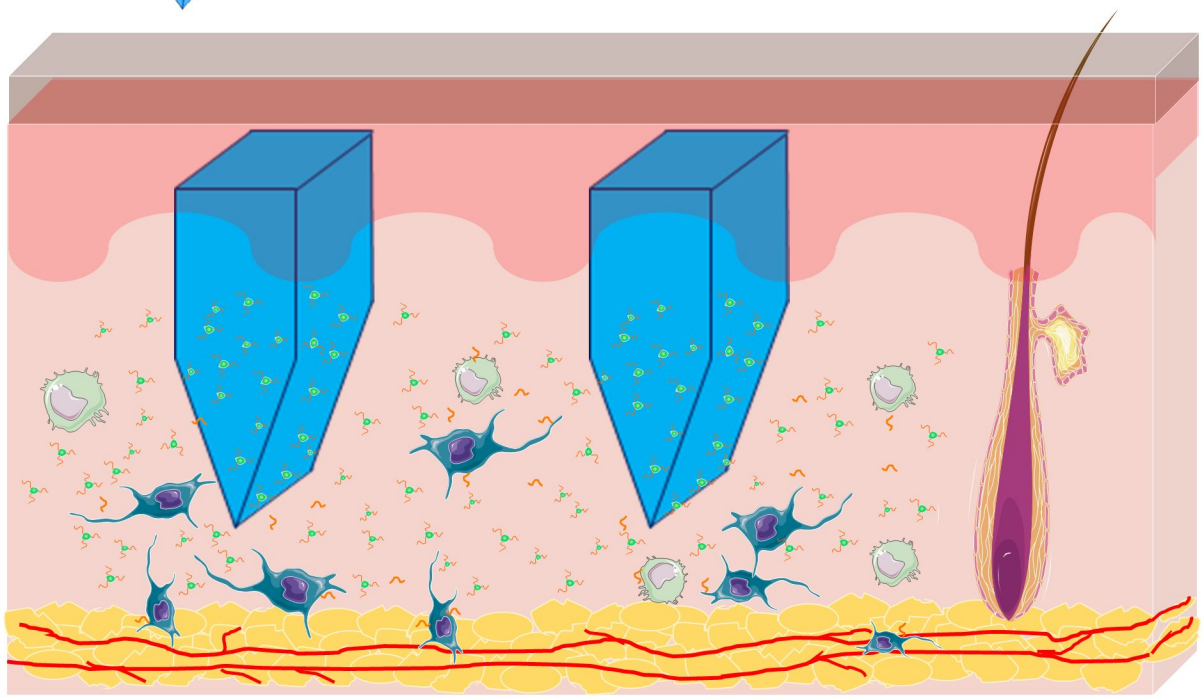
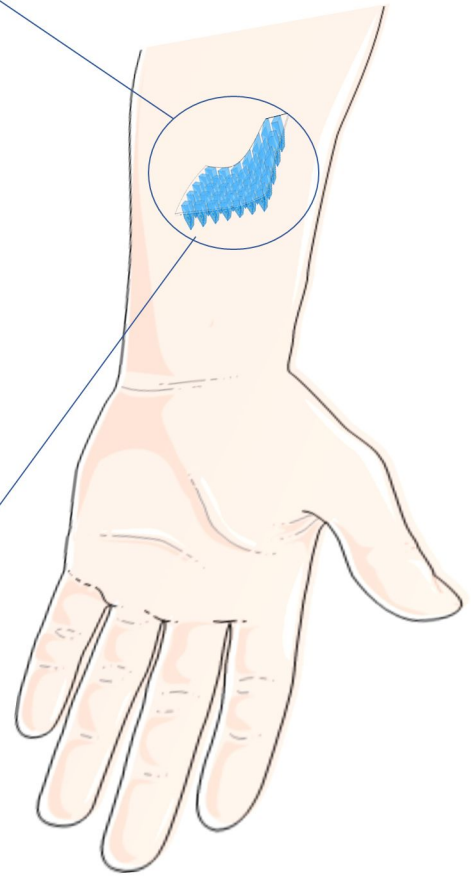
Microneedle patch





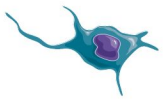


Antigen-loaded nanoparticles

Macrophage

Blood vessels

Antigens



Langerhans cell

Hair follicle

Microneedle patch

Adipose tissue