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An Idea of Using Microneedles for the Targeted Drug Delivery to Overcome the Blood Brain Barrier for the Treatment of Brain Diseases

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Abstract

Drug delivery and vaccination have become the most challenging issues recently in the patients who suffer from severe form of central nervous system diseases along with mild or severe bleeding disorders. In conventional drug delivery system, drug delivery through blood brain barrier remains a big challenge. Additionally, administration of such drugs by syringe injection has many limitations such as pain at the site of injection, infection, hemorrhage, anxiety as well as high cost and incompetency towards patients. Therefore, the proposed hypothesis focusses on the fabrication and development of solid, water soluble and biodegradable microneedles which aims to overcome the disadvantages of conventional drug delivery system. These microneedles loaded with calcium phosphate nanoparticles easily combine with mononuclear phagocytic cells (macrophages/monocytes) which serve as nanocarriers to deliver the drug at the site of interest for treating the inflammatory central nervous system diseases. The present idea expresses that microneedles can play a significant role to inject the ^{45}calcium phosphate nanoparticles (radioisotope labelled) sub-dermally where they are engulfed and processed by macrophages. These nanoparticles loaded macrophages from the peripheral circulation follow the chemokine gradient secreted from the brain parenchyma to eventually enter inside the brain parenchyma and reach the site of ailment. Moreover, they can later secrete these particles and engage themselves in mundane immune replication. In summary, this work explores the potential of microneedles combined with macrophages as a carrier for targeted drug delivery to the diseased area of the brain.

Keywords: Microneedles; Blood brain barrier; Anticancer drug; Macrophages; Targeted drug delivery; Calcium phosphate nanoparticles; Cerebralspinal fluid

Abbreviations: BBB: Blood Brain Barrier; BMP2: Bone Morphogenetic Protein 2; CaPNs: Calcium Phosphate Nanoparticles; CCR: Chemokine Receptors; CD62L: L-selectin; CNS: Central Nervous System; CCL-2 and CXCL-10: Chemokine Ligands, Hsp70: Heat Shock Protein 70; MAC-1: Macrophage Receptor; Ma/Mo: Macrophages/Monocytes; MNs: Microneedles; PSGPL1: P-Selectin Glycoprotein Ligand 1

Background Problems

For the past many decades, the use of syringe injection for drug deliveries has presented many drawbacks on the health of patients who are suffering from central nervous system (CNS) diseases accompanied by severe and mild form of blood disorders (such as AIDS, hepatitis, hemophilia, and sepsis) [1]. Moreover, the use of syringe presents a serious issue regarding probability of infection and inflammation at the site of injection together with occurrence of pain and anxiety, which has drawn attention towards some alternative mode of drug delivery and incompetency towards patients [2]. Additionally, the use of syringe also presents a serious issue regarding waste management [3]. In view of these constraints, we present an idea of using water soluble and biodegradable gelatin made solid microneedles (MNs) instead of syringe injection. The MNs are arrays of micro-tips ranging from 25-2000 μm in height, being attached to a base-supporting patch shown in Figure 1. MNs are short enough to just penetrate the skin barrier of stratum corneum which minimizes the invasion resulting in the significant reduction of the pain [4]. Moreover, the use of MNs doesn't require any professional training which makes them a fascinating tool in medicine. But, practical application of these MNs requires attention towards certain issues that are linked to the whole process of targeted drug delivery. For instance, blood brain barrier (BBB) is a significant area of concern due to its anatomical obstruction in the drug delivery [5,6]. The BBB is formed by tightly connected brain capillaries composed of endothelial cells with tight junctions, pericytes, astrocytes, and basal membrane, which protect the brain from hazardous substances and separates the brain from the circulation system [5]. Most of the CNS disorders, such as neuroinflammation, degeneration and tumors, are difficult to be treated, mainly because of the presence of BBB. Moreover, the restriction imposed on the infiltration of the drug molecules due to their size is the biggest challenge to allow successful delivery; since only small molecules that have a molecular weight less than 400 Da or are lipid soluble can cross the BBB. Thus, 95% of the molecules fail to reach at the desired site for the treatment [6]. In view, there occurs an urgent need to overcome the BBB for increasing the efficiency of treatments.

Several advances have been made to explore the properties of BBB for effective treatment of brain diseases, in which nanodrugs are specifically transferred by the nanoparticles as carriers and using non-invasive techniques to enhance brain drug uptake. Recently, the application of viral vectors for delivery in patients with brain diseases has been widely investigated into mouse models [7]. But, severe side

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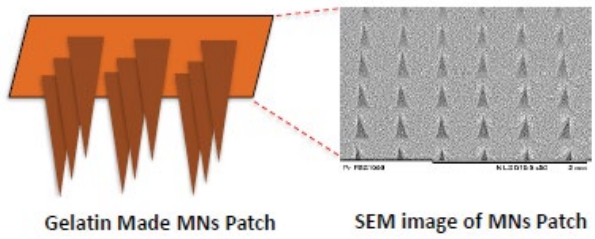

Figure 1: Schematic diagram of gelatin made MNs patch, left panel represents the typical structure of MNs patch, right panel shows the scanning electron microscope image of MNs patch.

effects have been reported (e.g. immune response and insertional mutagenesis), which limit's the use of virus vectors for the treatment of the brain diseases. Instead of using virus vectors, cell-based vehicles have also shown significant role in the targeted drug delivery [8-12]. These studies have reported that macrophage/monocytes (Ma/Mo) have a natural ability to penetrate the BBB and traffic through the brain-ventricular choroid plexus, hence, can travel the long distances [8]. The macrophages owing to their several advantages such as easily procurement from the patients and involving low risk due to their natural accumulation in response to brain diseases are being preferred these days as a drug delivery vectors in both diagnostic and therapeutic oncological applications [9]. However, chemical property of the drug which is loaded inside the macrophages has certain issues. In most of the cases, these drug particles are not biodegradable, hence, after being engulfed by the macrophages, particles usually remain in the brain for a long time [10]. Therefore, non-biodegradable materials which increase the likelihood of acute or chronic toxicity must be replaced by some alternative materials [11]. Hence, we propose the use of biocompatible calcium phosphates nanoparticles (CaPNs) as a nanodrug in the present work. MNs can be loaded with CaPNs which has been shown as a chemotherapeutic agent for the treatment of cancer [12-14], which can be delivered transdermally. Once the CaPNs releases into the peripheral circulation, macrophages can engulf, process and deliver them at site of ailment inside the brain.

Hypothesis

In the present hypothesis, we demonstrate the conception of utilizing MNs as a distribution platform for delivering the CaPNs to macrophages. Once the CaPNs are engulfed by Ma/Mo, these cells can carry these particles from the injected area to the inflammatory/diseased site of the brain. Here, the mechanism of hypothesis is described through the following steps: 1) Uptake of CaPNs by Ma/Mo through MNs injection; 2) Migration of CaPNs loaded Ma/Mo from skin to the brain parenchyma; 3) Mechanism of the CaPNs loaded Ma/Mo infiltration in brain overcoming BBB; and 4) Mechanism of drug release (CaPNs from the macrophages) at the inflammatory/diseased site of brain.

Detailed description of above mentioned steps is as follows:

1) Uptake of CaPNs by Ma/Mo, delivered through MNs transdermally: We use MNs-based transdermal delivery of CaPNs to assist the Ma/Mo cells for the targeted drug delivery. There are two possible routes of drug penetration across the intact skin, namely the transepidermal and transappendegeal pathways [15,16]. Ionic substrates follow the transepidermal pathway, which involves the passage of drug molecules through the stratum corneum and being released into the peripheral circulation. Since CaPNs have been released into the serum, ionic interaction enhances the adsorption of serum proteins on surface of CaPNs. Such serum proteins adsorbed on the surface of CaPNs are easily recognized by the macrophage surface receptors, hence, nanodrug gets engulfed by the macrophages [17]. Phagocytic recognition by the surface receptors of the macrophages (known as Opsonin proteins) is influenced by the both adsorbed protein composition and structure of the CaPNs [18].

2) Mechanism of CaPNs loaded Ma/Mo migration towards the brain parenchyma: In the brain, since there are no lymphatic vessels, interstitial brain fluids interact with peripheral fluid which circulates at the cribriform plate and choroid plexus. CaPNs loaded Ma/Mo present in the peripheral fluid can easily detect antigens originating from the brain. This detection triggers the peripheral control of brain immune functions especially in case of inflammation and brain malignancies, and brain parenchyma produces chemokines, forming chemokine gradients which mediate migration of macrophages into brain. The chemokine receptors (CCR) expressed on Ma/Mo such as CCR2, CCR1, CCR5, L-selectin (CD62L), macrophage receptor (MAC-1), P-selectin glycoprotein ligand 1 (PSGPL1) and other molecules promote adhesion of macrophages to endothelial cells of BBB and help in transmigration [19]. The CCR2–CCL2 (chemokine ligand) axis has been extensively reported as a chemokine pathway involved in the locomotion of peripheral Ma/Mo and infiltration into the brain through perivascular spaces [20].

3) Mechanism of the CaPNs loaded Ma/Mo infiltration in brain overcoming BBB: Most of the macrophages enter the brain at the post-capillary venules. Monocytes infiltrate the CNS by first migrating to BBB and adhering to its endothelial lining of blood vessels, and then cross into the brain parenchyma through perivascular spaces. Monocytes express several cell surface molecules, such as CD62L, MAC-1, PSGL1 and other molecules [21]. These cell surface molecules promote adhesion of Ma/Mo to endothelium in the CNS. High expression of CD62L on monocytes to gain access into the inflammatory sites. Moreover, another pathway for leukocyte migration into the brain is the blood-cerebrospinal fluid (CSF) barrier [22]. This CSF barrier expresses chemokines and adhesion molecules, mainly mediating transmigration of macrophages which has the natural ability to target the areas of pathology.

4) Mechanism of drug release (CaPNs from the macrophages) at the inflammatory/diseased site of brain: Macrophages have natural ability to reach the site of pathology hence, can deliver the drug at the specific diseased area. Once reached the target site they release CaPNs through the mechanism as follows: they transport these nanoparticles to the lysosomes which digest in the endosomes/lysosome complex, resulting in the high concentration of Ca^{+2} and PO_4^{-3} ions [23]. The complex will become inflated and get ruptured due to the change of osmotic pressure, thus, releasing the Ca^{+2} into the cytoplasm. Release of the excess Ca^{+2} ions from the dissolved CaPNs will also trigger exocytosis of Ca^{+2} into cellular matrix in the surrounding of infected area [8,14,24], which will lead the apoptotic events in to the infected cells [25]. Peripheral macrophages recruited in the brain also mundane the immune response by phagocytosis of infected cells and hence, amplify the immune mechanism significantly. In the case of malignancies macrophages not only help nanodrug to release at the tumor site, but also increase the depth of infiltration into the tumor cells. All the mechanism is illustrated in Figure 2, which is proposed to be tested on rat model of glioma.

Testing of Hypothesis

To test our hypothesis on rat model of brain tumor, we propose

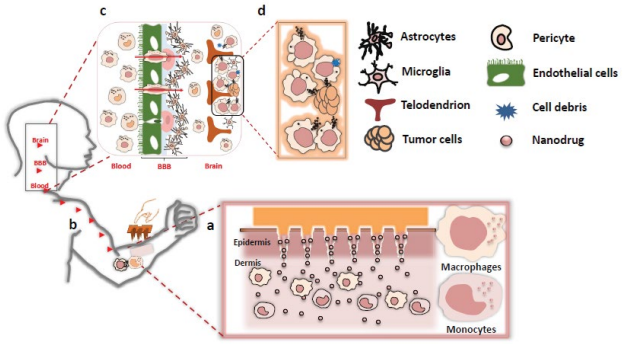

Figure 2: Schematic diagram explaining the mechanism of targeted drug delivery with MNs (a) transdermal delivery of nanodrug/CaPNs with MNs (b) nanodrug engulfed and carried to BBB by macrophages/monocytes (c) infiltration of nanodrug loaded macrophages inside the brain parenchyma (d) drug released by macrophages at tumor site and phagocytosis of tumor cells and cell debris.

synthesis of nanodrug which is made up of custom-made CaPNs by isotope labelling with 45calcium [26]. Size of CaPNs ranges from 100-200 nm [27]. We will make use of MNs as a delivery platform for the CaPNs. These nanoparticles will be incorporated inside the MNs. The most common method for fabricating nanodrug encapsulated biodegradable MNs is micro-molding. This procedure includes pouring of gelatin polymer mixed with CaPNs onto a silicon negative mold and followed by the centrifugation at 3000 rpm to compact the gelatin into mold cavities. Thereafter, silicon negative mold is further poured with pure gelatin solution to form the base substrate. The samples will be allowed to dry at room temperature for 1 day. Finally, the drugs-encapsulated MNs can be obtained by manually removing from the silicone mold using an adhesive tape. We will check the mechanical strength of a single MN by using the force analyzing machine to prevent its mechanical failure during the injection.

Numerous in vivo models of brain tumors have been developed in rats [28]. In this study, we propose in situ glioma rat model. Nontoxic concentrations of CaPNs will be given to the test group and vehicle gelatin will be given to the control group. Most of the drug molecules cannot cross the BBB because of their larger sizes, complex structures and lack of specific transporters. But, if engulfed by macrophages, the drug substances can be delivered at the targeted pathogenic brain area. Peripheral Ma/Mo cells carry drug from the site of drug injection and aggregate at the site of inflammation and malignancies in the brain parenchyma. Macrophages with the engulfed CaPNs reach the tumor sites and start their phagocytic action. The proposed nanodrug will be labeled with 45calcium radioisotope to enable continuous monitoring of its location through both single photon emission computed tomography (SPECT)/positron-emission tomography (PET) [29].

Many research groups have already suggested that the drugs containing nanoparticles can be released at the pathological sites through macrophages [8]. Drug release by the macrophages at the pathogenic site has been described via two processes. In the first mechanism, drug gets diffused in the cell and is released into the extracellular space using passive or active transport mechanism. Whereas, the other process suggests that the drug could be released by exocytosis into the extracellular environment. However, both these processes work in the synchronous manner to release the drug from macrophages. As a result, these two processes cause simultaneous release of drugs to amplify the immune response significantly, at the pathogenic site. SPECT/PET will be carried out every 2 hours to find out the location of Ma/Mo cells carrying the drug [30]. In addition, whether these macrophages cross the BBB into the brain parenchyma can be identified through the SPECT/PET imaging.

Additionally, to increase the permeability of the BBB to facilitate the scavenging action of peripheral Ma/Mo cells inside the brain, it is proposed that serotonin/bradykinin will be injected using MNs which has been reported to increase the permeability of BBB [8]. We will also monitor the serum protein profile of bone morphogenetic protein 2 (BMP2), chemokine ligands (Ccl2 and CXCL-10), and heat shock protein 70 (Hsp70) in control and test groups as a potential diagnostic tool of glioma [31]. Further, we will perform magnetic resonance imaging (MRI) for the estimation of tumor volume after the injection of nanodrug in control and experimental rat groups [32]. MR spectroscopy will also be performed to monitor the potential marker of glioma such as creatine phosphate continuously to monitor the effect of drug on tumor. The survival times of rats in each group would be calculated from the day 0 since tumor inoculation to the day of death. Later, the presence of the macrophages at the site of ailment inside the brain can be investigated with the immunohistochemistry of the brain tissue sections. Glioma sections, are then, stained with hematoxylin and eosin to evaluate the apoptosis of tumor cells. The whole mechanism of testing the hypothesis is pictorially illustrated in Figure 3 which systematically explains the encapsulation of nanodrug in MNs, delivery of drug in rats and all the processes followed throughout the experiment.

Implication of the Hypothesis

The development of MNs patch should necessarily be safe and bio-compatible. MNs can be one of the most efficient vehicle for the vaccination and targeted drug-delivery in the treatment of brain diseases. Moreover, painless vaccination along with reduced bio-hazards would make them more appealing than syringe injections. Issues or challenges such as amount of drug that should be loaded, number and length of microneedles in a single patch etc. that can influence efficient use of MNs will also be a matter of be concern [33]. Though MNs which are currently being used for drug delivery since made up of non-biodegradable materials such as silicon, ceramic, glass, metal, and organic materials (like polymer, hydrogel) have higher toughness in comparison to proposed MNs and allow facile insertion of needles into the skin; yet their waste management is still a major issue to be looked upon [34]. Moreover, the size of MNs imposes a restriction on their use for the delivery of molecules that are having excess dimensions on the micrometer scale.

But, this synthesis of MNs does not end the process, in fact it has prompted us to carry out the investigation regarding efficient drug delivery by the macrophages. And, the efficiency of drug delivery is determined by the ability of these macrophages to engulf the nanodrug and their number infiltrating the BBB. Surface receptors of these macrophages cause the phagocytosis of CaPNs and this process of

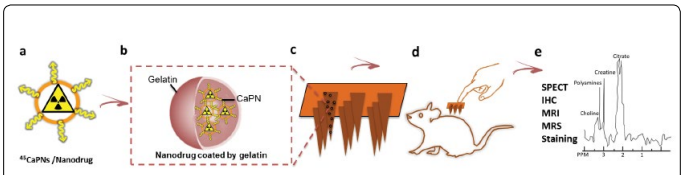

Figure 3: Schematic diagram for the testing of hypothesis on rat model of glioma (a) radioisotope Labelled ^{45}Calcium Phosphate Nanoparticles (45CaPNs) (b) Design and synthesis of biodegradable nanodrug/CaPN encapsulated microneedle patch (c) nanodrug loaded MNs patch. (d) *In vivo* drug delivery with MNs (e) *In vivo* nanodrug imaging and analysis techniques.

phagocytosis can be enhanced by conjugating the surface-receptor ligand-protein (which is known as opsonin i.e. C_3b) to the CaPNs [35]. Furthermore, efficiency of phagocytosis can also be improved through the gelatin used for encapsulating the CaPNs in this work. The positive charge of gelatin attracts the negatively charged macrophages and increases the conjugation which in turn improves the whole phagocytic mechanism.

In summary, the present work emphasizes the significance of these biodegradable MNs for effective and targeted drug delivery. The proposed hypothesis intends to achieve efficient transdermal injection of the drug along with enhanced ability of macrophages to engulf the drug and infiltrate the BBB. Such type of targeted drug delivery that can be made possible through these MNs, will certainly influence the futuristic treatment of brain diseases.

Competing Interests

The authors declare that they have no competing interest.

Authors Contributions

LA and MHC brain stormed and developed the Idea. LA and SKV drafted the manuscript. SKV, MHC and TS contributed in the development of idea and edited the manuscript. All the authors read and approved the final manuscript.

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